2017

Phenotyping Temperature-Sensitive Coronaviruses

Amani Eddins
Loyola University Chicago

LOYOLA UNIVERSITY CHICAGO

CHARACTERIZING TEMPERATURE-SENSITIVE CORONAVIRUSES

A THESIS SUBMITTED TO

THE FACULTY OF THE GRADUATE SCHOOL

IN CANDIDACY FOR THE DEGREE OF

MASTER OF SCIENCE

PROGRAM IN MICROBIOLOGY AND IMMUNOLOGY

BY

AMANI T. EDDINS

CHICAGO, ILLINOIS

DECEMBER 2017

ACKNOWLEDGMENTS

I would like to thank Dr. Susan Baker, PhD, for her guidance throughout this process. I would aslo like to thank Dr. Baker for pushing me to not only become a better scientist but also to remember no matter what in life you choose to do, ensure it makes you happy. Additionally, I would like to thank my lab family, Rob Mettleman, Matthew Hackbart, Armorant O'Brian, Xufang Deng, and Aaron Volk. I reference them as my lab family because they provided unwavering love and support as I went through this process, and will forever hold a special place in my heart.

I would also like to thank my committee members, Dr. Edward Campbell, and Dr. Kathrine Knight, for their enthusiasm and willingness to join my committee with such short notice.

Last but not least, I would like to thank all of my close family and friends who supported me throughout my journey at Loyola University Chicago. Most importantly, I want to thank my parents for all the love, support, and words of wisdom. Without them, none of this would have been possible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

CoV	Coronavirus
ARDS	Acute Respiratory Distress Syndrome
MHV	Murine Hepatitis Virus
TS	Temperature-sensitive
SARS	Severe Acute Respiratory Syndrome
MERS	Middle Eastern Respiratory Syndrome
PEDV	Porcine Epidemic Diarrhea Virus
NSP	Nonstructural Protein
ROS	Reactive oxygen Species
DBT	Delayed Brain Tumor Cells
FCS	Fetal Calf Serum
MEM	Minimum Essential Media
PFU	Plaque Forming Unit
ORF1A	Open Reading Frame

ABSTRACT

Coronaviruses (CoVs) can cause a range of symptoms; from a mild common cold to life threatening acute respiratory distress syndrome (ARDS) upon infection. CoVs provide an example of the importance of studying emerging viruses. These emerging viruses have the ability to be highly pathogenic and detrimental to the human population. Two prime examples of CoV emergence, SARS-CoV (Severe Acute Respiratory Syndrome) and MERS-CoV, (Middle Eastern Respiratory Syndrome), which exhibits the pandemic potential of emerging CoVs that gain human tropism. This human tropism is gained through mutations that allow for cross species and zoonotic transmission. If there was an outbreak of an emerging CoV strain, not only would that be deleterious to human health but could potentially accumulate billions in health care costs to treat infected patients. Given the increasing potential of an emerging CoV outbreak, there needs to be a state of urgency to develop vaccines that will help protect the human population against current and future circulating strains. The Coronavirdae family of viruses are unique because they contain a highly conserved gene arrangement and function within its viral genome. The purpose of this study is to identify a technique that will allow the identification of attenuating mutations that are present within a functionally conserved region. By identifying conserved targets of attenuation, I can identify mutations that can be applied to the development of a CoV vaccine strain for one or more CoVs. Currently, there are many ways in which we can identify attenuating mutations, and one such way is to identify temperature-sensitive (TS) mutants. A TS mutant will be able to confer a wild type phenotype when incubated under permissive conditions and will confer an attenuated phenotype when incubated in non-permissive conditions. By

chemically inducing mutations and then verifying mutations that confer a TS phenotype, I can identify mutants that contain TS mutations in their viral genome. By using genomic sequencing of these TS mutants, I am able to identify novel attenuating mutations in the CoV genome. TS phenotyping allows for the potential application of the attenuating mutation to current strains, and confer protection, but also identifies targets for mutation that may also attenuate future emerging CoVs. The results gained from this study confirm the use of TS phenotyping and sequencing as an advantageous tool to identify sites that confer attenuation. With the proof that CoVs have the capability to mutate to gain human tropism, resulting in deadly disease, we must begin to prepare for potential pandemic outbreak.

CHAPTER ONE

BACKGROUND

Literaure Review

There has been an ongoing battle between humans and viruses since both have occupied the earth. As obligate intracellular pathogens, viruses have only two main focuses: replicate and disseminate. Humans, however, have developed methods to counteract these viral infections. Our bodies as living organisms have two main focuses as well: identify the pathogen and eliminate it. Typically, our bodies' constantly adapting immune system has the ability to fight off viral infections by controlling the infection to keep them from causing major health issues. But there have been many examples in history in which viruses have won that battle causing major health issues. Within the last 100 years we have numerous documented examples of such viral success, such as the 1918 influenza outbreak resulting in millions of deaths[1], Human Immunodeficiency Virus (HIV) also killing millions[2], as well as SARS-CoV and MERS-CoV infections, which combined have killed thousands[3,4]. Although these RNA viruses are different in their characteristics they harbor, there is one common factor that aided their success to cause so much harm to the human population: un-anticipated emergence. When these viruses emerged from their host species into our naïve human population it resulted in the deaths of many. How can these pandemics be avoided one may wonder? The answer is simple: by studying emerging viruses and making vaccines that can protect against current and potentially emerging strains. One viral group that deserves such attention is coronaviruses, which in the last 15 years have had

two viral strains emerge into the human population with the potential to cause world-wide pandemics.

Coronavirus History and Classification.

CoVs were first isolated in 1965 from human organ cultures from the respiratory tract of an adult with a common cold. *Byone and Tyrell et al.* were able to determine the presence of an infectious agent by inoculating human volunteers with the media from these cultures, which resulted in colds developing in these patients[5]. Simultaneously, another group was able to grow a virus that seemed to have "unusual properties" from samples obtained from medical students who also had colds[6]. This virus was named 229E. In the late 1960's, *Tyrell et a*l. began to work with not only human strains but also many animal viruses, which included 229E, Mouse Hepatitis Virus (MHV) and a gastroenteritis virus of swine, which would later be named PEDV[5]. Looking at the various strains under the electron microscope he noted that the particles were pleomorphic, membrane coated and covered with club-like projections on the surface. This new family of viruses, having a crown-like appearance, was officially named the Coronaviridae. As further studies began to look more in depth at the pathology and the epidemiology of coronaviruses, the number of identified animal CoVs grew at a rapid rate. Researchers were able to isolate CoVs that caused disease in many animal species, which include but are not limited to cats, dogs, rabbits, mice, rats, chickens, turkeys and most importantly bats[7]. Fast forward to current day, using sequence analysis, researchers have been able to identify four subfamilies of CoVs that include, alpha, beta, gamma and delta CoVs (Figure 1). Currently, there are only six CoVs that infect humans: alpha Co's 229E, NL63 and beta CoV's OC34, HKU1, SARS-CoV, and MERS-CoV (Figure 1). Of the viruses that infect humans, the latter two listed are the most pathogenic.

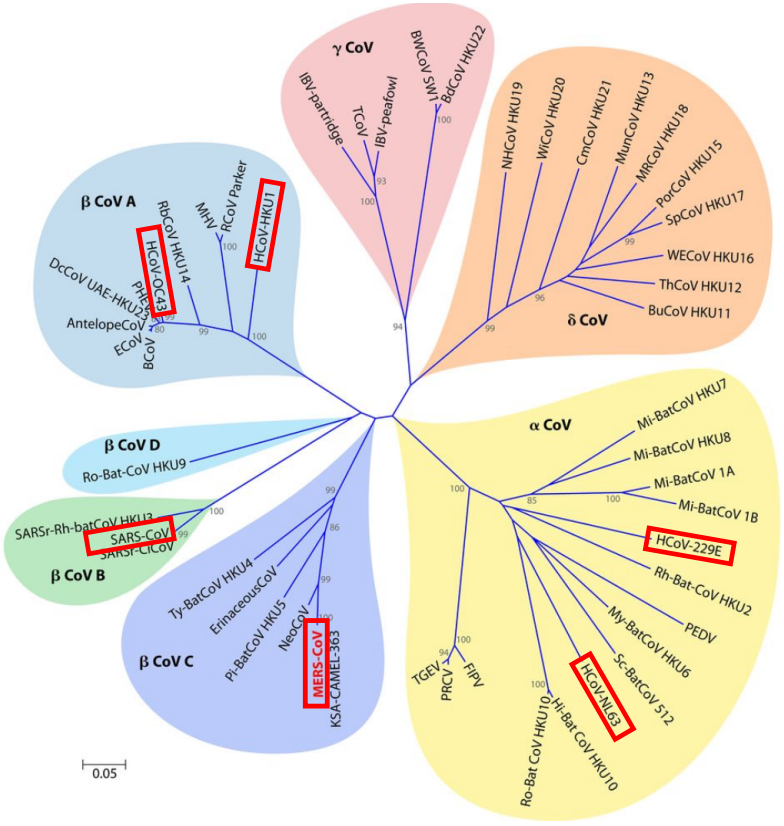


Figure 1. Phylogenic Tree of Coronavirus Family. Above depicts numerous strains of viruses that have currently been identified to belong to the four CoV subtypes: alpha, beta, gamma, and delta. Highlighted in the red boxes are the current strains that have been identified to infect humans. The phylogenic tree was comprised of partial nucleotide sequences of RNA-dependent polymerase. Modified from Chan et al.[8]

CoV Structure and Replication.

CoVs are spherical, enveloped viruses, which house a single-stranded, positive sense RNA genome. These enveloped virions are studded with spike proteins on the surface. The surface of CoVs are made up of membrane proteins and a host derived envelope. Some strains also contain a hemagglutinin-esterase protein that is needed for releasing the viral genome during infection. Inside this enveloped virion is the positive strand RNA genome covered in nucleocapsid protein (Figure 2).

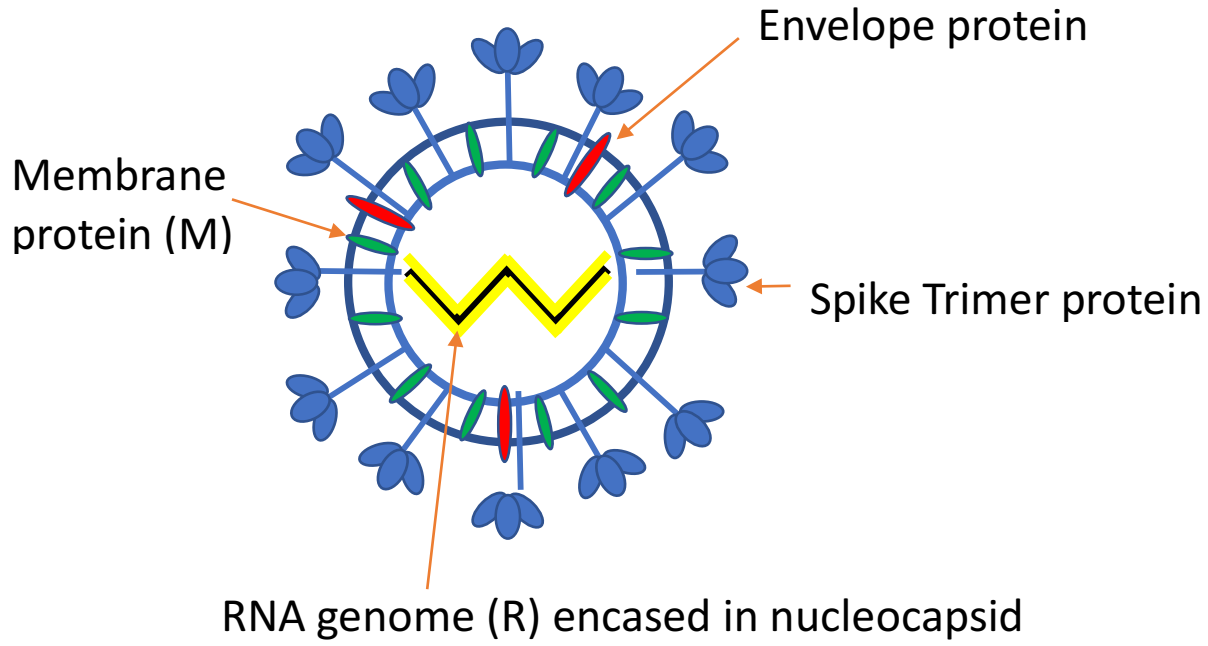


Figure 2. Schematic diagram of the typical virion structure for a Coronavirus. CoVs produce a spherical shaped virion that contains a single stranded RNA genome that is encased in nucleocapsid protein. (S) Spike protein is a transmembrane protein that forms the spike-like projections that interacts with host's surface receptor to allow endocytosis to occur. (M) Membrane houses the viral RNA. (E) Envelope protein that is derived from the host and also serves as a barrier for protection. (R) Viral RNA genome that encodes all of the structural and non-structural proteins used during the viral lifecycle to produce and infectious virion. (N) Nucleocapsid protein covers the viral genome and protects the RNA when injected into the host cell cytoplasm[9].

Upon viral infection, the virus enters a cell through receptor-mediated endocytosis or fuses directly at the cell membrane for entry into a host cell[1]. The genomic RNA, once released into the cytoplasm, is translated into two large polyproteins termed ORF1a/b, which encode the replication machinery needed for viral replication and are processed by the virus-encoded autocatalytic non-structural proteins. The genome also encodes structural and accessory proteins at the 3' end[10,11]. Although all of the functions of the non-structural proteins (NSPs) that CoV's genome encode have yet to be identified, it is known that some of the NSPs will interact with the host endoplasmic reticulum (ER) allowing the formation of double-membrane vesicles (DMVs).

Inside these DMVs, other NSPs combine to make replication complexes and allow for genomic replication. The structural proteins, viral genome, nucleocapsid and viral envelope are assembled at the ER-Golgi intermediate compartment. Once assembly of the new virion is complete, the newly infectious viral particle is released from the cell [12,13] (Figure 3).

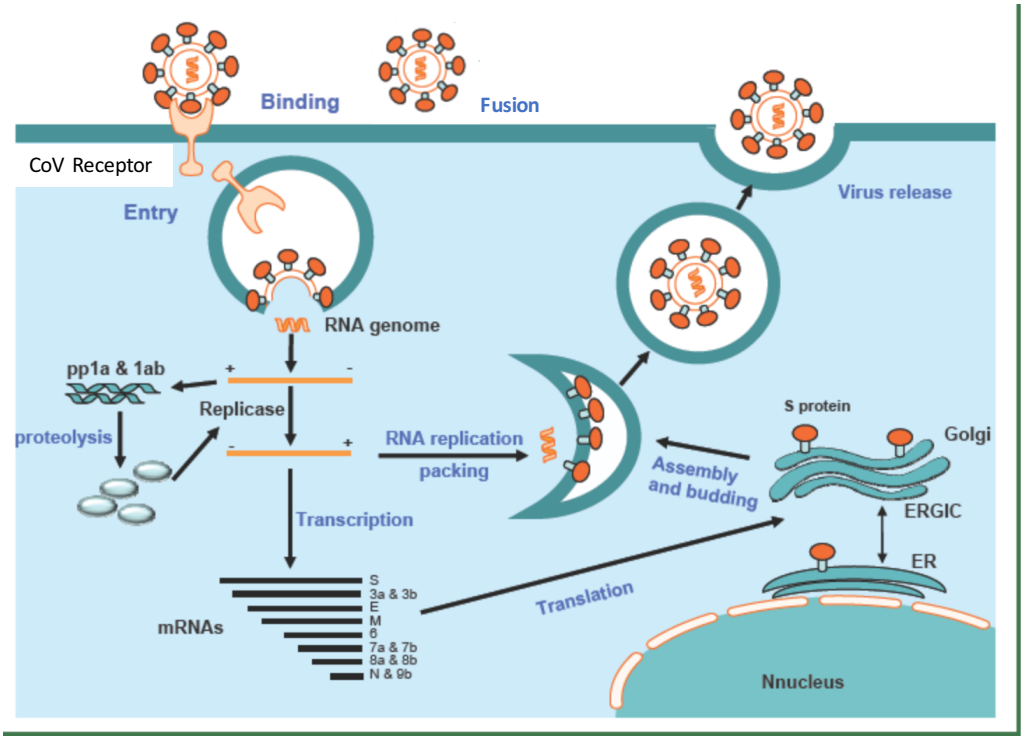


Figure 3. Coronavirus Replication Cycle. Depicted above is the general CoV replication cycle. Replication initiates with receptor mediated endocytosis, or fusing at the cell membrane. Upon entry into the cell the viral genome is released into the cytoplasm where the positive strand genome is translated to make two large polyproteins that encode the viral replicase machinery. Following the production of the replicase machinery, viral transcription occurs in double membrane vesicles derived from the ER-Golgi complex. Virions are packaged and matured within the double membrane vesicles, as they travel towards the host membrane. These vesicles that contain the infectious virion is then released from the cell. Modified from Zhu et al.[14]

Coronavirus Genome.

As members of the Nidovirales order, CoVs have a unique coding strategy: a majority of viral RNA is translated into two large polyproteins and the remainder of the viral genome is

transcribed into a nested set of subgenomic mRNAs[10,13]. The two polyproteins encode 16 non-structural proteins that make up the viral replicase-transciptase complex[15]. Containing a large viral genome is very unique, and this is able to be achieved because CoVs genome encodes an exoribonuclease that functions as a proofreading mechanism and allows for the maintenance of the large viral genome without accumulating deleterious mutations[16,17]. This large viral genome that is characteristic of CoVs is actually highly conserved amongst the CoV's subtypes (Figure 4). The conservation of gene order and function has been key in studying emerging viruses. This highly conserved gene order and function is the key component in many successful CoV infections. It can be appreciated that it is the accessory proteins and mutations in the viral spike that aid the CoV's ability to have a large range of host tropism[18]. However, it is the function of the polyproteins of the viral genome that aids in the efficient replication of these viruses[19]. These viruses have a conserved gene order and function because they arose from a common ancestor: the bat.

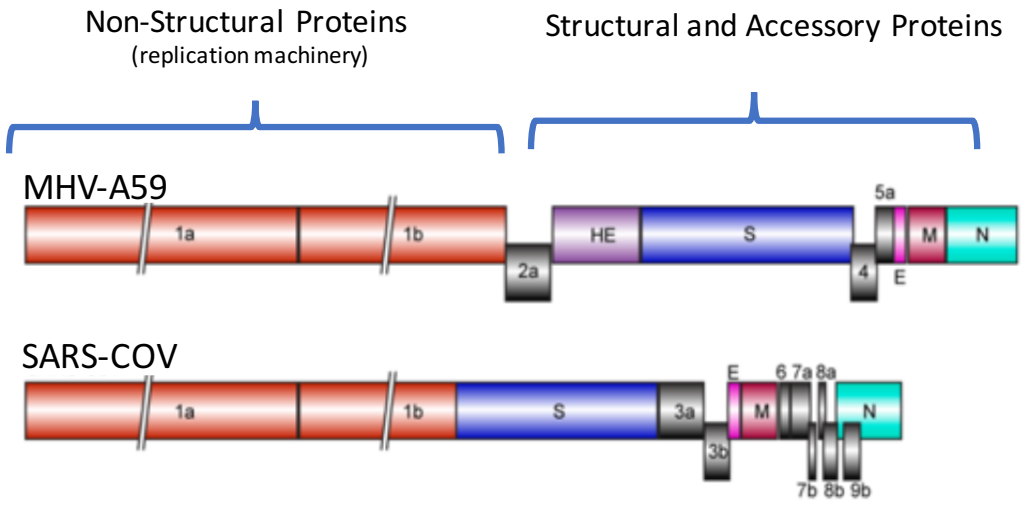


Figure 4. CoVs contain a conserved genome organization. Depicted above are two CoV genomes from two different strains of the virus. MHV is able to infect mice and SARS infects humans, yet they have a very similar genome organization. These two different viruses have very different host tropism but all retain similar replication machinery needed for successful viral infection. Although the other strains of CoVs are not depicted, these are a representation of the

conserved viral genomes organization present in the non-structural protein portion of their genome. Modified from Belouzard et al.[8]

Bats: Coronavirus's Natural Reservoir.

Although CoVs are dangerous due to their pathogenicity, another very important factor that should be considered is their high rate of zoonotic transmission. A study was done to determine which animal species harbored the most novel viral sequences and bats were identified as the number one candidate[20]. Bats are an advantageous host for viruses for a number of reasons. First, these mammals have the ability to occupy diverse niches, which allows these animals to interact with large colonies of other bats and animals[18,21]. The interactions with other bats allow for the transmission of different CoV's to co-infect a single bat, resulting in the opportunity for multiple quasi species to maintain a low-level of infection and recombine, resulting in populations of viruses that can infect a broad range of hosts[22]. Secondly, as humans continue to colonize more of the earth's natural landscape, we begin to force bats to have more interactions with species (i.e. humans and other animals) they may not commonly come into contact with. These interactions aid the transmission of CoVs from bats to other mammals, in which they would not readily infect. A prime example of this gain of host tropism is the emergence of SARS CoV and MERS CoV[22]. Although these CoVs originated in bats, due to mutations and interactions these viruses were able to gain tropism to allow them to infect an intermediate host (civet cats and camels) and ultimately gain human tropism[18,23,24] (Figure 5). Aside from these diverse niches, it also has to be taken into consideration that these animals are migratory in nature. This migratory behavior is very advantageous for an emerging virus, giving the virus the opportunity to have a broad geographical area in which these viruses potentially can disseminate.

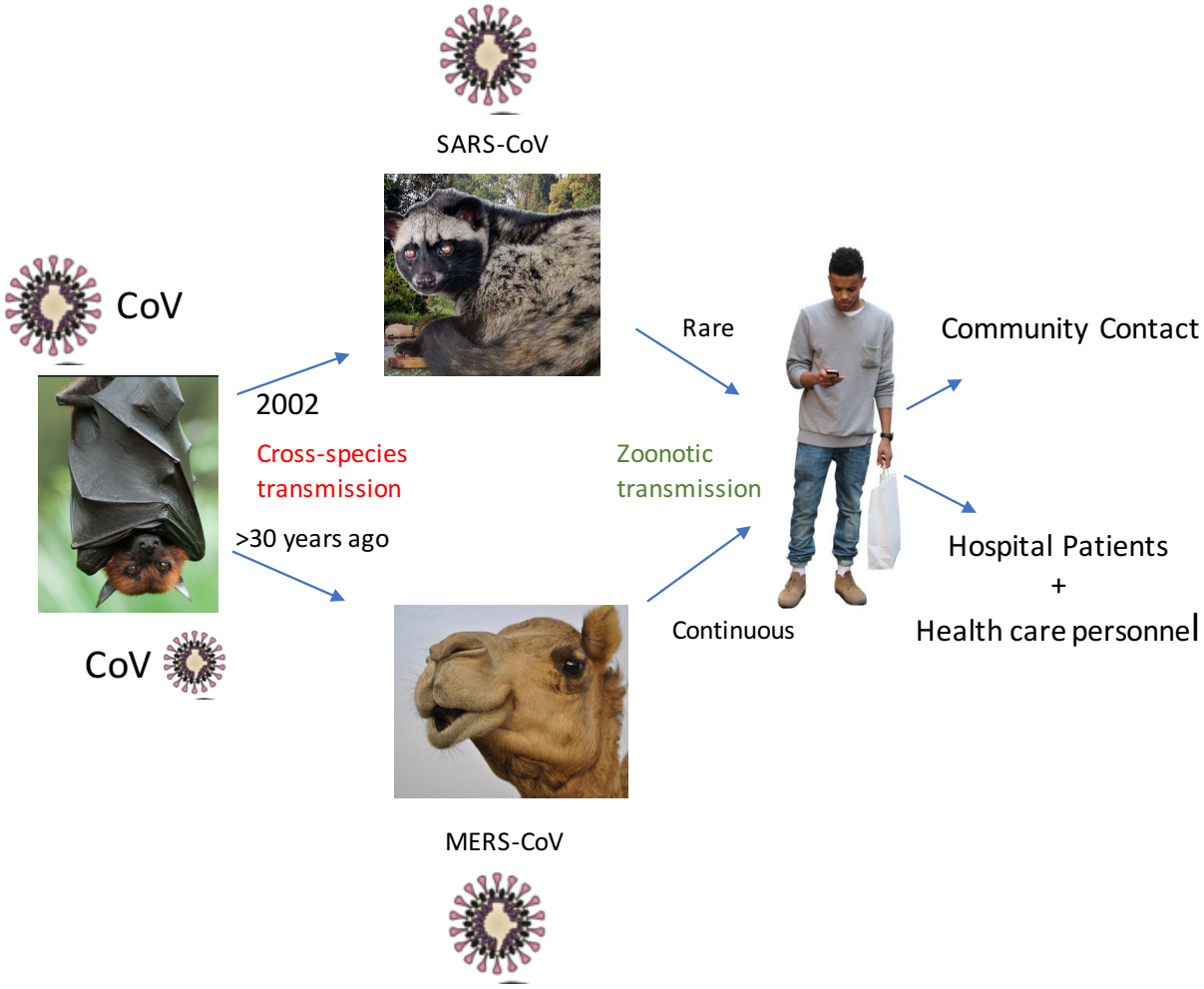


Figure 5. CoVs have the ability to gain human tropism. SARS and MERS to date have been the most pathogenic CoVs resulting in hundreds of deaths combined. These viruses emerged into humans by gaining mutations that allowed the viruses to have cross species transmission and ultimately, use these secondary reservoirs as zoonotic modes of transmission. Depicted above are the routes that SARS and MERS took to become introduced into the human population. Image modified from Wit et al.[11]

It has yet to be fully elucidated as to why bats have the ability to harbor more than one species of CoVs and result in an asymptomatic infection[24]. There are several proposed hypotheses as to why multiple CoV infections seem to have no deleterious effects on the bats they infect. First, it is has been proposed that bats are able to produce high levels of reactive

oxygen species (ROS) which controls genes that limit oxidative stress, resulting in reduced viral replication and pathogenesis[25,26]. Also, studies have shown that bats harbor a modified innate immune response that potentially also contributes to the diverse population of CoVs that these mammals harbor. Bats also constitutively express interferon subtypes that rationally could limit disease while permitting low-level viral infection to happen concurrently[27].

The Emergence of Potentially Pandemic Strains of CoVs.

In the 21st century, CoVs have proven to be a huge pathogenic threat. In November of 2002, the first known documented case of a novel CoV infection occurred in Foshan, China[28]. The patient was admitted with symptoms of fever, dry cough, and shortness of breath. These symptoms then resulted in severe respiratory complications, which exhibited symptoms of a deep lung infection. Upon initial diagnoses, medical care workers thought the patient had a novel form of influenza but upon testing the results were negative. By February of 2003 more than 300 individuals had been admitted to the hospital for the same symptoms with and unknown cause[11]. In March of 2003 the World Health Organization (WHO) established a network of laboratories that were able to identify the causative agent as the emergence of SARS-CoV. By the end of 2003, the SARS emergence resulted in 774 deaths[4]. The SARS CoV outbreak was contained by isolation of infected patients and their contacts.

A decade later in June of 2012 another novel CoV, MERS, was isolated from the sputum of an infected patient in Saudi Arabia[29]. MERS-CoV continued to emerge and spread outside of the Arabian Peninsula due to infected people traveling out of the country. Since then, there has been a slow spread of infection throughout the Middle East. As of April of 2016, there have been 1,728 confirmed cases of MERS, which resulted in 624 deaths in 27 countries[3]. Thus, the need to find a vaccine has become of the upmost importance.

Studying CoVs: Animal Model.

In order to study CoVs in a laboratory safe environment, researchers use the model animal CoV Mouse Hepatitis Virus (MHV). MHV is a beta CoV that causes illness in mice with a high mortality rate. Although the pathogenic characteristics differ from that of SARS and MERS, the high mortality is greater in MHV. MHV has more than 25 isolates, which have may different tissue tropism, but the incidence of enteric infection of MHV strains is most common[30,31]. Some strains of MHV can cause progressive demyelinating encephalitis in mice, which has also been used as an animal model for multiple sclerosis[32]. This CoV is readily found in laboratory settings and can be transmitted via fecal-oral, direct contact, aerosols and fomite transmission. Although vertical transmission has been observed in experimental infections, this does not occur in spontaneous infections[33,34]. In immune competent mice, MHV is very subclinical in its pathology[30,34]. Expression of disease phenotype is directly dependent upon the virus strain and host factors, which include age, genotype, and immune function[34]. Upon studying the histopathology of infected mice, the intestinal tracts exhibit syncytia in the mucosal epithelial tissue[34]. MHV aside from being a murine tropic virus is also a great model because it contains the highly conserved gene order and function that is characteristic of CoVs[19]. MHV is the most highly studied CoV in-vivo and in-vitro at the molecular level. The majority of research projects that use MHV as a model, focus on viral pathogenesis and understanding of how CoVs interact and alter host immune functions. With the development of a reverse genetics system for MHV, the isolation and study of individual mutations in certain viral genes can be assessed. I can then identify mutations that specifically effect virus-host interactions resulting in a better outcome for the host[35].

Identifying Attenuating Mutations.

Different methods are essential for identifying critical mutations that attenuate the virus. One such method of identifying deleterious mutations, is to induce mutations in the virus and screen for TS mutations. TS mutations are unique because under permissive conditions the mutations are able to confer a normal functioning gene product. In contrast, under non-permissive conditions the mutation will result in a gene product that displays a mutated phenotype. Identifying TS mutations can tell you many things about the pathogen. This method has been used primarily as a genetic tool. In 1965, *Edgar et al.* used TS phenotyping to identify the number of genes in a novel strain of bacteriophage T4D[36]. By using chemical mutagenesis and screening the mutant progeny for growth under different temperature conditions, this group was able to identify 37 individual genes the phage contained[36]. These mutations classically aid in the identification of essential genes in an unknown pathogen's genome. However, with the advancement in technology these TS mutations can now be induced and identified by sequencing down to the exact residue conferring the TS phenotype.

Complementation analysis can be used to verify that the mutant progeny observed from chemically inducing TS mutations are unique. Complementation analysis is a special and critical analysis that has been used throughout research in lieu of sequencing to identify not only the unique mutants but to also aid in predicting where in the genome these mutations may be located. *Edgar et al.*, after identifying the chemically induced strains of T4D bacteriophage were TS, used complementation to verify they were unique mutants and to begin to map where these mutations were located[36,37]. Complementation analysis involves the co-infection of two mutated phage that are grown under non-permissive conditions and assessed for successful phage production. Individually the phage are unable to grow at non-permissive conditions[38]. Thus, if

the two strains of phage contained the same mutations, they would not be able to complement or "over-come" the attenuating mutations because they are located in the same region. However, if the phage contains different mutations these mutant phage would be able to propagate infectious progeny because the phage were able to complement each other due to the mutations being located in different locations thus allowing the phage to "over-come" the attenuating mutations each phage contains individually.

Phenotyping CoVs: MHV.

In order to design a method to develop a vaccine strain that contains mutations that may help to protect against current and future strains emergence, we first must identify areas of attenuation in conserved regions of the viral genome. As I previously stated, the one common factor that aids in CoVs success in infection is also a chink in the armor, the highly conserved gene order and functions amongst the CoV family. By using different techniques, one may be able to identify key residues that attenuate the virus in an essential gene. For example, currently in vaccine development many attenuated strains contain a mutated structure/surface protein. Although this is typically the most common method, it also has to be taken into account that surface proteins tend to undergo a high rate of mutation to evade the immune response and are typically very tolerable of mutations. In contrast, finding a mutation in a conserved region that does not tolerate mutations presents the opportunity to potentially identify sites for attenuation that are common amongst current and potentially emerging CoV strains.

TS mutants of animal viruses have been very useful for studying many critical functions of individual viral genes [36,39,40]. These studies have allowed researchers to understand pathogenic, biochemical and pathological characteristics of these viruses with a goal to find an attenuating mutation that can be used to produce a vaccine strain. In 1983, *Koolen et al.* sought

to understand the viral genome of MHV[39]. Prior to his studies, not much was known about coronaviruses nor its genome, but by using this animal model, they sought to identify different viral genes and their roles in the viral life cycle[39]. In order to decipher the contents of the viral genome, a chemical mutagenesis was performed on over 1,284 viral clones[39]. To verify that these mutants were TS, the viruses were allowed to grow under three conditions (33, 37 or 40^{o}C)[39]. If the mutant viruses showed a defect or reduction in titer at 40^{o}C, these viruses were deemed TS[39]. Of these clones, they were able to identify 20 TS mutants, that were classified into two categories, RNA + or RNA -. If the virus was RNA-, the virus was unable to synthesize viral RNA at non-permissive temperatures (40^{o}C)[39]. RNA+ TS mutants were documented to still grow at all three temperatures, but were shown to have a reduction in titer as the temperature increased.

In 1990, *Baric et al.* sought to understand the function of the MHV polymerase genes to better understand how MHV transcription occurs [38]. Taking a similar approach as Koolen ten years earlier, chemical mutagenesis was performed to identify areas in the viral genome that tolerated mutations under permissive conditions[38]. Several chemical mutagenesis experiments were performed to induce mutations throughout the genome. 26 mutant viruses containing genetic mutations were isolated. These mutants were assessed for phenotypes that differ to that of Wild Type virus. All 26 mutant strains were reported to have reduced viral titers and defects in RNA synthesis during temperature shift experiments [38]. Using complementation analysis, this group determined the number of genes or genetic functions that were altered by the chemical mutagenesis. The Baric Lab identified six complementation groups, each representing a hypothesized area of mutation in the viral genome[38]. The temperature-dependent phenotype paired with the reduction in viral titer led the Baric Lab to hypothesize that the mutations were in

the viral replication machinery. Of these mutants that were derived, LA16 and NC2 stood out as potential candidates that should receive further studies. LA16 and NC2 are both hypothesized to have mutations in the replication machinery, thus giving them their documented TS phenotype. No further analysis was performed to identify the location of these mutations. By identifying the locations of these mutations, potentially I may potenitally be able to identify a novel target of attenuation that is conserved amongst many strains of CoVs. This information will be important in the development of a vaccine strain for protection from current and future stains of CoVs.

Aims and Hypothesis

Over the past 15 years, CoVs have exhibited both rapid and highly pathogenic emergences into the human population, eluding to the potential to cause a world-wide pandemic. Prime examples of this emergence were observed by the emergence of SARS-CoV in 2003[4], which resulted in 774 deaths, followed by the emergence of MERS-CoV, in 2012, which resulted in 624 deaths thus far[3]. These unanticipated zoonotic jumps into the human population highlight the need to produce common methods or identify conserved targets of attenuation that can be used for generating vaccines against diverse CoV species. Although CoVs are infamous for having diversity in host tropism, one common factor of CoVs is a highly-conserved genome order and function. It is important to identify attenuation mutations in conserved genes or residues because it allows for the development of vaccine strains or direct-acting antivirals that can act on current and future emerging CoVs. The goal of this study is to identify conserved targets of attenuation, with the intention of identifying a strategy to make vaccines that can protect against the current circulating strains but also for future coronavirus outbreaks. For my thesis, I propose to identify the TS phenotype of two previously derived TS mutants of MHV, NC2 and LA16, followed by deep genomic sequencing to identify the location of these mutations within the viral genome. I hypothesize that LA16 and NC2 harbor mutations in the viral polymerase machinery conferring their TS phenotype. In AIM 1, I will characterize LA16 and NC2 by performing plaque assays under three different conditions to verify the TS phenotype[40]. In AIM 2**,** I will identify the mutations present in the RNA genome of NC2 and LA16 by genomic deep sequencing. By identifying the mutations in the viral genome, I can further hypothesize whether individual or collective mutations confer a TS phenotype.

Significance

By conducting this study, we will gain information on novel mutations that lead to virus attenuation. TS phenotyping has become a key method in identifying novel gene functions of many unknown pathogens [36,37]. By identifying the mutations that result in a TS phenotype we can begin to investigate how mutations in certain viral genes affect virus-host interactions resulting in a better outcome for the host. In this proposal, we will combine classic genetic phenotyping techniques with current genomic sequencing to identify novel attenuating mutations. By confirming the TS phenotype of these mutant strains of a model CoV MHV, we will be able to confirm the accuracy and reproducibility that chemical mutagenesis can offer as well as establish the stability of the progeny that come from this technique. With the advancement of sequencing we can pin-point mutations that are located in highly conserved regions of the viral genome with much more accuracy than that of classic complementation and gene mapping techniques. Additionally, the information obtained in this study will allow the identification of sites in conserved genes that are important for replication. In the future, this information could be applied to a variety of CoVs to determine if the mutant viruses could be used as vaccines for protection from current and more importantly future strains of CoVs.

CHAPTER TWO

MATERIALS AND METHODS

Virus and Cell lines

Delayed Brain Tumor (DBT) cells were cultured in minimal essential medium (MEM) (Fisher) supplemented with 2.2g $NaHCO_3$, 5% TPB, 2% Penicillin/Streptomycin, 2% L-Glutamine and 5% heat inactivated Fetal Calf Serum (FCS). Cells were incubated at 37^{o}C in 5% CO_2 enriched environment. Media, used for plaque assays, is made of 2X MEM supplemented with $NaHCO_3$, 5% TPB, 2% Penicillin/Streptomycin, 2% L-Glutamine and 2% FCS. During plaque assays cells were incubated in 5% CO_2 at either 33, 37, or 40 ^{o}C. Typically, cells were passaged 1:3 using trypsin-EDTA to disperse cells from culture plates. Our laboratory stock of WT MHV-A59 has been sequenced and propogated in DBT cells[35]. LA16 and NC2 viral stocks were graciously obtained from the Baric Lab[41].

Thawing of Viral Stocks

To thaw the frozen viral stocks, vials which contained supernatant from virally infected cells were flash thawed by incubating in the 37 ^{o}C water bath until 90% thawed. During the incubation period vials were constantly swirled in bath to ensure even thawing and were placed directly on ice once 90% of contents were no longer frozen. Once fully thawed, vials were vortexed to equally distribute the virions within supernatant.

Performing Plaque Assays

Cells are plated into a six-well tissue culture dish at a 5 x 10^5 cells/well confluency. These cells are plated in complete MEM supplemented as indicated above. The cells are then placed into the 37^{o}C incubator for at least 18 hours prior to infection to allow cells to evenly form a monolayer on the bottom of the well. A ten-fold dilution was performed as indicated in Figure 6. Dilutions were performed in incomplete MEM on ice. Culturing media was aspirated from each well and cells were washed in PBS. 300 µl of viral dilution is applied to the well. Cells were incubated at 37 ^{o}C, 5% CO_2 for one hour, rocking plates gently every 15 minutes. During the absorption step, a 1:1 ratio of 2X-MEM and 0.4% Noble Agar was mixed. After the one hour incubation period the inoculum was removed and 2 ml of the 2X-MEM and Noble agar mix was added to each well. The plates were allowed to sit for ten minutes at room temperature (RT) to solidify prior to being moved to the appropriate incubator. Plates were allowed to incubate at the determined temperature for 48 hours. After 48 hours, 2 ml of 3.7% Formaldehyde-PBS solution was added to each well and incubated for 30 minutes at RT. The Formaldehyde-PBS solution/agar solution was removed and plates were washed with tap water. 1 ml of 0.1% Crystal Violet solution is added and allowed to incubate at RT for 15 mins on a rocker. Crystal violet was removed and plates rinsed and allowed to dry overnight. Plaques were then counted by hand.

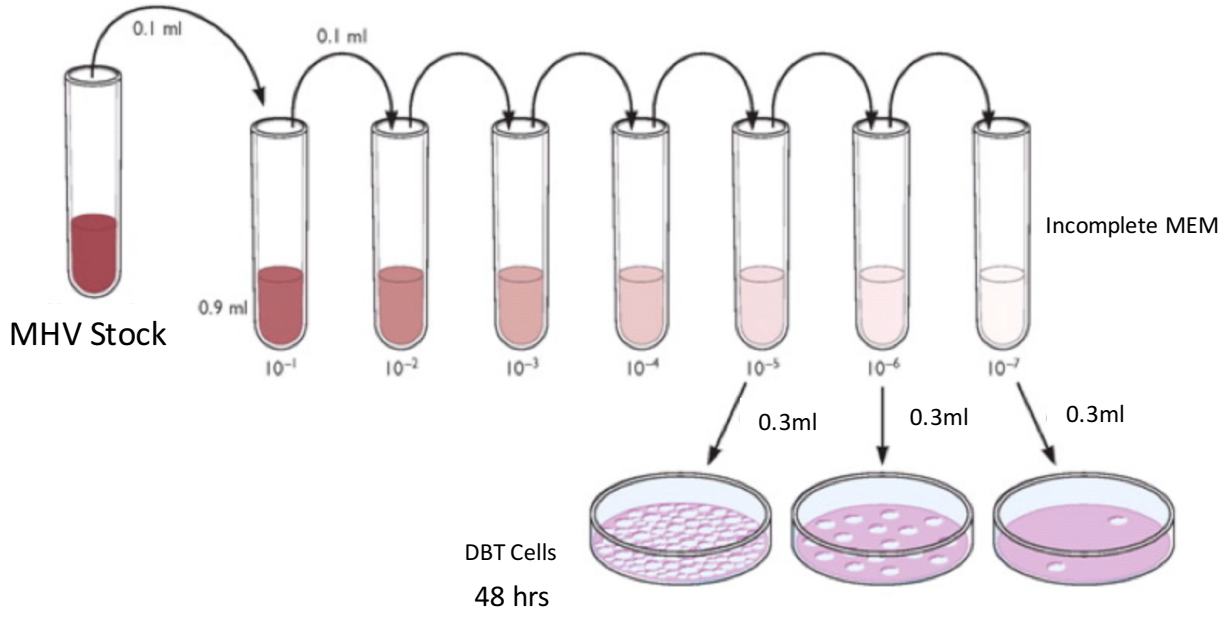


Figure 6. Diluting Viral stock for plaque assay. In order to accurately determine viral titer serial dilutions are performed and applied to DBT cells in sequential order. Viral titer is back calculated based on the number of plaques produced after incubation. One plaque represents the identification of a successful viral infection indicated by the appearance of plaques. Modified from Dulbecco et al[42,43].

Calculating Viral Titer

In order to calculate viral titer, individual plaques were hand counted and documented. Using the following equation, the viral titer was able to be calculated using the amount of inoculum added and the number of plaques produced (Equation 1). By multiplying the number of plaques by the dilution they were formed by 1000ul divided by the amount of inoculum will result in the viral titer.

$$\textbf{(Eq 1)} \qquad \frac{(\#\ \boldsymbol{Plaques})\ \boldsymbol{x}\ \frac{\mathbf{1}}{\boldsymbol{dilution}}\boldsymbol{x}\ (\mathbf{1000}\boldsymbol{ul/ml})}{\boldsymbol{volume\ plated}\ (\boldsymbol{ml})} = \textbf{pfu/ml = Viral Titer}$$

Imaging and Quantifying Plaques

Plaques were photographed after fixation. Images were taken after the indicated incubation time and temperatures using a consistent magnification with a Zeiss Stemi 2000-C dissecting microscope. Photos were then uploaded to Microsoft Photoshop. Once uploaded the lasso tool was used to outline the clear boarder of the plaques. Once the plaque was outlined the image tab was selected and from the drop-down menu the analysis option was highlighted and the record measurement tab was selected. Upon selection of this option, the number of pixels are calculated within the outlined plaque. These represent the area in which the plaque occupies and is used as the area of the plaque. Five individual plaques were assessed at random for each indicated temperature for each strain.

Genomic Deep Sequencing and Sequencing Analysis

In order to obtain viral genomic sequences, DBT cells were infected with LA16 or NC2. After 48 hours of incubation in 33^{o}C, the cellular supernatant is harvested. Supernatant is centrifuged at 5,000g for ten minutes. The viral supernatant was then added to naïve DBT cells to assess the presence of infectious virus production, verified by plaque formation. 1 ml of the cleared viral supernatant was shipped to Kansas State University's Integrated Genomics Facility in which deep genomic sequencing was performed[44]. The consensus nucleotide sequence was provided in a word processor format which was entered into a genomic sequence file in Clone Manager. Once imputed into Clone Manager, the nucleotide sequence alignment function was applied to compare the sequences of LA16, NC2 and WT MHV (MHV full genome geneID reference accession number: NC_001846). The Blossum 65 Alignment function within Clone

Manager was applied to compare and identify amino acid differences between LA16, NC2 and WT sequences.

CHAPTER THREE

RESULTS

Validation of Temperature-sensitive Phenotype of NC2 and LA16

To determine if the viral stocks of LA16 and NC2 obtained from the Baric lab were TS, plaque assays were performed at 33, 37 and 40^{o}C. DBT cells were infected with serial dilutions of the viral stock. The cells were incubated at different temperatures for 48 hours prior to fixation (Figure 7). Temperature sensitivity was assessed by viral titer and plaque morphology at the different temperatures. As a positive control a WT MHV infection was performed as well. It has been well documented that WT MHV, under all three conditions, is able to maintain the same viral titer, but exhibits an increase in plaque size[35,40,41,45]. The increase in plaque size is due to the increase in metabolic rate of the cells under these conditions, resulting in an increase in viral replication, i.e. plaque size. WT MHV at 33, 37 and 40^{o}C exhibit normal plaque morphology with a slight increase in plaque size as the temperature increases (Figure 7A). Although the plaque size increases slightly, it can be appreciated that the number of plaques present remains constant. The viral titer was maintained at 6.5 x $10^{6} \pm 0.40$ pfu/ml (Figure 7B). The images of WT MHV at 33, 37 and 40^{o}C were all taken at the 10^{5} dilution in which the plaques were able to be visualized individually.

Assessing Temperature-sensitive Phenotype of LA16

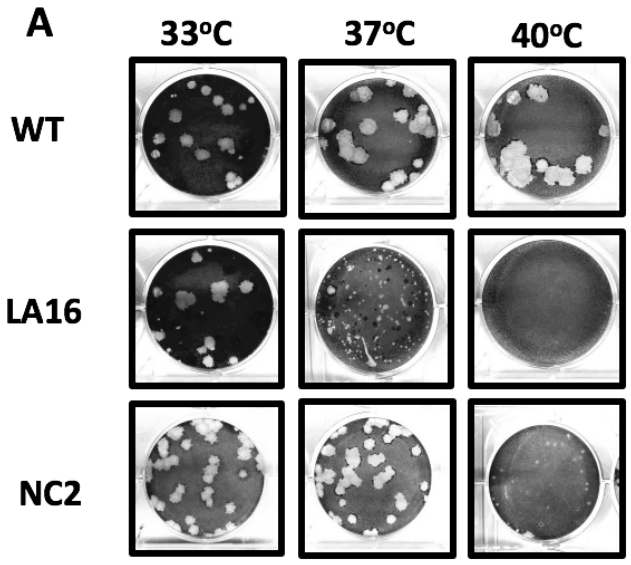
When assessing TS mutant LA16 for replication and plaque formation at 33^{o}C, it can be noted that the plaques have the same morphology as that of WT,in which the plaques appear to be the same size and shape (Figure 7A). The representative image of LA16 at 33^{0}C was taken of

a 10^5 dilution allowing the visualization of individual plaques. The viral titer at 33^0C was 1.2 x 10^7 pfu/ml (Figure 7B). The titer of LA16 at 33°C is similar as the literature documented titer, in which *Baric et al*. documented LA16 to have a 4.9 x 10^7 pfu/ml titer at 33 °C (Figure 7B)[41]. This conservation of viral titer speaks to the integrity of the samples. When assessing LA16 at 37°C the morphology of the plaques can be appreciated at a 1:1000 dilution (Figure 7A). At this temperature, the plaques have a heterogeneous morphology that appear to range from small to pin-point in size. LA16 has a viral titer of 5.3 x 10^4 pfu/ml at 37°C (Figure 7B). LA16's titer is v similar to the documented titer of 2.7 x 10^4 pfu/ml when shifted to 37 °C[41]. A reduction in titer was observed at 37°C compared to the titer at 33°C. Upon assessing the titer of LA16 at 40°C, I was unable to be detect any plaques using our assay, which has the sensitivity to detect 100 pfu/ml. This lack of detection at 40°C as well as the reduction in viral titer further validates that LA16 has retained its TS phenotype as documented in the literature.

Assessing TS Phenotype of NC2

In order to validate that NC2 is TS, as documented in the literature, a plaque assay was performed at different temperatures in which the morphology and viral titer were assessed. At 33°C, NC2 plaque morphology is very similar to that of WT at 33°C. These plaques appeared rather round in shape and the representative image was taken at 10^5 dilution, which was the same as LA16 and WT (Figure 7A). When assessing the viral titer of NC2 at 33°C, the titer was calculated at 1.04 x 10^7 pfu/ml (Figure 7B). This titer correlates with the viral titer documented in the literature as 5.6 x 10^7 pfu/ml[41]. Upon incubating NC2 at 37°C, the plaques maintained their size and similar titer, resembling that of WT. The representative image of NC2 at 37°C (Figure 7A) was taken at a 10^5 dilution. Although there was a ten-fold decrease in the viral titer, the morphology of the plaques remained constant. At 37°C NC2 has a titer of 10^6 pfu/ml. When NC2

was incubated at 40°C, the plaques uniformly changed to a pin-point morphology (Figure 7A). Upon checking the titer of NC2 at 40°C, the titer was 5.3 x 10^4 pfu/ml (Figure 7B). This titer was slightly higher than the documented titer which was 4.0 x 10^3 pfu/ml[41]. The reduction of viral titer and change in plaque morphology, upon the increase of incubation temperature validates the integrity of NC2 as TS, as documented in the literature.



B

Virus	Temperature Sensitive Phenotype	Viral Titer (pfu/ml)		
		33°C	37°C	40°C
WT	NO	10^6	10^6	10^6
LA16	YES	10^7	10^4 *pinpoint	$>10^2$
NC2	YES	10^7	10^6	10^4 *pinpoint

Figure 7. Comparison of Murine Coronavirus strains LA16 and NC2 to asses for Temperature-sensitive defects in replication. (A) Visual representations of plaques that were generated by WT, LA16, and NC2 after incubation in the indicated temperatures. DBT cells were infected with WT, NC2 or LA16 incubated at the indicated temperature and stained 48 hours post infection. These images were representative data from two independent experiments. (B) Summary of the characteristics of WT, LA16, NC2 at different temperatures. Plaque assays

were performed using DBT cell and were allowed to incubate at the indicated temperature (33, 37 or 40°C) for 48 hours.

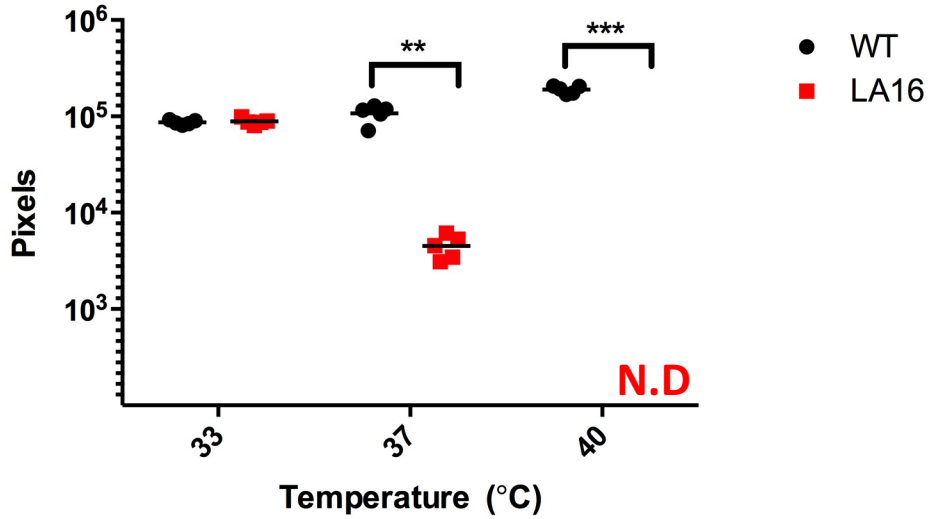


Figure 8. Analysis of WT and LA16 plaque size at different temperatures. Plaque assays were performed using DBT cells. DBT cells were infected with LA16 or WT virus, incubated at the indicated temperature and fixed 48 post-infection.Five plaques were chosen at random and photographed using a fixed camera. These images were analyzed using Adobe Photoshop. Photoshop was used to quantify the number of pixels present in the plaque, which is used to quantify plaque area. Statistical analysis was performed using Student's T test. (**p:>0.005. ***p:>0.0005)

LA16 MHV Exhibits a Reduction in Plaque Size

In order to further assess the TS phenotype of LA16, the plaque areas from the plaque assay shown in Figure 7A were quantified. DBT cells were inoculated with LA16 and WT MHV, and incubated at the indicated temperature for 48 hours. After fixation, images were taken using a camera set at a fixed magnification. These images were then assessed in Adobe Photoshop. Using the lasso tool in Photoshop the outline of plaque was traced. Once the plaque was outlined, Photoshop was able to quantify the amount of pixels within the outlined area corresponding to the area that plaque occupies. Five individual plaques were chosen at random and assessed at each indicated temperature. In order to ensure the plaques were chosen at random, upon fixingthe camera at the correct setting the first five plaques that were unobstructed

were selected and assessed. Upon comparing WT and LA16 plaque sizes at 33^{o}C, the plaques were very similar in size (Figure 8). This also correlates with the morphology shown in Figure 7A. When comparing the plaque size of WT and LA16 at 37^{o}C, LA16 has a significant decrease in plaque size. The reduction in plaque size correlates with the change in morphology (Figure 7A) and a decrease in viral titer (Figure 7B). Due to the heterogeneous population of plaques produced during incubation, the small and entirely cleared plaque population were quantified. LA16 failed to produce plaques within the detection limit of the plaque assay when incubated at 40^{o}C. This data further verify that LA16 is TS.

NC2 MHV May Contain Mutations That Confer a Reduction in Plaque Size

To further validate the TS phenotype of NC2, the plaque area from the plaque assay shown in Figure 7A was quantified. DBT cells were inoculated with NC2 and incubated at the indicated temperature for 48 hours. WT was incubated at 40^{o}C for 48 hours as a positive control. These assays were fixed and images were taken using a fixed camera. These images were then assessed in Photoshop. Using the lasso tool in Photoshop the outline of plaque was traced. Once the plaque was outlined, Photoshop was able to quantify the area with the outlined area. Five individual plaques were chosen at random and assessed at each indicated temperature. The average of plaque sizes produced by NC2 are identified at differnet temperatures as shown in Figure 9A. When assessing the average of the plaques NC2 produces it can be appreciated that the plaque size was maintained from 33 ^{o}C to 37 ^{o}C. Upon incubating NC2 at 40^{o}C, there was a drastic reduction in plaque size (Figure 9A). When comparing the change in plaque size at 40^{o}C, NC2 had a significant reduction in plaque size compared to WT (Figure 9B). This also correlates with the decrease in titer and change in plaque morphology (Figure 7A). From this preliminary

data, I can further validate NC2 is TS and potentially contains different mutations than that of LA16.

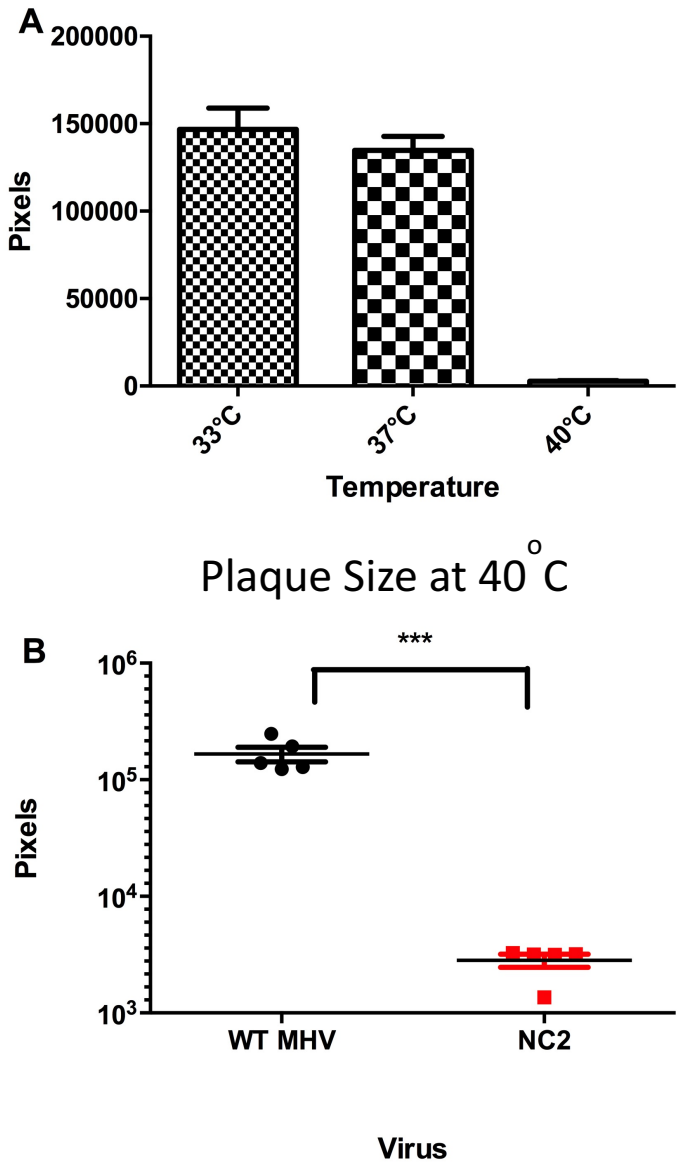


Figure 9. Preliminary Data Quantifying NC2 Plaque Size. (A) Quantification of the average plaque size of five individual plaques of NC2. Plaques size was quantified using Adobe Photoshop. DBT cells were infected with NC2 or WT virus, and were incubated at the indicated temperature and fixed 48 hours post-infection. (B) Quantification of plaque size for NC2 and WT MHV at 40°C. Plaques were incubated for 48 hours prior to fixation and quantification. Statistical Analysis was performed using Students T-test. (p***0.0005)

Deep Sequencing of LA16 and NC2 Identifies Multiple Mutations in ORF1A

Previous data from the Baric lab led to the hypothesis that mutations that conferred the TS phenotype of LA16 and NC2 were located in ORF1A[41]. Both mutants are classified in the RNA- group, which has a defect in viral RNA synthesis[39,41]. The data indicated the RNA synthesis defect furthers supports the hypothesis that these mutations may be located in ORF1A which contains genes essential for viral replication. Sequencing samples were obtained from the supernatant of DBT cells infected with LA16, NC2 or WT at 33^{o}C and incubated for 48 hours prior to harvesting. These samples were harvested from the infections incubated at 33^{o}C to avoid selecting for mutations that were not originally present in the Baric stock. Selective pressure occurs when growing the viruses at non-permissive conditions. The samples were then cleared of cellular debris by centrifugation and sent to a collaborator for genomic deep sequencing. Upon receiving the sequencing data, the results were analyzed using Clone Manager function which allowed for the alignment of the nucleotide sequence. I also used Blossum 65 to align the three genomic sequences at the amino acid level, allowing the identification of nucleotides that result in amino acid changes (Figure 10).

In analyzing the data, I found that LA16 and NC2 collectively contained 13 nucleotide changes in ORF1A. Although there were 13 changes, only eight of these mutations resulted in amino acid changes. There were two mutations conserved between both strains, which I hypothesize may have been part of the original strain of viruses that these mutants were derived from. LA16 contains two unique mutations that resulted in amino acid changes. These mutations were located in Nsp3 and Nsp10, genes which have been shown to be important in viral replication[40,45]. The first unique mutation in LA16 was located in Nsp3, the viral protease. This mutation change was in the second position of the codon, which resulted in an “a” nucleotide

substituted for a “g”. This substitution resulted in the change of the amino acid from glycine to glutamic acid. The second unique mutation was located in Nsp10, whose function has been hypothesized to be a regulator of RNA synthesis by controlling polyprotein processing[46]. This mutation resulted in a nucleotide change located in the second nucleotide position for a codon that coded for a cysteine. Additionally, this mutation resulted in an amino acid that was changed from a cysteine to a tryptophan.

In analyzing the data from NC2, it was noted there were four unique mutations that resulted in amino acid changes. These mutations were located in Nsp3, Nsp4 and Nsp5. Of these four unique mutations, three were conservative, resulting in a substitution of amino acids that have conserved properties. However, there was one unique mutation that was located in Nsp3. This mutation occurred in the first position of the codon, resulting in an amino acid change from Lysine to Glutamic Acid. The sequencing data reveal that LA16 and N2 both contain mutations in ORF1A, may be responsible for the TS phenotype observed in each strain.

Figure 10. Genomic Sequence Alignment of WT, LA16 and NC2 of ORF1A. Using the Blossum 65 (clone manager) function the amino acid sequence was determined and was used to identify amino acid changes resulting from a change in the nucleotide sequence. (Black) The WT reference strain nucleotide sequence. (Green)The corresponding amino acids associated with the WT nucleotide sequence directly beneath the WT codon. (Highlighted nucleotide codon) Represents the codon in WT that was mutated in LA16 or NC2. (Red)Represents the mutated nucleotide within the highlighted codon that was changed. (Blue) Indicates a nucleotide mutation that resulted in a silent mutation. (Purple) Nucleotide change that resulted in an amino acid change. (Purple letter) Amino acid change. The sequence starts at the first methionine in ORF1A. This corresponds to nucleotide 210 in the full genomic sequence.

```
WT   1  atg gca aag atg ggc aaa tac ggt ctc ggc ttc aaa tgg gcc cca gaa ttt cca tgg atg ctt ccg aac
          m   a   k   m   g   k   y   g   l   g   f   k   w   a   p   e   f   p   w   m   l   p   n

    70  gca tcg gag aag ttg ggt aac cct gag agg tca gag gag gat ggg ttt tgc ccc tct gct gcg caa gaa
          a   s   e   k   l   g   n   p   e   r   s   e   e   d   g   f   c   p   s   a   a   q   e

   139  ccg aaa gtt aaa gga aaa act ttg gtt aat cac gtg agg gtg aat tgt agc cgg ctt cca gct ttg gaa
          p   k   v   k   g   k   t   l   v   n   h   v   r   v   n   c   s   r   l   p   a   l   e

   208  tgc tgt gtt cag tct gcc ata atc cgt gat att ttt gta gat gag gat ccc cag aag gtg gag gcc tca
          c   c   v   q   s   a   i   i   r   d   i   f   v   d   e   d   p   q   k   v   e   a   s

   277  act atg atg gca ttg cag ttc ggt agt gcc gtc ttg gtt aag cca tcc aag cgc ttg tct att cag gca
          t   m   m   a   l   q   f   g   s   a   v   l   v   k   p   s   k   r   l   s   i   q   a

   346  tgg act aat ttg ggt gtg ctt ccc aaa aca gct gcc atg ggg ttg ttc aag cgc gtc tgc ctg tgt aac
          w   t   n   l   g   v   l   p   k   t   a   a   m   g   l   f   k   r   v   c   l   c   n

   415  acc agg gag tgc tct tgt gac gcc cac gtg gcc ttt cac ctt ttt acg gtc caa ccc gat ggt gta tgc
          t   r   e   c   s   c   d   a   h   v   a   f   h   l   f   t   v   q   p   d   g   v   c

   484  ctg ggt aat ggc cgt ttt ata ggc tgg ttc gtt cca gtc aca gcc ata ccg gag tat gcg aag cag tgg
          l   g   n   g   r   f   i   g   w   f   v   p   v   t   a   i   p   e   y   a   k   q   w

   553  ttg caa ccc tgg tcc atc ctt ctt cgt aag ggt ggt aac aaa ggg tct gtg aca tcc ggc cac ttc cgc
          l   q   p   w   s   i   l   l   r   k   g   g   n   k   g   s   v   t   s   g   h   f   r

   622  cgc gct gtt acc atg cct gtg tat gac ttt aat gta gag gat gct tgt gag gag gtt cat ctt aac ccg
          r   a   v   t   m   p   v   y   d   f   n   v   e   d   a   c   e   e   v   h   l   n   p

   691  aag ggt aag tac tcc tgc aag gcg tat gct ctt ctt aag ggc tat cgc ggt gtt aag ccc atc ctg ttt
          k   g   k   y   s   c   k   a   y   a   l   l   k   g   y   r   g   v   k   p   i   l   f

   760  gtg gac cag tat ggt tgc gac tat act gga tgt ctc gcc aag ggt ctt gag gac tat ggc gat ctc acc
          v   d   q   y   g   c   d   y   t   g   c   l   a   k   g   l   e   d   y   g   d   l   t

   829  ttg agt gag atg aag gag ttg ttc cct gtg tgg cgt gac tcc ttg gat agt gaa gtc ctt gtg gct tgg
          l   s   e   m   k   e   l   f   p   v   w   r   d   s   l   d   s   e   v   l   v   a   w

   898  cac gtt gat cga gat cct cgg gct gct atg cgt ctg cag act ctt gct act gta cgt tgc att gat tat
          h   v   d   r   d   p   r   a   a   m   r   l   q   t   l   a   t   v   r   c   i   d   y

   967  gtg ggc caa ccg acc gag gat gtg gtg gat gga gat gtg gta gtg cgt gag cct gct cat ctt ctc gca
          v   g   q   p   t   e   d   v   v   d   g   d   v   v   v   r   e   p   a   h   l   l   a

  1036  gcc aat gcc att gtt aaa aga ctc ccc cgt ttg gtg gag act atg ctg tat acg gat tcg tcc gtt aca
          a   n   a   i   v   k   r   l   p   r   l   v   e   t   m   l   y   t   d   s   s   v   t

  1105  gaa ttc tgt tat aaa acc aag ctg tgt gaa tgc ggt ttt atc acg cag ttt ggc tat gtg gat tgt tgt
          e   f   c   y   k   t   k   l   c   e   c   g   f   i   t   q   f   g   y   v   d   c   c

  1174  ggt gac acc tgc gat ttt cgt ggg tgg gtt gcc ggc aat atg atg gat ggc ttt cca tgt cca ggg tgt
          g   d   t   c   d   f   r   g   w   v   a   g   n   m   m   d   g   f   p   c   p   g   c

  1243  acc aaa aat tat atg ccc tgg gaa ttg gag gcc cag tca tca ggt gtt ata cca gaa gga ggt gtt cta
          t   k   n   y   m   p   w   e   l   e   a   q   s   s   g   v   i   p   e   g   g   v   l

  1312  ttc act cag agc act gat aca gtg aat cgt gag tcc ttt aag ctc tac ggt cat gct gtt gtg cct ttt
```

```
          f   t   q   s   t   d   t   v   n   r   e   s   f   k   l   y   g   h   a   v   v   p   f

  1381  ggt tct gct gtg tat tgg agc cct tgc cca ggt atg tgg ctt cca gta att tgg tct tct gtt aag tca
          g   s   a   v   y   w   s   p   c   p   g   m   w   l   p   v   i   w   s   s   v   k   s

  1450  tac tct ggt ttg act tat aca gga gta gtt ggt tgt aag gca att gtt caa gag aca gac gct ata tgt
          y   s   g   l   t   y   t   g   v   v   g   c   k   a   i   v   q   e   t   d   a   i   c

  1519  cgt tct ctg tat atg gat tat gtc cag cac aag tgt ggc aat ctc gag cag aga gct atc ctt gga ttg
          r   s   l   y   m   d   y   v   q   h   k   c   g   n   l   e   q   r   a   i   l   g   l

  1588  gac gat gtc tat cat aga cag ttg ctt gtg aat agg ggt gac tat agt ctc ctc ctt gag aat gtg gat
          d   d   v   y   h   r   q   l   l   v   n   r   g   d   y   s   l   l   l   e   n   v   d

  1657  ttg ttt gtt aag cgg cgc gct gaa ttt gct tgc aaa ttc gcc acc tgt gga gat ggt ctt gta ccc ctc
          l   f   v   k   r   r   a   e   f   a   c   k   f   a   t   c   g   d   g   l   v   p   l

  1726  cta cta gat ggt tta gtg ccc cgc agt tat tat ttg att aag agt ggt caa gct ttc acc tct atg atg
          l   l   d   g   l   v   p   r   s   y   y   l   i   k   s   g   q   a   f   t   s   m   m

  1795  gtt aat ttt agc cat gag gtg act gac atg tgt atg gac atg gct tta ttg ttc atg cat gat gtt aaa
          v   n   f   s   h   e   v   t   d   m   c   m   d   m   a   l   l   f   m   h   d   v   k

  1864  gtg gcc act aag tat gtt aag aag gtt act ggc aaa ctg gcc gtg cgc ttt aaa gcg ttg ggt gta gcc
          v   a   t   k   y   v   k   k   v   t   g   k   l   a   v   r   f   k   a   l   g   v   a

  1933  gtt gtc aga aaa att act gaa tgg ttt gat tta gcc gtg gac att gct gct agt gcc gct gga tgg ctt
          v   v   r   k   i   t   e   w   f   d   l   a   v   d   i   a   a   s   a   a   g   w   l

  2002  tgc tac cag ctg gta aat ggc tta ttt gca gtg gcc aat ggt gtt ata acc ttt gta cag gag gtg cct
          c   y   q   l   v   n   g   l   f   a   v   a   n   g   v   i   t   f   v   q   e   v   p

  2071  gag ctt gtc aag aat ttt gtt gac aag ttc aag gca ttt ttc aag gtt ttg atc gac tct atg tcg gtt
          e   l   v   k   n   f   v   d   k   f   k   a   f   f   k   v   l   i   d   s   m   s   v

  2140  tct atc ttg tct gga ctt act gtt gtc aag act gcc tca aat agg gtg tgt ctt gct ggc agt aag gtt
          s   i   l   s   g   l   t   v   v   k   t   a   s   n   r   v   c   l   a   g   s   k   v

  2209  tat gaa gtt gtg cag aaa tct ttg tct gca tat gtt atg cct gtg ggt tgc agt gaa gcc act tgt ttg
          y   e   v   v   q   k   s   l   s   a   y   v   m   p   v   g   c   s   e   a   t   c   l
LA16                                                                      ..c
NC2                                                                       ..c
  2278  gtg ggt gag att gaa cct gca gtt ttt gaa gat gat gtt gtt gat gtg gtt aaa gcc cca tta aca tat
          v   g   e   i   e   p   a   v   f   e   d   d   v   v   d   v   v   k   a   p   l   t   y

  2347  caa ggc tgt tgt aag cca ccc act tct ttc gag aag att tgt att gtg gat aaa ttg tat atg gcc aag
          q   g   c   c   k   p   p   t   s   f   e   k   i   c   i   v   d   k   l   y   m   a   k

  2416  tgt ggt gat caa ttt tac cct gtg gtt gtt gat aac gac act gtt ggc gtg tta gat cag tgc tgg agg
          c   g   d   q   f   y   p   v   v   v   d   n   d   t   v   g   v   l   d   q   c   w   r

  2485  ttt ccc tgt gcg ggc aag aaa gtc gag ttt aac gac aag ccc aaa gtc agg aag ata ccc tcc acc cgt
          f   p   c   a   g   k   k   v   e   f   n   d   k   p   k   v   r   k   i   p   s   t   r

  2554  aag att aag atc acc ttc gca ctg gat gcg acc ttt gat agt gtt ctt tcg aag gcg tgt tca gag ttt
          k   i   k   i   t   f   a   l   d   a   t   f   d   s   v   l   s   k   a   c   s   e   f

  2623  gaa gtt gat aaa gat gtt aca ttg gat gag ctg ctt gat gtt gtg ctt gac gca gtt gag agt acg ctc
          e   v   d   k   d   v   t   l   d   e   l   l   d   v   v   l   d   a   v   e   s   t   l

  2692  agc cct tgt aag gag cat gat gtg ata ggc aca aaa gtt tgt gct tta ctt gat agg ttg gca gga gat
          s   p   c   k   e   h   d   v   i   g   t   k   v   c   a   l   l   d   r   l   a   g   d

  2761  tat gtc tat ctt ttt gat gag gga ggc gat gaa gtg atc gcc ccg agg atg tat tgt tcc ttt tct gct
          y   v   y   l   f   d   e   g   g   d   e   v   i   a   p   r   m   y   c   s   f   s   a

  2830  cct gat gat gaa gac tgc gtt gca gcg gat gtt gta gat gca gat gaa aac caa gat gat gat gct gaa
          p   d   d   e   d   c   v   a   a   d   v   v   d   a   d   e   n   q   d   d   d   a   e

  2899  gac tca gca gtc ctt gtc gct gat acc caa gaa gag gac ggc gtt gcc aag ggg cag gtt gag gcg gat
          d   s   a   v   l   v   a   d   t   q   e   e   d   g   v   a   k   g   q   v   e   a   d

  2968  tcg gaa att tgc gtt gcg cat act ggt agt caa gaa gaa ttg gct gag cct gat gct gtc gga tct caa
          s   e   i   c   v   a   h   t   g   s   q   e   e   l   a   e   p   d   a   v   g   s   q

  3037  act ccc atc gcc tct gct gag gaa acc gaa gtc gga gag gca agc gac agg gaa ggg att gct gag gcg
```

```
          t   p   i   a   s   a   e   e   t   e   v   g   e   a   s   d   r   e   g   i   a   e   a

  3106  aag gca act gtg tgt gct gat gct gta gat gcc tgc ccc gat caa gtg gag gca ttt gaa att gaa aag
          k   a   t   v   c   a   d   a   v   d   a   c   p   d   q   v   e   a   f   e   i   e   k

  3175  gtt gaa gac tct atc ttg gat gag ctt caa act gaa ctt aat gcg cca gcg gac aag acc tat gag gat
          v   e   d   s   i   l   d   e   l   q   t   e   l   n   a   p   a   d   k   t   y   e   d

  3244  gtc ttg gca ttc gat gcc gta tgc tca gag gcg ttg tct gca ttc tat gct gtg ccg agt gat gag acg
          v   l   a   f   d   a   v   c   s   e   a   l   s   a   f   y   a   v   p   s   d   e   t

  3313  cac ttt aaa gtg tgt gga ttc tat tcg cct gct ata gag cgc act aat tgt tgg ctg cgt tct act ttg
          h   f   k   v   c   g   f   y   s   p   a   i   e   r   t   n   c   w   l   r   s   t   l

  3382  ata gta atg cag agt cta cct ttg gaa ttt aaa gac ttg gag atg caa aag ctc tgg ttg tct tac aag
          i   v   m   q   s   l   p   l   e   f   k   d   l   e   m   q   k   l   w   l   s   y   k

  3451  gcc ggc tat gac caa tgc ttt gtg gac aaa cta gtt aag agc gtg ccc aag tct att atc ctt cca caa
          a   g   y   d   q   c   f   v   d   k   l   v   k   s   v   p   k   s   i   i   l   p   q

  3520  ggt ggt tat gtg gca gat ttt gcc tat ttc ttt cta agc cag tgt agc ttt aaa gct tat gct aac tgg
          g   g   y   v   a   d   f   a   y   f   f   l   s   q   c   s   f   k   a   y   a   n   w

  3589  cgt tgt tta gag tgt gac atg gag tta aag ctt caa ggc ttg gac gcc atg ttt ttc tat ggg gac gtt
          r   c   l   e   c   d   m   e   l   k   l   q   g   l   d   a   m   f   f   y   g   d   v

  3658  gtg tct cat atg tgc aag tgt ggt aat agc atg acc ttg ttg tct gca gat ata ccc tac act ttg cat
          v   s   h   m   c   k   c   g   n   s   m   t   l   l   s   a   d   i   p   y   t   l   h

  3727  ttt gga gtg cga gat gat aag ttt tgc gct ttt tac acg cca aga aag gtc ttt agg gct gct tgt gcg
          f   g   v   r   d   d   k   f   c   a   f   y   t   p   r   k   v   f   r   a   a   c   a

  3796  gta gat gtt aat gat tgt cac tct atg gct gta gta gag ggc aag caa att gat ggt aaa gtg gtt acc
          v   d   v   n   d   c   h   s   m   a   v   v   e   g   k   q   i   d   g   k   v   v   t

  3865  aaa ttt att ggt gac aaa ttt gat ttt atg gtg ggt tac ggg atg aca ttt agt atg tct cct ttt gaa
          k   f   i   g   d   k   f   d   f   m   v   g   y   g   m   t   f   s   m   s   p   f   e

  3934  ctc gcc cag tta tat ggt tca tgt ata aca cca aat gtt tgt ttt gtt aaa gga gat gtt ata aag gtt
          l   a   q   l   y   g   s   c   i   t   p   n   v   c   f   v   k   g   d   v   i   k   v

  4003  gtt cgc tta gtt aat gct gaa gtc att gtt aac cct gct aat ggg cgt atg gct cat ggt gca ggt gtt
          v   r   l   v   n   a   e   v   i   v   n   p   a   n   g   r   m   a   h   g   a   g   v

  4072  gca ggt gct ata gct gaa aag gcg ggc agt gct ttt att aaa gaa acc tcc gat atg gtg aag gct cag
          a   g   a   i   a   e   k   a   g   s   a   f   i   k   e   t   s   d   m   v   k   a   q
LA16                          ...
NC2                           g..(E)
  4141  ggc gtt tgc cag gtt ggt gaa tgc tat gaa tct gcc ggt ggt aag tta tgt aaa aag gtg ctt aac att
          g   v   c   q   v   g   e   c   y   e   s   a   g   g   k   l   c   k   k   v   l   n   i

  4210  gta ggg cca gat gcg cga ggg cat ggc aag caa tgc tat tca ctt tta gag cgt gct tat cag cat att
          v   g   p   d   a   r   g   h   g   k   q   c   y   s   l   l   e   r   a   y   q   h   i

  4279  aat aag tgt gac aat gtt gtc act act tta att tcg gct ggt ata ttt agt gtg cct act gat gtc tcc
          n   k   c   d   n   v   v   t   t   l   i   s   a   g   i   f   s   v   p   t   d   v   s

  4348  cta act tac tta ctt ggt gta gtg aca aag aat gtc att ctt gtc agt aac aac cag gat gat ttt gat
          l   t   y   l   l   g   v   v   t   k   n   v   i   l   v   s   n   n   q   d   d   f   d

  4417  gtg ata gag aag tgt cag gtg acc tcc gtt gct ggt acc aaa gcg cta tca ctt caa ttg gcc aaa aat
          v   i   e   k   c   q   v   t   s   v   a   g   t   k   a   l   s   l   q   l   a   k   n

  4486  ttg tgc cgt gat gta aag ttt gtg acg aat gca tgt agt tcg ctt ttt agt gaa tct tgc ttt gtc tca
          l   c   r   d   v   k   f   v   t   n   a   c   s   s   l   f   s   e   s   c   f   v   s

  4555  agc tat gat gtg ttg cag gaa gtt gaa gcg ctg cga cat gat ata caa ttg gat gat gat gct cgt gtc
          s   y   d   v   l   q   e   v   e   a   l   r   h   d   i   q   l   d   d   d   a   r   v

  4624  ttt gtg cag gct aat atg gac tgt ctg ccc aca gac tgg cgt ctc gtt aac aaa ttt gat agt gtt gat
          f   v   q   a   n   m   d   c   l   p   t   d   w   r   l   v   n   k   f   d   s   v   d

  4693  ggt gtt aga acc att aag tat ttt gaa tgc ccg ggc ggg att ttt gta tcc agc cag ggc aaa aag ttt
          g   v   r   t   i   k   y   f   e   c   p   g   g   i   f   v   s   s   q   g   k   k   f

  4762  ggt tat gtt cag aat ggt tca ttt aag gag gcg agt gtt agc caa ata agg gct tta ctc gct aat aag
```

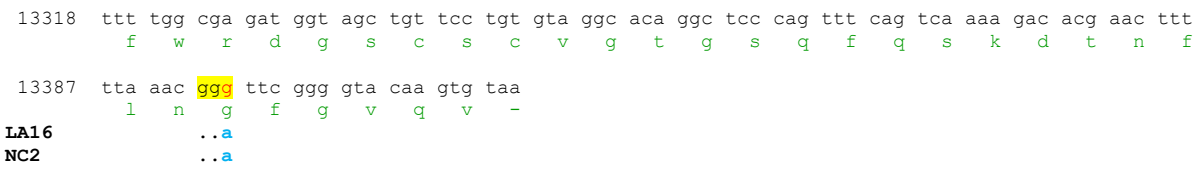
```
          g   y   v   q   n   g   s   f   k   e   a   s   v   s   q   i   r   a   l   l   a   n   k

  4831  gtt gat gtc ttg tgt act gtt gat ggt gtt aac ttc cgc tcc tgc tgc gta gca gag ggt gaa gtt ttt
          v   d   v   l   c   t   v   d   g   v   n   f   r   s   c   c   v   a   e   g   e   v   f

  4900  ggc aag aca tta ggt tca gtc ttt tgt gat ggc ata aat gtc acc aaa gtt agg tgt agt gcc att tac
          g   k   t   l   g   s   v   f   c   d   g   i   n   v   t   k   v   r   c   s   a   i   y

  4969  aag ggt aag gtt ttc ttt cag tac agt gat ttg tcc gag gca gat ctt gtg gct gtt aaa gat gcc ttt
          k   g   k   v   f   f   q   y   s   d   l   s   e   a   d   l   v   a   v   k   d   a   f

  5038  ggt ttt gat gaa cca caa ctg ctg aag tac tac act atg ctt ggc atg tgt aag tgg tca gta gtt gtt
          g   f   d   e   p   q   l   l   k   y   y   t   m   l   g   m   c   k   w   s   v   v   v
LA16                                                                                  c..(P)
NC2                                                                                   c..(P)
  5107  tgt ggc aat tat ttt gct ttc aag cag tca aat aat aat tgc tat ata aat gtg gca tgt tta atg ctg
          c   g   n   y   f   a   f   k   q   s   n   n   n   c   y   i   n   v   a   c   l   m   l

  5176  caa cac ttg agt tta aag ttt cct aag tgg caa tgg caa gag gct tgg aac gag ttc cgc tct ggt aaa
          q   h   l   s   l   k   f   p   k   w   q   w   q   e   a   w   n   e   f   r   s   g   k

  5245  cca cta agg ttt gtg tcc ttg gta tta gca aag ggc agc ttt aaa ttt aat gaa cct tct gat tct atc
          p   l   r   f   v   s   l   v   l   a   k   g   s   f   k   f   n   e   p   s   d   s   i

  5314  gat ttt atg cgt gtg gtg cta cgt gaa gca gat ttg agt ggt gcc acg tgc aat ttg gaa ttt gtt tgt
          d   f   m   r   v   v   l   r   e   a   d   l   s   g   a   t   c   n   l   e   f   v   c

  5383  aaa tgt ggt gtg aag caa gag cag cgc aaa ggt gtt gac gct gtt atg cat ttt ggt acg ttg gat aaa
          k   c   g   v   k   q   e   q   r   k   g   v   d   a   v   m   h   f   g   t   l   d   k

  5452  ggt gat ctt gtc agg ggt tat aat atc gca tgt acg tgc ggt agt aaa ctt gtg cat tgc acc caa ttt
          g   d   l   v   r   g   y   n   i   a   c   t   c   g   s   k   l   v   h   c   t   q   f

  5521  aac gta cca ttt tta att tgc tcc aac aca cca gag ggt agg aaa ctg ccc gac gat gtt gtt gca gct
          n   v   p   f   l   i   c   s   n   t   p   e   g   r   k   l   p   d   d   v   v   a   a

  5590  aat att ttt act ggt ggt agt gtg ggc cat tac acg cat gtg aaa tgt aaa ccc aag tac cag ctt tat
          n   i   f   t   g   g   s   v   g   h   y   t   h   v   k   c   k   p   k   y   q   l   y

  5659  gat gct tgt aat gtt aat aag gtt tcg gag gct aag ggt aat ttt acc gat tgc ctc tac ctt aaa aat
          d   a   c   n   v   n   k   v   s   e   a   k   g   n   f   t   d   c   l   y   l   k   n

  5728  tta aag caa act ttt tcg tct gtg ctg acg act ttt tat tta gat gat gta aag tgt gtg gag tat aag
          l   k   q   t   f   s   s   v   l   t   t   f   y   l   d   d   v   k   c   v   e   y   k

  5797  cca gat tta tcg cag tat tac tgt gag tct ggt aaa tat tat aca aaa ccc att att aag gcc caa ttt
          p   d   l   s   q   y   y   c   e   s   g   k   y   y   t   k   p   i   i   k   a   q   f

  5866  aga aca ttt gag aag gtt gat ggt gtc tat acc aac ttt aaa ttg gtg gga cat agt att gct gaa aaa
          r   t   f   e   k   v   d   g   v   y   t   n   f   k   l   v   g   h   s   i   a   e   k

  5935  ctc aat gct aag ctg gga ttt gat tgt aat tct ccc ttt gtg gag tat aaa att aca gag tgg cca aca
          l   n   a   k   l   g   f   d   c   n   s   p   f   v   e   y   k   i   t   e   w   p   t

  6004  gct act gga gat gtg gtg ttg gct agt gat gat ttg tat gta agt cgg tac tca agc ggg tgc att act
          a   t   g   d   v   v   l   a   s   d   d   l   y   v   s   r   y   s   s   g   c   i   t
LA16                                                          ..g
NC2                                                           ..g
  6073  ttt ggt aaa ccg gtt gtc tgg ctt ggc cat gag gaa gca tcg ctg aaa tct ctc aca tat ttt aat aga
          f   g   k   p   v   v   w   l   g   h   e   e   a   s   l   k   s   l   t   y   f   n   r

  6142  cct agt gtc gtt tgt gaa aat aaa ttt aat gtg ttg ccc gtt gat gtc agt gaa ccc acg gac aag ggg
          p   s   v   v   c   e   n   k   f   n   v   l   p   v   d   v   s   e   p   t   d   k   g

  6211  cct gtg cct gct gca gtc ctt gtt acc ggc gtc cct gga gct gat gcg tca gct ggt gcc ggt att gcc
          p   v   p   a   a   v   l   v   t   g   v   p   g   a   d   a   s   a   g   a   g   i   a

  6280  aag gag caa aaa gcc tgt gct tct gct agt gtg gag gat cag gtt gtt acg gag gtt cgt caa gag cca
          k   e   q   k   a   c   a   s   a   s   v   e   d   q   v   v   t   e   v   r   q   e   p

  6349  tct gtt tca gct gct gat gtc aaa gag gtt aaa ttg aat ggt gtt aaa aag cct gtt aag gtg gaa ggt
          s   v   s   a   a   d   v   k   e   v   k   l   n   g   v   k   k   p   v   k   v   e   g

  6418  agt gtg gtt gtt aat gat ccc act agc gaa acc aaa gtt gtt aaa agt ttg tct att gtt gat gtc tat
          s   v   v   v   n   d   p   t   s   e   t   k   v   v   k   s   l   s   i   v   d   v   y
```

```
  6487  gat atg ttc ctg aca ggg tgt aag tat gtg gtt tgg act gct aat gag ttg tct cga cta gta aat tca
          d   m   f   l   t   g   c   k   y   v   v   w   t   a   n   e   l   s   r   l   v   n   s

  6556  ccg act gtt agg gag tat gtg aag tgg ggt aag gga aag att gta aca ccc gct aag ttg ttg ttg tta
          p   t   v   r   e   y   v   k   w   g   k   g   k   i   v   t   p   a   k   l   l   l   l
LA16                                          .t.(M)
NC2                                           .t.(M)
  6625  aga gat gag aag caa gag ttc gta gcg cca aaa gta gtc aag gcg aaa gct att gcc tgc tat tgt gct
          r   d   e   k   q   e   f   v   a   p   k   v   v   k   a   k   a   i   a   c   y   c   a

  6694  gtg aag tgg ttt ctc ctc tat tgt ttt agt tgg ata aag ttt aat act gat aat aag gtt ata tac acc
          v   k   w   f   l   l   y   c   f   s   w   i   k   f   n   t   d   n   k   v   i   y   t

  6763  aca gaa gta gct tca aag ctt act ttc aag ttg tgc tgt ttg gcc ttt aag aat gcc tta cag acg ttt
          t   e   v   a   s   k   l   t   f   k   l   c   c   l   a   f   k   n   a   l   q   t   f

  6832  aat tgg agc gtt gtg tct agg ggc ttt ttc cta gtt gca acg gtc ttt tta tta tgg ttt aac ttt ttg
          n   w   s   v   v   s   r   g   f   f   l   v   a   t   v   f   l   l   w   f   n   f   l

  6901  tat gct aat gtt att ttg agt gac ttc tat ttg cct aat att ggg cct ctc cct acg ttt gtg gga cag
          y   a   n   v   i   l   s   d   f   y   l   p   n   i   g   p   l   p   t   f   v   g   q

  6970  ata gtt gcg tgg ttt aag act aca ttt ggt gtg tca acc atc tgt gat ttc tac cag gtg acg gat ttg
          i   v   a   w   f   k   t   t   f   g   v   s   t   i   c   d   f   y   q   v   t   d   l

  7039  ggc tat aga agt tcg ttt tgt aat gga agt atg gta tgt gaa cta tgc ttc tca ggt ttt gat atg ctg
          g   y   r   s   s   f   c   n   g   s   m   v   c   e   l   c   f   s   g   f   d   m   l
LA16                                  .a.(E)
NC2                                   ...
  7108  gac aac tat gat gct ata aat gtt gtt caa cac gtt gta gat agg cgt ttg tcc ttt gac tat att agc
          d   n   y   d   a   i   n   v   v   q   h   v   v   d   r   r   l   s   f   d   y   i   s

  7177  cta ttt aaa tta gta gtt gag ctt gta atc ggc tac tct ctt tat act gtg tgc ttc tac cca ctg ttt
          l   f   k   l   v   v   e   l   v   i   g   y   s   l   y   t   v   c   f   y   p   l   f

  7246  gtc ctt att gga atg cag ttg ttg acc aca tgg ttg cct gaa ttc ttt atg ctg gag act atg cat tgg
          v   l   i   g   m   q   l   l   t   t   w   l   p   e   f   f   m   l   e   t   m   h   w

  7315  agt gct cgt ttg ttt gtg ttt gtt gcc aat atg ctt cca gct ttt acg tta ctg cga ttt tac atc gtg
          s   a   r   l   f   v   f   v   a   n   m   l   p   a   f   t   l   l   r   f   y   i   v

  7384  gtg aca gct atg tat aag gtc tat tgt ctt tgt aga cat gtt atg tat gga tgt agt aag cct ggt tgc
          v   t   a   m   y   k   v   y   c   l   c   r   h   v   m   y   g   c   s   k   p   g   c

  7453  ttg ttt tgt tat aag aga aac cgt agt gtc cgt gtt aag tgt agc acc gtt gtt ggt ggt tca cta cgc
          l   f   c   y   k   r   n   r   s   v   r   v   k   c   s   t   v   v   g   g   s   l   r

  7522  tat tac gat gta atg gct aac ggc ggc aca ggt ttc tgt aca aag cac cag tgg aac tgt ctt aat tgc
          y   y   d   v   m   a   n   g   g   t   g   f   c   t   k   h   q   w   n   c   l   n   c

  7591  aat tcc tgg aaa cca ggc aat aca ttc ata act cat gaa gca gcg gcg gac ctc tct aag gag ttg aaa
          n   s   w   k   p   g   n   t   f   i   t   h   e   a   a   a   d   l   s   k   e   l   k

  7660  cgc cct gtg aat cca aca gat tct gct tat tac tcg gtc aca gag gtt aag cag gtt ggt tgt tcc atg
          r   p   v   n   p   t   d   s   a   y   y   s   v   t   e   v   k   q   v   g   c   s   m

  7729  cgt ttg ttc tac gag aga gat gga cag cgt gtt tat gat gat gtt aat gct agt ttg ttt gtg gac atg
          r   l   f   y   e   r   d   g   q   r   v   y   d   d   v   n   a   s   l   f   v   d   m

  7798  aat ggt ctg ctg cat tct aaa gtt aaa ggt gtg cct gaa acg cat gtt gtg gtt gtt gag aat gaa gct
          n   g   l   l   h   s   k   v   k   g   v   p   e   t   h   v   v   v   v   e   n   e   a

  7867  gat aaa gct ggt ttt ctc ggc gcc gca gtg ttt tat gca caa tcg ctc tac aga cct atg ttg atg gtg
          d   k   a   g   f   l   g   a   a   v   f   y   a   q   s   l   y   r   p   m   l   m   v

  7936  gaa aag aaa tta ata act acc gcc aac act ggt ttg tct gtt agt cga act atg ttt gac ctt tat gta
          e   k   k   l   i   t   t   a   n   t   g   l   s   v   s   r   t   m   f   d   l   y   v

  8005  gat tca ttg ctg aac gtc ctc gac gtg gat cgc aag agt cta aca agt ttt gta aat gct gcg cac aac
          d   s   l   l   n   v   l   d   v   d   r   k   s   l   t   s   f   v   n   a   a   h   n

  8074  tct cta aag gag ggt gtt cag ctt gaa caa gtt atg gat acc ttt att ggc tgt gcc cga cgt aag tgt
          s   l   k   e   g   v   q   l   e   q   v   m   d   t   f   i   g   c   a   r   r   k   c

  8143  gct ata gat tct gat gtt gaa acc aag tct att acc aag tcc gtc atg tcg gca gta aat gct ggc gtt
          a   i   d   s   d   v   e   t   k   s   i   t   k   s   v   m   s   a   v   n   a   g   v
```

```
  8212  gat ttt acg gat gag agt tgt aat aac ttg gtg cct acc tat gtt aaa agt gac act atc gtt gca gcc
          d   f   t   d   e   s   c   n   n   l   v   p   t   y   v   k   s   d   t   i   v   a   a

  8281  gat ttg ggt gtt ctt att cag aat aat gct aag cat gta cag gct aat gtt gct aaa gcc gct aat gtg
          d   l   g   v   l   i   q   n   n   a   k   h   v   q   a   n   v   a   k   a   a   n   v

  8350  gct tgc att tgg tct gtg gat gct ttt aac cag cta tct gct gac tta cag cat agg ctg cga aaa gca
          a   c   i   w   s   v   d   a   f   n   q   l   s   a   d   l   q   h   r   l   r   k   a

  8419  tgt tca aaa act ggc ttg aag att aag ctt act tat aat aag cag gag gca aat gtt cct att tta act
          c   s   k   t   g   l   k   i   k   l   t   y   n   k   q   e   a   n   v   p   i   l   t

  8488  aca ccg ttc tct ctt aaa ggg ggc gct gtt ttt agt aga atg tta caa tgg ttg ttt gtt gct aat ttg
          t   p   f   s   l   k   g   g   a   v   f   s   r   m   l   q   w   l   f   v   a   n   l

  8557  att tgt ttc att gtg ttg tgg gcc ctt atg cca aca tat gca gtg cac aaa tcg gat atg cag ttg cct
          i   c   f   i   v   l   w   a   l   m   p   t   y   a   v   h   k   s   d   m   q   l   p

  8626  tta tat gcc agt ttt aaa gtt ata gat aat ggt gtg cta agg gat gtg tct gtt act gac gca tgc ttc
          l   y   a   s   f   k   v   i   d   n   g   v   l   r   d   v   s   v   t   d   a   c   f

  8695  gca aac aaa ttt aat caa ttt gat caa tgg tat gag tct act ttt ggt ctt gct tat tac cgc aac tct
          a   n   k   f   n   q   f   d   q   w   y   e   s   t   f   g   l   a   y   y   r   n   s

  8764  aag gct tgt cct gtt gtg gtt gct gta ata gat caa gac att ggc cat acc tta ttt aat gtt cct acc
          k   a   c   p   v   v   v   a   v   i   d   q   d   i   g   h   t   l   f   n   v   p   t

  8833  aca gtt tta aga tat gga ttt cat gtg ttg cat ttt ata acc cat gca ttt gct act gat agc gtg cag
          t   v   l   r   y   g   f   h   v   l   h   f   i   t   h   a   f   a   t   d   s   v   q

  8902  tgt tac acg cca cat atg caa atc ccc tat gat aat ttc tat gct agt ggt tgc gtg ttg tca tcc ctc
          c   y   t   p   h   m   q   i   p   y   d   n   f   y   a   s   g   c   v   l   s   s   l

  8971  tgt act atg ctt gcg cat gca gat gga acc ccg cat cct tat tgt tat aca ggg ggt gtt atg cac aat
          c   t   m   l   a   h   a   d   g   t   p   h   p   y   c   y   t   g   g   v   m   h   n

  9040  gcc tct ctg tat agt tct ttg gct cct cat gtc cgt tat aac ctg gct agt tca aat ggt tat ata cgt
          a   s   l   y   s   s   l   a   p   h   v   r   y   n   l   a   s   s   n   g   y   i   r

  9109  ttt ccc gaa gtg gtt agt gaa ggc att gtg cgt gtt gtg cgc act cgc tct atg acc tac tgc agg gtt
          f   p   e   v   v   s   e   g   i   v   r   v   v   r   t   r   s   m   t   y   c   r   v

  9178  ggt tta tgt gag gag gcc gag gag ggt atc tgc ttt aat ttt aat cgt tca tgg gta ttg aac aac ccg
          g   l   c   e   e   a   e   e   g   i   c   f   n   f   n   r   s   w   v   l   n   n   p

  9247  tat tat agg gcc atg cct gga act ttt tgt ggt agg aat gct ttt gat tta ata cat caa gtt tta gga
          y   y   r   a   m   p   g   t   f   c   g   r   n   a   f   d   l   i   h   q   v   l   g

  9316  gga tta gtg cgg cct att gat ttc ttt gcc tta acg gcg agt tca gtg gct ggt gct atc ctt gca att
          g   l   v   r   p   i   d   f   f   a   l   t   a   s   s   v   a   g   a   i   l   a   i

  9385  att gtc gtt ttg gct ttc tat tat tta ata aag ctt aaa cgt gcc ttt ggt gac tac act agt gtt gtg
          i   v   v   l   a   f   y   y   l   i   k   l   k   r   a   f   g   d   y   t   s   v   v

  9454  gtt atc aat gta att gtg tgg tgt ata aat ttt ctg atg ctt ttt gtg ttt cag gtt tat ccc aca ttg
          v   i   n   v   i   v   w   c   i   n   f   l   m   l   f   v   f   q   v   y   p   t   l

  9523  tct tgt tta tat gct tgt ttt tat ttc tac aca acg ctt tat ttc cct tcg gag ata agt gtt gtt atg
          s   c   l   y   a   c   f   y   f   y   t   t   l   y   f   p   s   e   i   s   v   v   m

  9592  cat ttg caa tgg ctt gtc atg tat ggt gct att atg ccc ttg tgg ttt tgc att att tac gtg gca gtc
          h   l   q   w   l   v   m   y   g   a   i   m   p   l   w   f   c   i   i   y   v   a   v

  9661  gtt gtt tca aac cat gca ttg tgg ttg ttc tct tac tgc cgc aaa att ggt acc gag gtt cgt agt gac
          v   v   s   n   h   a   l   w   l   f   s   y   c   r   k   i   g   t   e   v   r   s   d

  9730  ggc aca ttt gag gaa atg gcc ctt act acc ttt atg att act aaa gaa tct tat tgt aag ttg aaa aat
          g   t   f   e   e   m   a   l   t   t   f   m   i   t   k   e   s   y   c   k   l   k   n
LA16                                                      ...
NC2                                                       ..g(S)
  9799  tct gtt tct gat gtt gct ttt aac agg tac ttg agt ctt tat aac aag tat cgt tat ttt agt ggc aaa
          s   v   s   d   v   a   f   n   r   y   l   s   l   y   n   k   y   r   y   f   s   g   k
LA16                      ...
NC2                       .g.(G)
  9868  atg gat act gcc gct tat aga gag gct gcc tgt tca caa ctg gca aag gca atg gaa aca ttt aac cat
```

```
          m   d   t   a   a   y   r   e   a   a   c   s   q   l   a   k   a   m   e   t   f   n   h

 9937  aat aat ggt aat gat gtt ctc tat cag cct cca acc gcc tct gtt act aca tca ttt tta cag tct ggt
          n   n   g   n   d   v   l   y   q   p   p   t   a   s   v   t   t   s   f   l   q   s   g

10006  ata gtg aag atg gtg tcg ccc acc tct aaa gtg gag cct tgt att gtt agt gtt act tat ggt aac atg
          i   v   k   m   v   s   p   t   s   k   v   e   p   c   i   v   s   v   t   y   g   n   m

10075  aca ctt aat ggg ttg tgg ttg gat gat aaa gtt tat tgc cca aga cat gtt atc tgt tct tca gct gac
          t   l   n   g   l   w   l   d   d   k   v   y   c   p   r   h   v   i   c   s   s   a   d

10144  atg aca gac cct gat tat cct aat ttg ctt tgt aga gtg aca tca agt gat ttt tgt gtt atg tct ggt
          m   t   d   p   d   y   p   n   l   l   c   r   v   t   s   s   d   f   c   v   m   s   g
LA16                                      ...
NC2                                       a..(I)
10213  cgt atg agc ctt act gta atg tct tat caa atg cag ggc tgc caa ctt gtt ttg act gtt aca ctg caa
          r   m   s   l   t   v   m   s   y   q   m   q   g   c   q   l   v   l   t   v   t   l   q

10282  aat cct aac acg cct aag tat tcc ttc ggt gtt gtt aag cct ggt gag aca ttt act gta ctg gct gca
          n   p   n   t   p   k   y   s   f   g   v   v   k   p   g   e   t   f   t   v   l   a   a

10351  tac aat ggc aga cct caa gga gcc ttc cat gtt acg ctt cgt agt agc cat acc ata aag ggc tcc ttt
          y   n   g   r   p   q   g   a   f   h   v   t   l   r   s   s   h   t   i   k   g   s   f

10420  cta tgt gga tcc tgc ggt tct gta gga tat gtt tta act ggc gat agt gta cga ttt gtt tat atg cat
          l   c   g   s   c   g   s   v   g   y   v   l   t   g   d   s   v   r   f   v   y   m   h

10489  cag cta gag ttg agt act ggt tgt cat acc ggt act gac ttt agt ggg aac ttt tat ggt ccc tat aga
          q   l   e   l   s   t   g   c   h   t   g   t   d   f   s   g   n   f   y   g   p   y   r

10558  gat gcg caa gtt gta caa ttg cct gtt cag gat tat acg cag act gtt aat gtt gta gct tgg ctt tat
          d   a   q   v   v   q   l   p   v   q   d   y   t   q   t   v   n   v   v   a   w   l   y

10627  gct gct att ttt aac aga tgc aac tgg ttt gtg caa agt gat agt tgt tcc ctg gag gag ttt aat gtt
          a   a   i   f   n   r   c   n   w   f   v   q   s   d   s   c   s   l   e   e   f   n   v

10696  tgg gct atg acc aat ggt ttt agc tca atc aaa gcc gat ctt gtc ttg gat gcg ctt gct tct atg aca
          w   a   m   t   n   g   f   s   s   i   k   a   d   l   v   l   d   a   l   a   s   m   t
LA16                                      ..t
NC2                                       ..t
10765  ggc gtt aca gtt gaa cag gtg ttg gcc gct att aag agg ctg cat tct gga ttc cag ggc aaa caa att
          g   v   t   v   e   q   v   l   a   a   i   k   r   l   h   s   g   f   q   g   k   q   i

10834  tta ggt agt tgt gtg ctt gaa gat gag ctg aca cca agt gat gtt tat caa caa cta gct ggt gtc aag
          l   g   s   c   v   l   e   d   e   l   t   p   s   d   v   y   q   q   l   a   g   v   k

10903  cta cag tca aag cgc aca aga gtt ata aaa ggt aca tgt tgc tgg ata ttg gct tca acg ttt ttg ttc
          l   q   s   k   r   t   r   v   i   k   g   t   c   c   w   i   l   a   s   t   f   l   f

10972  tgt agc att atc tca gca ttt gta aaa tgg act atg ttt atg tat gtt act acc cat atg ttg gga gtg
          c   s   i   i   s   a   f   v   k   w   t   m   f   m   y   v   t   t   h   m   l   g   v

11041  aca ttg tgt gca ctt tgt ttt gta agc ttt gct atg ttg ttg atc aag cat aag cat ttg tat tta act
          t   l   c   a   l   c   f   v   s   f   a   m   l   l   i   k   h   k   h   l   y   l   t

11110  atg tat att atg cct gtg tta tgc aca ctg ttt tac acc aac tat ttg gtt gtg tac aaa cag agt ttt
          m   y   i   m   p   v   l   c   t   l   f   y   t   n   y   l   v   v   y   k   q   s   f

11179  aga ggt cta gct tat gct tgg ctt tca cac ttt gtc cct gct gta gat tat aca tat atg gat gaa gtt
          r   g   l   a   y   a   w   l   s   h   f   v   p   a   v   d   y   t   y   m   d   e   v

11248  tta tat ggt gtt gtg ttg cta gta gct atg gtg ttt gtt acc atg cgt agc ata aac cac gac gtc ttt
          l   y   g   v   v   l   l   v   a   m   v   f   v   t   m   r   s   i   n   h   d   v   f

11317  tct att atg ttc ttg gtt ggt aga ctt gtc agc ctg gta tcc atg tgg tat ttt gga gcc aat tta gag
          s   i   m   f   l   v   g   r   l   v   s   l   v   s   m   w   y   f   g   a   n   l   e

11386  gaa gag gta cta ttg ttc ctc aca tcc cta ttt ggc acg tac aca tgg act act atg ttg tca ttg gct
          e   e   v   l   l   f   l   t   s   l   f   g   t   y   t   w   t   t   m   l   s   l   a

11455  acc gct aag gtt att gct aaa tgg ttg gct gtg aat gtc ttg tac ttc aca gac gta ccg caa att aaa
          t   a   k   v   i   a   k   w   l   a   v   n   v   l   y   f   t   d   v   p   q   i   k

11524  tta gtt ctt ttg agc tac ttg tgt att ggt tat gtg tgt tgt tgt tat tgg gga atc ttg tca ctc ctt
          l   v   l   l   s   y   l   c   i   g   y   v   c   c   c   y   w   g   i   l   s   l   l
```

```
11593  aat agc att ttt agg atg cca ttg ggc gtc tac aat tat aaa atc tcc gtt cag gag tta cgt tat atg
         n   s   i   f   r   m   p   l   g   v   y   n   y   k   i   s   v   q   e   l   r   y   m

11662  aat gct aat ggc ttg cgc cca cct aga aat agt ttt gag gcc ctg atg ctt aat ttt aag ctg ttg gga
         n   a   n   g   l   r   p   p   r   n   s   f   e   a   l   m   l   n   f   k   l   l   g

11731  att ggt ggt gtg cca gtc att gaa gta tct caa att caa tca aga ttg acg gat gtt aaa tgt gct aat
         i   g   g   v   p   v   i   e   v   s   q   i   q   s   r   l   t   d   v   k   c   a   n

11800  gtt gtg ttg ctt aat tgc ctc cag cac ttg cat att gca tct aat tct aag ttg tgg cag tat tgt agt
         v   v   l   l   n   c   l   q   h   l   h   i   a   s   n   s   k   l   w   q   y   c   s

11869  act ttg cac aat gaa ata ctg gct aca tct gat ttg agc gtg gcc ttc gat aag ttg gct cag ctc tta
         t   l   h   n   e   i   l   a   t   s   d   l   s   v   a   f   d   k   l   a   q   l   l

11938  gtt gtt tta ttt gct aat cca gca gca gtg gat agc aag tgc ctt gca agt att gaa gaa gtg agc gat
         v   v   l   f   a   n   p   a   a   v   d   s   k   c   l   a   s   i   e   e   v   s   d

12007  gat tac gtt cgc gac aat act gtc ttg caa gcc tta cag agt gaa ttt gtt aat atg gct agc ttc gtt
         d   y   v   r   d   n   t   v   l   q   a   l   q   s   e   f   v   n   m   a   s   f   v

12076  gag tat gaa ctt gct aag aag aat cta gat gag gct aag gct agc ggc tct gcc aat caa cag cag att
         e   y   e   l   a   k   k   n   l   d   e   a   k   a   s   g   s   a   n   q   q   q   i

12145  aag cag cta gag aag gcg tgt aat att gct aag tca gca tat gag cgc gac aga gct gtt gct cgt aag
         k   q   l   e   k   a   c   n   i   a   k   s   a   y   e   r   d   r   a   v   a   r   k

12214  ctg gaa cgt atg gct gat tta gct ctt aca aac atg tat aaa gaa gct aga att aat gat aag aag agt
         l   e   r   m   a   d   l   a   l   t   n   m   y   k   e   a   r   i   n   d   k   k   s

12283  aag gta gtg tct gca ttg caa acc atg ctc ttt agt atg gtg cgt aag cta gat aac caa gct ctt aat
         k   v   v   s   a   l   q   t   m   l   f   s   m   v   r   k   l   d   n   q   a   l   n

12352  tct att tta gat aat gca gtt aag ggt tgt gta cct ttg aat gca ata cca tca ttg act tcg aac act
         s   i   l   d   n   a   v   k   g   c   v   p   l   n   a   i   p   s   l   t   s   n   t

12421  ctg act ata ata gtg cca gat aag cag gtt ttt gat cag gtt gtg gat aat gtg tat gtc acc tat gct
         l   t   i   i   v   p   d   k   q   v   f   d   q   v   v   d   n   v   y   v   t   y   a

12490  ggg aat gta tgg cat ata cag ttt att caa gat gct gat ggt gct gtt aaa caa ttg aat gag ata gat
         g   n   v   w   h   i   q   f   i   q   d   a   d   g   a   v   k   q   l   n   e   i   d

12559  gtt aat tca acc tgg cct cta gtc att gct gca aat agg cat aat gaa gtg tct act gtt gtt ttg cag
         v   n   s   t   w   p   l   v   i   a   a   n   r   h   n   e   v   s   t   v   v   l   q

12628  aac aat gag ttg atg cct cag aag ttg aga act cag gtt gtc aat agt ggc tca gat atg aat tgt aat
         n   n   e   l   m   p   q   k   l   r   t   q   v   v   n   s   g   s   d   m   n   c   n

12697  act cct acc cag tgt tac tat aat act act ggc acg ggt aag att gtg tat gct ata ctt agt gac tgt
         t   p   t   q   c   y   y   n   t   t   g   t   g   k   i   v   y   a   i   l   s   d   c

12766  gat ggt ctc aag tac act aag ata gta aaa gaa gat gga aat tgt gtt gtt ttg gaa ttg gat cct ccc
         d   g   l   k   y   t   k   i   v   k   e   d   g   n   c   v   v   l   e   l   d   p   p
LA16           ...
NC2            ..g
12835  tgt aag ttt tct gtt cag gat gtg aag ggc ctt aaa att aag tac ctt tac ttt gtg aag ggg tgt aat
         c   k   f   s   v   q   d   v   k   g   l   k   i   k   y   l   y   f   v   k   g   c   n

12904  aca ctg gct aga ggc tgg gtt gta ggc acc tta tcc tcg aca gtg aga ttg cag gcg ggt acg gca act
         t   l   a   r   g   w   v   v   g   t   l   s   s   t   v   r   l   q   a   g   t   a   t

12973  gag tat gcc tcc aac tct gca ata ctg tcg ctg tgt gcg ttt tct gta gat cct aag aaa acg tac ttg
         e   y   a   s   n   s   a   i   l   s   l   c   a   f   s   v   d   p   k   k   t   y   l

13042  gat tat ata aaa cag ggt gga gtt ccc gtt act aat tgt gtt aag atg tta tgt gac cat gct ggc act
         d   y   i   k   q   g   g   v   p   v   t   n   c   v   k   m   l   c   d   h   a   g   t
LA16                                                                       .a.(Y)
NC2
13111  ggt atg gcc att act att aag ccg gag gca acc act aat cag gat tct tat ggt ggt gct tcc gtt tgt
         g   m   a   i   t   i   k   p   e   a   t   t   n   q   d   s   y   g   g   a   s   v   c

13180  ata tat tgc cgc tcg cgt gtt gaa cat cca gat gtt gat gga ttg tgc aaa tta cgc ggc aag ttt gtc
         i   y   c   r   s   r   v   e   h   p   d   v   d   g   l   c   k   l   r   g   k   f   v

13249  caa gtg ccc tta ggc ata aaa gat cct gtg tca tat gtg ttg acg cat gat gtt tgt cag gtt tgt ggc
         q   v   p   l   g   i   k   d   p   v   s   y   v   l   t   h   d   v   c   q   v   c   g
```



Comparing Mutations Located in ORF1A from LA16 and NC2

To compare the mutations identified in the viral genome (Figure 10) a table was constructed (Table 1). Table 1 lists the mutated nucleotide position in the viral genome, the amino acid change and the type of mutation that resulted. The nucleotide position that was entered into the table was obtained from the sequence of ORF1A that starts with the first "atg" present in the first open reading frame. This corresponds to nucleotide number 210 in the full viral genome that is located in appendix. In assessing the location of the mutations, LA16 contains mutations in Nsp3 and Nsp10. LA16 has shown to have a more significant TS phenotype, assessed by a reduction in titer and plaque size, indicating further that these mutations in Nsp3 and Nsp10 are important (Figure 7&8). It is interesting that of the six mutations identified in NC2 that resulted in an amino acid change, three of these mutations are conservative. Conservative mutations are defined as mutations that result in an amino acid change which conserves the same amino acid charge but has a slightly different structure (Table 1). These results indicate NC2 and LA16 contain different attenuating mutations. The role of these mutations in conferring the TS phenotype could be evaluated using reverse genetics.

Virus	Nsp mutation location	Mutated Nucleotide Positon	Mutated Amino Acid	
LA16	Nsp3	t 5090 c	S→P	Polar to Nonpolar
	Nsp3	a 6587 t	K→M	Positively Charged to Nonpolar
	Nsp3	g 7064 a	G→E	Polar to Negatively Charged
	Nsp10	g 13094 a	G→Y	Polar to Nonpolar
NC2	Nsp3	a 4090 g	K→E	Positively Charged to Negatively Charged
	Nsp3	t 5090c	S→P	Polar to Nonpolar
	Nsp3	a 6587 t	K→M	Positively Charged to Nonpolar
	Nsp4	c 9770 g	T→S	Conservative
	Nsp4	c 9815g	A→G	Conservative
	Nsp5	c 10171 a	L→I	Conservative

TABLE 1. Table of nucleotide and amino acid changes in the genomic sequences of LA16 and NC2. Genomic sequence from ORF1A was analyzed and the nucleotide changes that resulted in amino acid changes are listed. Silent mutations are documented in Figure 10. Nucleotide position, amino acid position, non-structural protein location and mutation type are classified. The location of the nucleotide changes corresponds to the nucleotide sequence of ORF1A and not to the entire genomic nucleotide sequence.

CHAPTER FOUR

DISCUSSION AND FUTURE DIRECTIONS

Using TS phenotyping has become a staple in virology to better understand many unknown pathogens. TS mutants of pathogens allow for the identification of critical genes or residues which can be identified as targets for potential vaccine development. In assessing the TS phenotype for LA16 and NC2, it was observed that at 33^{o}C, both viruses were able to maintain a WT-like plaque size and viral titer (Figure 7A, B). Upon incubating LA16 at 37 ^{o}C, I observed a drastic change in plaque morphology (Figure 7A). When assessing the plaque morphology, I found a heterogeneous population of plaques formed. These plaques range from small to pin-point in size. *Schaad et al.* hypothesized that LA16 may contain two different mutations that confer the TS phenotype documented[41]. This heterogeneity does seem to further support why LA16 was placed into two complementation groups indicating multiple mutations in different areas of the viral genome. This change in morphology was also associated with a reduction in viral titer which also helps to support the hypothesis that LA16 confers a TS phenotype. Upon quantifying the plaque size of LA16 at 37 ^{o}C (Figure 8), there was a significant decrease in plaque size when compared to WT, further supporting that the change in morphology may be due to the mutations identified in ORF1A. LA16 failed to produce any plaques within the detection limit of the plaque assay when incubated at 40^{o}C. When analyzing the sequence of LA16 two unique mutations were identified. The first mutation was observed in Nsp3 which resulted in the substation of a polar amino acid to a negatively charged amino acid. This could

have an effect on the Nsp3 protein stability or structure, but further analysis is required. The second mutation found was located in Nsp10. Currently the documented function of Nsp10 is to act as a regulator that aides in the efficency of the polyprotein processing[45]. The amino acid that was mutated has been identified a one of several critical residues involved in Nsp10 stability.

When assessing the TS phenotype of NC2, I saw that NC2 was able to retain WT-like plaques at both 33 ^{o}C and 37 ^{o}C (Figure 7A). This was obviously different than the morphology depicted by LA16 at 37 ^{o}C. It was only when NC2 was incubated at 40 ^{o}C was the TS phenotype able to be observed. This reduction in plaque size also correlates with the reduction in titer (Figure 7B). It can be appreciated that although NC2 did present a reduction in plaque size at 40^{o}C, there was a homogenous morphology amongst the plaques formed. They were all pin-point sized plaques (Figure 7A). Upon the quantification of the plaques observed, I noted NC2 produced significantly smaller plaques compared to WT. The genomic sequencing analysis revealed NC2 contained four unique mutations, however, three of the four mutations were conservative. The major mutation observed was located in Nsp3, the viral protease. This mutation conferred a substitution of a positively charged amino acid to a negatively charged amino acid (Table 1). Since the conservative mutations are located around the same region, these mutations could potentially be compensating for the initial Nsp3 mutation. These changes in structure potentially could be to help counter act the deleterious mutation, thus allowing NC2 to not exhibit as drastic of TS phenotype when assessed at 37 ^{o}C (Figure 7, 8). To further support my hypothesis that NC2 may have secondary mutations that were induced to stabilize the initial attenuating mutation, it can be seen clearly that half of the mutations found in NC2 were conservative mutation changes If my hypothesis is true, it may explain why NC2 has much less of a significant TS phenotype than that of LA16.

Interestingly, my data supports the previous studies of *Schaad et al* [41]. Using complementation analysis *Schaad et al.* was able to categorize over 20 TS mutants based on the location of TS mutations throughout the viral genome (Table 2). Complementation groups A and B were mapped to contain mutations in ORF1A. ORF1A was the region where I identified the nucleotide changes that are predicted to be responsible for causing the TS phenotype. Over twenty years later is seems that this hypothesis was correct when assessing the genomic sequencing data. NC2 and LA16 both contained important mutations in Nsp3, supporting the rationale for grouping these two mutant strains together.

Complementation Group	Mutants
A	LA3, LA6, LA16, NC8, NC9, NC13
B	LA16, NC2, NC11
C	LA8, LA9 ,LA14 ,NC1,NC3,NC10
D	LA10
E	LA18,NC4,NC12
F	LA7,LA12,LA13,NC5,NC6

TABLE 2. Table of TS mutants that have been catorgized based on the location of their mutations within the viral genome. Over 2,000 TS mutants were produced following the chemical mtagensis of WT MHV. Using complementaiotn analysis *Schaad et al.* identified 24 mutants that contained unique mutations[41]. Highlighted in red are the two mutants that were further characterized in my study.

It is important to note that LA16 was the only virus that was added to two complementation groups. *Schaad et al.* hypothesized that this mutant strain actually contained two significant muations in ORF1A. This hypothesis does strongly correlate with the images that were taken of LA16 at 37^{o}C. In assessing the images in Figure 7 you can see that there seem to be two populations of plaques that formed. These two distict populations may represent viruses that contain one of the two hypothesized mutations in ORF1A. It is important to note that although

this visual representation does seem to further support previous hypothesis, however further analysis will need to be performed.

Temparture-sensitive phenotyping has aided in identifying the function of unknown CoV Nsps. *Donaldson et al.* used TS phenotyping to identify a novel attenuating mutation in another TS mutant that *Schaad et al* identified, LA6[41,45]. Deep sequencing of the viral genome revealed a mutation in Nsp10. In further assessing the crystal structure of Nsp10, the mutation that conferred the TS phenotype was located in an undefined region of the structure[45]. The amino acids located within the undefined region are thought to be critical to maintaining protein stability[45]. In comparing the location of the mutation in LA16, I found the mutation is located within this undefined region as well. Therefore, it is possible that the mutation in LA16 also affects protein stability.

The sequencing results reported in this study revealed that there are many mutations located within ORF1A of LA16 and NC2. In order to assess the phenotype of the mutations individually, these mutations can be investigated using the MHV reverse genetic system[35]. A reverse-genetics system allows the study of individual mutations by allowing the manipulation of a RNA viral genome, using classic genetic techniques[35]. Mutations are introduced into a cDNA version of the RNA genome and using transcription and electroporation, one can produce a "designer virus" that has the desired mutation. The production of this designer virus allows one to study the phenotype that a single mutation may confer. This same system has also been successfully used to identify TS mutation in LA6, as described above[45]. Hopefully, the information obtained in this study will allow the identification of mutations in conserved genes that can be applied to a variety of CoVs resulting in protection fro m current and more importantly, future strains of CoVs.

APPENDIX:

SUPPLEMENTAL FIGURES

Figure S1. Full Genomic Sequence Alignment of WT, LA16 and NC2. Using Clone Manager I compared the full genomic sequence of LA16, NC2, and WT MHV. Nucleotides that are mutated from the WT reference strain are highlighted in orange. Mutations were found throughout the viral genome however in this study I focused on the mutations located in ORF1A.

```
MHV IC NC_001846      1 tataagagtgattggcgtccgtacgtaccctctcaactctaaaactcttgtagtttaaat
LA16 #39              1 -.....................................................
NC2 #3                1 -.....................................................

MHV IC NC_001846     61 ctaatctaaactttataaacggcacttcctgcgtgtccatgcccgcgggcctggtcttgt
LA16 #39             60 .....................................................
NC2 #3               60 .....................................................

MHV IC NC_001846    121 catagtgctgacatttgtagttccttgactttcgttctctgccagtgacgtgtccattcg
LA16 #39            120 .....................................................
NC2 #3              120 .....................................................

MHV IC NC_001846    181 gcgccagcagcccacccataggttgcataatggcaaagatgggcaaatacggtctcggct
LA16 #39            180 .....................................................
NC2 #3              180 .....................................................

MHV IC NC_001846    241 tcaaatgggccccagaatttccatggatgcttccgaacgcatcggagaagttgggtaacc
LA16 #39            240 .....................................................
NC2 #3              240 .....................................................

MHV IC NC_001846    301 ctgagaggtcagaggaggatgggttttgcccctctgctgcgcaagaaccgaaagttaaag
LA16 #39            300 .....................................................
NC2 #3              300 .....................................................

MHV IC NC_001846    361 gaaaaactttggttaatcacgtgagggtgaattgtagccggcttccagctttggaatgct
LA16 #39            360 .....................................................
NC2 #3              360 .....................................................

MHV IC NC_001846    421 gtgttcagtctgccataatccgtgatatttttgtagatgaggatccccagaaggtggagg
LA16 #39            420 .....................................................
NC2 #3              420 .....................................................

MHV IC NC_001846    481 cctcaactatgatggcattgcagttcggtagtgccgtcttggttaagccatccaagcgct
LA16 #39            480 .....................................................
NC2 #3              480 .....................................................

MHV IC NC_001846    541 tgtctattcaggcatggactaatttgggtgtgcttcccaaaacagctgccatggggttgt
LA16 #39            540 .....................................................
NC2 #3              540 .....................................................

MHV IC NC_001846    601 tcaagcgcgtctgcctgtgtaacaccagggagtgctcttgtgacgcccacgtggcctttc
LA16 #39            600 .....................................................
NC2 #3              600 .....................................................
```

```
MHV IC NC_001846    661 acctttttacggtccaacccgatggtgtatgcctgggtaatggccgttttataggctggt
LA16 #39            660 ............................................................
NC2 #3              660 ............................................................

MHV IC NC_001846    721 tcgttccagtcacagccataccggagtatgcgaagcagtggttgcaaccctggtccatcc
LA16 #39            720 ............................................................
NC2 #3              720 ............................................................

MHV IC NC_001846    781 ttcttcgtaagggtggtaacaaagggtctgtgacatccggccacttccgccgcgctgtta
LA16 #39            780 ............................................................
NC2 #3              780 ............................................................

MHV IC NC_001846    841 ccatgcctgtgtatgactttaatgtagaggatgcttgtgaggaggttcatcttaacccga
LA16 #39            840 ............................................................
NC2 #3              840 ............................................................

MHV IC NC_001846    901 agggtaagtactcctgcaaggcgtatgctcttcttaagggctatcgcggtgttaagccca
LA16 #39            900 ............................................................
NC2 #3              900 ............................................................

MHV IC NC_001846    961 tcctgtttgtggaccagtatggttgcgactatactggatgtctcgccaagggtcttgagg
LA16 #39            960 ............................................................
NC2 #3              960 ............................................................

MHV IC NC_001846   1021 actatggcgatctcaccttgagtgagatgaaggagttgttccctgtgtggcgtgactcct
LA16 #39           1020 ............................................................
NC2 #3             1020 ............................................................

MHV IC NC_001846   1081 tggatagtgaagtccttgtggcttggcacgttgatcgagatcctcgggctgctatgcgtc
LA16 #39           1080 ............................................................
NC2 #3             1080 ............................................................

MHV IC NC_001846   1141 tgcagactcttgctactgtacgttgcattgattatgtgggccaaccgaccgaggatgtgg
LA16 #39           1140 ............................................................
NC2 #3             1140 ............................................................

MHV IC NC_001846   1201 tggatggagatgtggtagtgcgtgagcctgctcatcttctcgcagccaatgccattgtta
LA16 #39           1200 ............................................................
NC2 #3             1200 ............................................................

MHV IC NC_001846   1261 aaagactcccccgtttggtggagactatgctgtatacggattcgtccgttacagaattct
LA16 #39           1260 ............................................................
NC2 #3             1260 ............................................................

MHV IC NC_001846   1321 gttataaaaccaagctgtgtgaatgcggttttatcacgcagtttggctatgtggattgtt
LA16 #39           1320 ............................................................
NC2 #3             1320 ............................................................

MHV IC NC_001846   1381 gtggtgacacctgcgattttcgtgggtgggttgccggcaatatgatggatggctttccat
LA16 #39           1380 ............................................................
NC2 #3             1380 ............................................................

MHV IC NC_001846   1441 gtccagggtgtaccaaaaattatatgccctgggaattggaggcccagtcatcaggtgtta
LA16 #39           1440 ............................................................
NC2 #3             1440 ............................................................

MHV IC NC_001846   1501 taccagaaggaggtgttctattcactcagagcactgatacagtgaatcgtgagtccttta
LA16 #39           1500 ............................................................
NC2 #3             1500 ............................................................
```

```
MHV IC NC_001846   1561 agctctacggtcatgctgttgtgccttttggttctgctgtgtattggagcccttgcccag
LA16 #39           1560 ....................................................
NC2 #3             1560 ....................................................

MHV IC NC_001846   1621 gtatgtggcttccagtaatttggtcttctgttaagtcatactctggtttgacttatacag
LA16 #39           1620 ....................................................
NC2 #3             1620 ....................................................

MHV IC NC_001846   1681 gagtagttggttgtaaggcaattgttcaagagacagacgctatatgtcgttctctgtata
LA16 #39           1680 ....................................................
NC2 #3             1680 ....................................................

MHV IC NC_001846   1741 tggattatgtccagcacaagtgtggcaatctcgagcagagagctatccttggattggacg
LA16 #39           1740 ....................................................
NC2 #3             1740 ....................................................

MHV IC NC_001846   1801 atgtctatcatagacagttgcttgtgaataggggtgactatagtctcctccttgagaatg
LA16 #39           1800 ....................................................
NC2 #3             1800 ....................................................

MHV IC NC_001846   1861 tggatttgtttgttaagcggcgcgctgaatttgcttgcaaattcgccacctgtggagatg
LA16 #39           1860 ....................................................
NC2 #3             1860 ....................................................

MHV IC NC_001846   1921 gtcttgtacccctcctactagatggtttagtgccccgcagttattatttgattaagagtg
LA16 #39           1920 ....................................................
NC2 #3             1920 ....................................................

MHV IC NC_001846   1981 gtcaagctttcacctctatgatggttaattttagccatgaggtgactgacatgtgtatgg
LA16 #39           1980 ....................................................
NC2 #3             1980 ....................................................

MHV IC NC_001846   2041 acatggctttattgttcatgcatgatgttaaagtggccactaagtatgttaagaaggtta
LA16 #39           2040 ....................................................
NC2 #3             2040 ....................................................

MHV IC NC_001846   2101 ctggcaaactggccgtgcgctttaaagcgttgggtgtagccgttgtcagaaaaattactg
LA16 #39           2100 ....................................................
NC2 #3             2100 ....................................................

MHV IC NC_001846   2161 aatggtttgatttagccgtggacattgctgctagtgccgctggatggctttgctaccagc
LA16 #39           2160 ....................................................
NC2 #3             2160 ....................................................

MHV IC NC_001846   2221 tggtaaatggcttatttgcagtggccaatggtgttataacctttgtacaggaggtgcctg
LA16 #39           2220 ....................................................
NC2 #3             2220 ....................................................

MHV IC NC_001846   2281 agcttgtcaagaattttgttgacaagttcaaggcatttttcaaggttttgatcgactcta
LA16 #39           2280 ....................................................
NC2 #3             2280 ....................................................

MHV IC NC_001846   2341 tgtcggtttctatcttgtctggacttactgttgtcaagactgcctcaaatagggtgtgtc
LA16 #39           2340 ....................................................
NC2 #3             2340 ....................................................

MHV IC NC_001846   2401 ttgctggcagtaaggtttatgaagttgtgcagaaatctttgtctgcatatgttatgcctg
LA16 #39           2400 ....................................................
NC2 #3             2400 ....................................................
```

```
MHV IC NC_001846   2461 tgggttgcagtgaagccacttgtttggtgggtgagattgaacctgcagtttttgaagatg
LA16 #39           2460 .........c.....................................................
NC2 #3             2460 .........c.....................................................

MHV IC NC_001846   2521 atgttgttgatgtggttaaagccccattaacatatcaaggctgttgtaagccacccactt
LA16 #39           2520 ............................................................
NC2 #3             2520 ............................................................

MHV IC NC_001846   2581 ctttcgagaagatttgtattgtggataaattgtatatggccaagtgtggtgatcaatttt
LA16 #39           2580 ............................................................
NC2 #3             2580 ............................................................

MHV IC NC_001846   2641 accctgtggttgttgataacgacactgttggcgtgttagatcagtgctggaggtttccct
LA16 #39           2640 ............................................................
NC2 #3             2640 ............................................................

MHV IC NC_001846   2701 gtgcgggcaagaaagtcgagtttaacgacaagcccaaagtcaggaagataccctccaccc
LA16 #39           2700 ............................................................
NC2 #3             2700 ............................................................

MHV IC NC_001846   2761 gtaagattaagatcaccttcgcactggatgcgacctttgatagtgttctttcgaaggcgt
LA16 #39           2760 ............................................................
NC2 #3             2760 ............................................................

MHV IC NC_001846   2821 gttcagagtttgaagttgataaagatgttacattggatgagctgcttgatgttgtgcttg
LA16 #39           2820 ............................................................
NC2 #3             2820 ............................................................

MHV IC NC_001846   2881 acgcagttgagagtacgctcagcccttgtaaggagcatgatgtgataggcacaaaagttt
LA16 #39           2880 ............................................................
NC2 #3             2880 ............................................................

MHV IC NC_001846   2941 gtgctttacttgataggttggcaggagattatgtctatctttttgatgagggaggcgatg
LA16 #39           2940 ............................................................
NC2 #3             2940 ............................................................

MHV IC NC_001846   3001 aagtgatcgccccgaggatgtattgttccttttctgctcctgatgatgaagactgcgttg
LA16 #39           3000 ............................................................
NC2 #3             3000 ............................................................

MHV IC NC_001846   3061 cagcggatgttgtagatgcagatgaaaaccaagatgatgatgctgaagactcagcagtcc
LA16 #39           3060 ............................................................
NC2 #3             3060 ............................................................

MHV IC NC_001846   3121 ttgtcgctgatacccaagaagaggacggcgttgccaaggggcaggttgaggcggattcgg
LA16 #39           3120 ............................................................
NC2 #3             3120 ............................................................

MHV IC NC_001846   3181 aaatttgcgttgcgcatactggtagtcaagaagaattggctgagcctgatgctgtcggat
LA16 #39           3180 ............................................................
NC2 #3             3180 ............................................................

MHV IC NC_001846   3241 ctcaaactcccatcgcctctgctgaggaaaccgaagtcggagaggcaagcgacagggaag
LA16 #39           3240 ............................................................
NC2 #3             3240 ............................................................

MHV IC NC_001846   3301 ggattgctgaggcgaaggcaactgtgtgtgctgatgctgtagatgcctgccccgatcaag
LA16 #39           3300 ............................................................
NC2 #3             3300 ............................................................
```

```
MHV IC NC_001846   3361 tggaggcatttgaaattgaaaaggttgaagactctatcttggatgagcttcaaactgaac
LA16 #39           3360 ............................................................
NC2 #3             3360 ............................................................

MHV IC NC_001846   3421 ttaatgcgccagcggacaagacctatgaggatgtcttggcattcgatgccgtatgctcag
LA16 #39           3420 ............................................................
NC2 #3             3420 ............................................................

MHV IC NC_001846   3481 aggcgttgtctgcattctatgctgtgccgagtgatgagacgcactttaaagtgtgtggat
LA16 #39           3480 ............................................................
NC2 #3             3480 ............................................................

MHV IC NC_001846   3541 tctattcgcctgctatagagcgcactaattgttggctgcgttctactttgatagtaatgc
LA16 #39           3540 ............................................................
NC2 #3             3540 ............................................................

MHV IC NC_001846   3601 agagtctacctttggaatttaaagacttggagatgcaaaagctctggttgtcttacaagg
LA16 #39           3600 ............................................................
NC2 #3             3600 ............................................................

MHV IC NC_001846   3661 ccggctatgaccaatgctttgtggacaaactagttaagagcgtgcccaagtctattatcc
LA16 #39           3660 ............................................................
NC2 #3             3660 ............................................................

MHV IC NC_001846   3721 ttccacaaggtggttatgtggcagattttgcctatttctttctaagccagtgtagcttta
LA16 #39           3720 ............................................................
NC2 #3             3720 ............................................................

MHV IC NC_001846   3781 aagcttatgctaactggcgttgtttagagtgtgacatggagttaaagcttcaaggcttgg
LA16 #39           3780 ............................................................
NC2 #3             3780 ............................................................

MHV IC NC_001846   3841 acgccatgtttttctatggggacgttgtgtctcatatgtgcaagtgtggtaatagcatga
LA16 #39           3840 ............................................................
NC2 #3             3840 ............................................................

MHV IC NC_001846   3901 ccttgttgtctgcagatataccctacactttgcattttggagtgcgagatgataagtttt
LA16 #39           3900 ............................................................
NC2 #3             3900 ............................................................

MHV IC NC_001846   3961 gcgctttttacacgccaagaaaggtctttagggctgcttgtgcggtagatgttaatgatt
LA16 #39           3960 ............................................................
NC2 #3             3960 ............................................................

MHV IC NC_001846   4021 gtcactctatggctgtagtagagggcaagcaaattgatggtaaagtggttaccaaattta
LA16 #39           4020 ............................................................
NC2 #3             4020 ............................................................

MHV IC NC_001846   4081 ttggtgacaaatttgattttatggtgggttacgggatgacatttagtatgtctcctttg
LA16 #39           4080 ............................................................
NC2 #3             4080 ............................................................

MHV IC NC_001846   4141 aactcgcccagttatatggttcatgtataacaccaaatgtttgttttgttaaaggagatg
LA16 #39           4140 ............................................................
NC2 #3             4140 ............................................................

MHV IC NC_001846   4201 ttataaaggttgttcgcttagttaatgctgaagtcattgttaaccctgctaatgggcgta
LA16 #39           4200 ............................................................
NC2 #3             4200 ............................................................
```

```
MHV IC NC_001846   4261 tggctcatggtgcaggtgttgcaggtgctatagctgaaaaggcgggcagtgcttttatta
LA16 #39           4260 ............................................................
NC2 #3             4260 ...........................................g................

MHV IC NC_001846   4321 aagaaacctccgatatggtgaaggctcagggcgtttgccaggttggtgaatgctatgaat
LA16 #39           4320 ............................................................
NC2 #3             4320 ............................................................

MHV IC NC_001846   4381 ctgccggtggtaagttatgtaaaaaggtgcttaacattgtagggccagatgcgcgagggc
LA16 #39           4380 ............................................................
NC2 #3             4380 ............................................................

MHV IC NC_001846   4441 atggcaagcaatgctattcacttttagagcgtgcttatcagcatattaataagtgtgaca
LA16 #39           4440 ............................................................
NC2 #3             4440 ............................................................

MHV IC NC_001846   4501 atgttgtcactactttaatttcggctggtatatttagtgtgcctactgatgtctccctaa
LA16 #39           4500 ............................................................
NC2 #3             4500 ............................................................

MHV IC NC_001846   4561 cttacttacttggtgtagtgacaaagaatgtcattcttgtcagtaacaaccaggatgatt
LA16 #39           4560 ............................................................
NC2 #3             4560 ............................................................

MHV IC NC_001846   4621 ttgatgtgatagagaagtgtcaggtgacctccgttgctggtaccaaagcgctatcacttc
LA16 #39           4620 ............................................................
NC2 #3             4620 ............................................................

MHV IC NC_001846   4681 aattggccaaaaatttgtgccgtgatgtaaagtttgtgacgaatgcatgtagttcgcttt
LA16 #39           4680 ............................................................
NC2 #3             4680 ............................................................

MHV IC NC_001846   4741 ttagtgaatcttgctttgtctcaagctatgatgtgttgcaggaagttgaagcgctgcgac
LA16 #39           4740 ............................................................
NC2 #3             4740 ............................................................

MHV IC NC_001846   4801 atgatatacaattggatgatgatgctcgtgtctttgtgcaggctaatatggactgtctgc
LA16 #39           4800 ............................................................
NC2 #3             4800 ............................................................

MHV IC NC_001846   4861 ccacagactggcgtctcgttaacaaatttgatagtgttgatggtgttagaaccattaagt
LA16 #39           4860 ............................................................
NC2 #3             4860 ............................................................

MHV IC NC_001846   4921 attttgaatgcccgggcgggatttttgtatccagccagggcaaaaagtttggttatgttc
LA16 #39           4920 ............................................................
NC2 #3             4920 ............................................................

MHV IC NC_001846   4981 agaatggttcatttaaggaggcgagtgttagccaaataagggctttactcgctaataagg
LA16 #39           4980 ............................................................
NC2 #3             4980 ............................................................

MHV IC NC_001846   5041 ttgatgtcttgtgtactgttgatggtgttaacttccgctcctgctgcgtagcagagggtg
LA16 #39           5040 ............................................................
NC2 #3             5040 ............................................................

MHV IC NC_001846   5101 aagtttttggcaagacattaggttcagtcttttgtgatggcataaatgtcaccaaagtta
LA16 #39           5100 ............................................................
NC2 #3             5100 ............................................................
```

```
MHV IC NC_001846   5161 ggtgtagtgccatttacaagggtaaggttttctttcagtacagtgatttgtccgaggcag
LA16 #39           5160 ....................................................
NC2 #3             5160 ....................................................

MHV IC NC_001846   5221 atcttgtggctgttaaagatgcctttggttttgatgaaccacaactgctgaagtactaca
LA16 #39           5220 ....................................................
NC2 #3             5220 ....................................................

MHV IC NC_001846   5281 ctatgcttggcatgtgtaagtggtcagtagttgtttgtggcaattattttgctttcaagc
LA16 #39           5280 ......................c.............................
NC2 #3             5280 ......................c.............................

MHV IC NC_001846   5341 agtcaaataataattgctatataaatgtggcatgtttaatgctgcaacacttgagtttaa
LA16 #39           5340 ....................................................
NC2 #3             5340 ....................................................

MHV IC NC_001846   5401 agtttcctaagtggcaatggcaagaggcttggaacgagttccgctctggtaaaccactaa
LA16 #39           5400 ....................................................
NC2 #3             5400 ....................................................

MHV IC NC_001846   5461 ggtttgtgtccttggtattagcaaagggcagctttaaatttaatgaaccttctgattcta
LA16 #39           5460 ....................................................
NC2 #3             5460 ....................................................

MHV IC NC_001846   5521 tcgattttatgcgtgtggtgctacgtgaagcagatttgagtggtgccacgtgcaatttgg
LA16 #39           5520 ....................................................
NC2 #3             5520 ....................................................

MHV IC NC_001846   5581 aatttgtttgtaaatgtggtgtgaagcaagagcagcgcaaaggtgttgacgctgttatgc
LA16 #39           5580 ....................................................
NC2 #3             5580 ....................................................

MHV IC NC_001846   5641 attttggtacgttggataaaggtgatcttgtcaggggttataatatcgcatgtacgtgcg
LA16 #39           5640 ....................................................
NC2 #3             5640 ....................................................

MHV IC NC_001846   5701 gtagtaaacttgtgcattgcacccaatttaacgtaccatttttaatttgctccaacacac
LA16 #39           5700 ....................................................
NC2 #3             5700 ....................................................

MHV IC NC_001846   5761 cagagggtaggaaactgcccgacgatgttgttgcagctaatatttttactggtggtagtg
LA16 #39           5760 ....................................................
NC2 #3             5760 ....................................................

MHV IC NC_001846   5821 tgggccattacacgcatgtgaaatgtaaacccaagtaccagctttatgatgcttgtaatg
LA16 #39           5820 ....................................................
NC2 #3             5820 ....................................................

MHV IC NC_001846   5881 ttaataaggtttcggaggctaagggtaattttaccgattgcctctaccttaaaaatttaa
LA16 #39           5880 ....................................................
NC2 #3             5880 ....................................................

MHV IC NC_001846   5941 agcaaacttttcgtctgtgctgacgacttttttatttagatgatgtaaagtgtgtggagt
LA16 #39           5940 ....................................................
NC2 #3             5940 ....................................................

MHV IC NC_001846   6001 ataagccagatttatcgcagtattactgtgagtctggtaaatattatacaaaacccatta
LA16 #39           6000 ....................................................
NC2 #3             6000 ....................................................
```

```
MHV IC NC_001846   6061 ttaaggcccaatttagaacatttgagaaggttgatggtgtctataccaactttaaattgg
LA16 #39           6060 ..........................................................
NC2 #3             6060 ..........................................................

MHV IC NC_001846   6121 tgggacatagtattgctgaaaaactcaatgctaagctgggatttgattgtaattctccct
LA16 #39           6120 ..........................................................
NC2 #3             6120 ..........................................................

MHV IC NC_001846   6181 ttgtggagtataaaattacagagtggccaacagctactggagatgtggtgttggctagtg
LA16 #39           6180 ..........................................................
NC2 #3             6180 ..........................................................

MHV IC NC_001846   6241 atgatttgtatgtaagtcggtactcaagcgggtgcattacttttggtaaaccggttgtct
LA16 #39           6240 ............g.............................................
NC2 #3             6240 ............g.............................................

MHV IC NC_001846   6301 ggcttggccatgaggaagcatcgctgaaatctctcacatattttaatagacctagtgtcg
LA16 #39           6300 ..........................................................
NC2 #3             6300 ..........................................................

MHV IC NC_001846   6361 tttgtgaaaataaatttaatgtgttgcccgttgatgtcagtgaacccacggacaaggggc
LA16 #39           6360 ..........................................................
NC2 #3             6360 ..........................................................

MHV IC NC_001846   6421 ctgtgcctgctgcagtccttgttaccggcgtccctggagctgatgcgtcagctggtgccg
LA16 #39           6420 ..........................................................
NC2 #3             6420 ..........................................................

MHV IC NC_001846   6481 gtattgccaaggagcaaaaagcctgtgcttctgctagtgtggaggatcaggttgttacgg
LA16 #39           6480 ..........................................................
NC2 #3             6480 ..........................................................

MHV IC NC_001846   6541 aggttcgtcaagagccatctgtttcagctgctgatgtcaaagaggttaaattgaatggtg
LA16 #39           6540 ..........................................................
NC2 #3             6540 ..........................................................

MHV IC NC_001846   6601 ttaaaaagcctgttaaggtggaaggtagtgtggttgttaatgatcccactagcgaaacca
LA16 #39           6600 ..........................................................
NC2 #3             6600 ..........................................................

MHV IC NC_001846   6661 aagttgttaaaagtttgtctattgttgatgtctatgatatgttcctgacagggtgtaagt
LA16 #39           6660 ..........................................................
NC2 #3             6660 ..........................................................

MHV IC NC_001846   6721 atgtggtttggactgctaatgagttgtctcgactagtaaattcaccgactgttagggagt
LA16 #39           6720 ..........................................................
NC2 #3             6720 ..........................................................

MHV IC NC_001846   6781 atgtgaagtggggtaagggaaagattgtaacacccgctaagttgttgttgttaagagatg
LA16 #39           6780 ..............t...........................................
NC2 #3             6780 ..............t...........................................

MHV IC NC_001846   6841 agaagcaagagttcgtagcgccaaaagtagtcaaggcgaaagctattgcctgctattgtg
LA16 #39           6840 ..........................................................
NC2 #3             6840 ..........................................................

MHV IC NC_001846   6901 ctgtgaagtggtttctcctctattgttttagttggataaagtttaatactgataataagg
LA16 #39           6900 ..........................................................
NC2 #3             6900 ..........................................................
```

```
MHV IC NC_001846   6961 ttatatacaccacagaagtagcttcaaagcttactttcaagttgtgctgtttggccttta
LA16 #39           6960 ............................................................
NC2 #3             6960 ............................................................

MHV IC NC_001846   7021 agaatgccttacagacgtttaattggagcgttgtgtctaggggctttttcctagttgcaa
LA16 #39           7020 ............................................................
NC2 #3             7020 ............................................................

MHV IC NC_001846   7081 cggtctttttattatggtttaacttttgtatgctaatgttattttgagtgacttctatt
LA16 #39           7080 ............................................................
NC2 #3             7080 ............................................................

MHV IC NC_001846   7141 tgcctaatattgggcctctccctacgtttgtgggacagatagttgcgtggtttaagacta
LA16 #39           7140 ............................................................
NC2 #3             7140 ............................................................

MHV IC NC_001846   7201 catttggtgtgtcaaccatctgtgatttctaccaggtgacggatttgggctatagaagtt
LA16 #39           7200 ............................................................
NC2 #3             7200 ............................................................

MHV IC NC_001846   7261 cgttttgtaatggaagtatggtatgtgaactatgcttctcaggttttgatatgctggaca
LA16 #39           7260 ............a...............................................
NC2 #3             7260 ............................................................

MHV IC NC_001846   7321 actatgatgctataaatgttgttcaacacgttgtagataggcgtttgtcctttgactata
LA16 #39           7320 ............................................................
NC2 #3             7320 ............................................................

MHV IC NC_001846   7381 ttagcctatttaaattagtagttgagcttgtaatcggctactctctttatactgtgtgct
LA16 #39           7380 ............................................................
NC2 #3             7380 ............................................................

MHV IC NC_001846   7441 tctacccactgtttgtccttattggaatgcagttgttgaccacatggttgcctgaattct
LA16 #39           7440 ............................................................
NC2 #3             7440 ............................................................

MHV IC NC_001846   7501 ttatgctggagactatgcattggagtgctcgtttgtttgtgtttgttgccaatatgcttc
LA16 #39           7500 ............................................................
NC2 #3             7500 ............................................................

MHV IC NC_001846   7561 cagcttttacgttactgcgattttacatcgtggtgacagctatgtataaggtctattgtc
LA16 #39           7560 ............................................................
NC2 #3             7560 ............................................................

MHV IC NC_001846   7621 tttgtagacatgttatgtatggatgtagtaagcctggttgcttgttttgttataagagaa
LA16 #39           7620 ............................................................
NC2 #3             7620 ............................................................

MHV IC NC_001846   7681 accgtagtgtccgtgttaagtgtagcaccgttgttggtggttcactacgctattacgatg
LA16 #39           7680 ............................................................
NC2 #3             7680 ............................................................

MHV IC NC_001846   7741 taatggctaacggcggcacaggtttctgtacaaagcaccagtggaactgtcttaattgca
LA16 #39           7740 ............................................................
NC2 #3             7740 ............................................................

MHV IC NC_001846   7801 attcctggaaaccaggcaatacattcataactcatgaagcagcggcggacctctctaagg
LA16 #39           7800 ............................................................
NC2 #3             7800 ............................................................
```

```
MHV IC NC_001846   7861 agttgaaacgccctgtgaatccaacagattctgcttattactcggtcacagaggttaagc
LA16 #39           7860 ............................................................
NC2 #3             7860 ............................................................

MHV IC NC_001846   7921 aggttggttgttccatgcgtttgttctacgagagagatggacagcgtgtttatgatgatg
LA16 #39           7920 ............................................................
NC2 #3             7920 ............................................................

MHV IC NC_001846   7981 ttaatgctagtttgtttgtggacatgaatggtctgctgcattctaaagttaaaggtgtgc
LA16 #39           7980 ............................................................
NC2 #3             7980 ............................................................

MHV IC NC_001846   8041 ctgaaacgcatgttgtggttgttgagaatgaagctgataaagctggttttctcggcgccg
LA16 #39           8040 ............................................................
NC2 #3             8040 ............................................................

MHV IC NC_001846   8101 cagtgttttatgcacaatcgctctacagacctatgttgatggtggaaaagaaattaataa
LA16 #39           8100 ............................................................
NC2 #3             8100 ............................................................

MHV IC NC_001846   8161 ctaccgccaacactggtttgtctgttagtcgaactatgtttgacctttatgtagattcat
LA16 #39           8160 ............................................................
NC2 #3             8160 ............................................................

MHV IC NC_001846   8221 tgctgaacgtcctcgacgtggatcgcaagagtctaacaagttttgtaaatgctgcgcaca
LA16 #39           8220 ............................................................
NC2 #3             8220 ............................................................

MHV IC NC_001846   8281 actctctaaaggagggtgttcagcttgaacaagttatggataccttattggctgtgccc
LA16 #39           8280 ............................................................
NC2 #3             8280 ............................................................

MHV IC NC_001846   8341 gacgtaagtgtgctatagattctgatgttgaaaccaagtctattaccaagtccgtcatgt
LA16 #39           8340 ............................................................
NC2 #3             8340 ............................................................

MHV IC NC_001846   8401 cggcagtaaatgctggcgttgattttacggatgagagttgtaataacttggtgcctacct
LA16 #39           8400 ............................................................
NC2 #3             8400 ............................................................

MHV IC NC_001846   8461 atgttaaaagtgacactatcgttgcagccgatttgggtgttcttattcagaataatgcta
LA16 #39           8460 ............................................................
NC2 #3             8460 ............................................................

MHV IC NC_001846   8521 agcatgtacaggctaatgttgctaaagccgctaatgtggcttgcatttggtctgtggatg
LA16 #39           8520 ............................................................
NC2 #3             8520 ............................................................

MHV IC NC_001846   8581 cttttaaccagctatctgctgacttacagcataggctgcgaaaagcatgttcaaaaactg
LA16 #39           8580 ............................................................
NC2 #3             8580 ............................................................

MHV IC NC_001846   8641 gcttgaagattaagcttacttataataagcaggaggcaaatgttcctattttaactacac
LA16 #39           8640 ............................................................
NC2 #3             8640 ............................................................

MHV IC NC_001846   8701 cgttctctcttaaaggggcgctgtttttagtagaatgttacaatggttgtttgttgcta
LA16 #39           8700 ............................................................
NC2 #3             8700 ............................................................
```

```
MHV IC NC_001846   8761 atttgatttgtttcattgtgttgtgggcccttatgccaacatatgcagtgcacaaatcgg
LA16 #39           8760 ............................................................
NC2 #3             8760 ............................................................

MHV IC NC_001846   8821 atatgcagttgcctttatatgccagttttaaagttatagataatggtgtgctaagggatg
LA16 #39           8820 ............................................................
NC2 #3             8820 ............................................................

MHV IC NC_001846   8881 tgtctgttactgacgcatgcttcgcaaacaaatttaatcaatttgatcaatggtatgagt
LA16 #39           8880 ............................................................
NC2 #3             8880 ............................................................

MHV IC NC_001846   8941 ctacttttggtcttgcttattaccgcaactctaaggcttgtcctgttgtggttgctgtaa
LA16 #39           8940 ............................................................
NC2 #3             8940 ............................................................

MHV IC NC_001846   9001 tagatcaagacattggccataccttatttaatgttcctaccacagttttaagatatggat
LA16 #39           9000 ............................................................
NC2 #3             9000 ............................................................

MHV IC NC_001846   9061 ttcatgtgttgcattttataacccatgcatttgctactgatagcgtgcagtgttacacgc
LA16 #39           9060 ............................................................
NC2 #3             9060 ............................................................

MHV IC NC_001846   9121 cacatatgcaaatcccctatgataatttctatgctagtggttgcgtgttgtcatccctct
LA16 #39           9120 ............................................................
NC2 #3             9120 ............................................................

MHV IC NC_001846   9181 gtactatgcttgcgcatgcagatggaaccccgcatccttattgttatacaggggtgtta
LA16 #39           9180 ............................................................
NC2 #3             9180 ............................................................

MHV IC NC_001846   9241 tgcacaatgcctctctgtatagttctttggctcctcatgtccgttataacctggctagtt
LA16 #39           9240 ............................................................
NC2 #3             9240 ............................................................

MHV IC NC_001846   9301 caaatggttatatacgttttcccgaagtggttagtgaaggcattgtgcgtgttgtgcgca
LA16 #39           9300 ............................................................
NC2 #3             9300 ............................................................

MHV IC NC_001846   9361 ctcgctctatgacctactgcagggttggtttatgtgaggaggccgaggagggtatctgct
LA16 #39           9360 ............................................................
NC2 #3             9360 ............................................................

MHV IC NC_001846   9421 ttaattttaatcgttcatgggtattgaacaacccgtattatagggccatgcctggaactt
LA16 #39           9420 ............................................................
NC2 #3             9420 ............................................................

MHV IC NC_001846   9481 tttgtggtaggaatgcttttgatttaatacatcaagttttaggaggattagtgcggccta
LA16 #39           9480 ............................................................
NC2 #3             9480 ............................................................

MHV IC NC_001846   9541 ttgatttctttgccttaacggcgagttcagtggctggtgctatccttgcaattattgtcg
LA16 #39           9540 ............................................................
NC2 #3             9540 ............................................................

MHV IC NC_001846   9601 ttttggctttctattatttaataaagcttaaacgtgcctttggtgactacactagtgttg
LA16 #39           9600 ............................................................
NC2 #3             9600 ............................................................
```

```
MHV IC NC_001846   9661 tggttatcaatgtaattgtgtggtgtataaattttctgatgctttttgtgtttcaggttt
LA16 #39           9660 ............................................................
NC2 #3             9660 ............................................................

MHV IC NC_001846   9721 atcccacattgtcttgtttatatgcttgtttttatttctacacaacgctttatttccctt
LA16 #39           9720 ............................................................
NC2 #3             9720 ............................................................

MHV IC NC_001846   9781 cggagataagtgttgttatgcatttgcaatggcttgtcatgtatggtgctattatgccct
LA16 #39           9780 ............................................................
NC2 #3             9780 ............................................................

MHV IC NC_001846   9841 tgtggttttgcattatttacgtggcagtcgttgtttcaaaccatgcattgtggttgttct
LA16 #39           9840 ............................................................
NC2 #3             9840 ............................................................

MHV IC NC_001846   9901 cttactgccgcaaaattggtaccgaggttcgtagtgacggcacatttgaggaaatggccc
LA16 #39           9900 ............................................................
NC2 #3             9900 ............................................................

MHV IC NC_001846   9961 ttactacctttatgattactaaagaatcttattgtaagttgaaaaattctgtttctgatg
LA16 #39           9960 ............................................................
NC2 #3             9960 ..................g.........................................

MHV IC NC_001846  10021 ttgcttttaacaggtacttgagtctttataacaagtatcgttattttagtggcaaaatgg
LA16 #39          10020 ............................................................
NC2 #3            10020 ...g........................................................

MHV IC NC_001846  10081 atactgccgcttatagagaggctgcctgttcacaactggcaaaggcaatggaaacattta
LA16 #39          10080 ............................................................
NC2 #3            10080 ............................................................

MHV IC NC_001846  10141 accataataatggtaatgatgttctctatcagcctccaaccgcctctgttactacatcat
LA16 #39          10140 ............................................................
NC2 #3            10140 ............................................................

MHV IC NC_001846  10201 ttttacagtctggtatagtgaagatggtgtcgcccacctctaaagtggagccttgtattg
LA16 #39          10200 ............................................................
NC2 #3            10200 ............................................................

MHV IC NC_001846  10261 ttagtgttacttatggtaacatgacacttaatgggttgtggttggatgataaagtttatt
LA16 #39          10260 ............................................................
NC2 #3            10260 ............................................................

MHV IC NC_001846  10321 gcccaagacatgttatctgttcttcagctgacatgacagaccctgattatcctaatttgc
LA16 #39          10320 ............................................................
NC2 #3            10320 ............................................................a

MHV IC NC_001846  10381 tttgtagagtgacatcaagtgatttttgtgttatgtctggtcgtatgagccttactgtaa
LA16 #39          10380 ............................................................
NC2 #3            10380 ............................................................

MHV IC NC_001846  10441 tgtcttatcaaatgcagggctgccaacttgttttgactgttacactgcaaaatcctaaca
LA16 #39          10440 ............................................................
NC2 #3            10440 ............................................................

MHV IC NC_001846  10501 cgcctaagtattccttcggtgttgttaagcctggtgagacatttactgtactggctgcat
LA16 #39          10500 ............................................................
NC2 #3            10500 ............................................................
```

```
MHV IC NC_001846  10561 acaatggcagacctcaaggagccttccatgttacgcttcgtagtagccataccataaagg
LA16 #39          10560 ............................................................
NC2 #3            10560 ............................................................

MHV IC NC_001846  10621 gctcctttctatgtggatcctgcggttctgtaggatatgttttaactggcgatagtgtac
LA16 #39          10620 ............................................................
NC2 #3            10620 ............................................................

MHV IC NC_001846  10681 gatttgtttatatgcatcagctagagttgagtactggttgtcataccggtactgacttta
LA16 #39          10680 ............................................................
NC2 #3            10680 ............................................................

MHV IC NC_001846  10741 gtgggaacttttatggtccctatagagatgcgcaagttgtacaattgcctgttcaggatt
LA16 #39          10740 ............................................................
NC2 #3            10740 ............................................................

MHV IC NC_001846  10801 atacgcagactgttaatgttgtagcttggctttatgctgctatttttaacagatgcaact
LA16 #39          10800 ............................................................
NC2 #3            10800 ............................................................

MHV IC NC_001846  10861 ggtttgtgcaaagtgatagttgttccctggaggagtttaatgtttgggctatgaccaatg
LA16 #39          10860 ............................................................
NC2 #3            10860 ............................................................

MHV IC NC_001846  10921 gttttagctcaatcaaagccgatcttgtcttggatgcgcttgcttctatgacaggcgtta
LA16 #39          10920 ............t...............................................
NC2 #3            10920 ............t...............................................

MHV IC NC_001846  10981 cagttgaacaggtgttggccgctattaagaggctgcattctggattccagggcaaacaaa
LA16 #39          10980 ............................................................
NC2 #3            10980 ............................................................

MHV IC NC_001846  11041 ttttaggtagttgtgtgcttgaagatgagctgacaccaagtgatgtttatcaacaactag
LA16 #39          11040 ............................................................
NC2 #3            11040 ............................................................

MHV IC NC_001846  11101 ctggtgtcaagctacagtcaaagcgcacaagagttataaaaggtacatgttgctggatat
LA16 #39          11100 ............................................................
NC2 #3            11100 ............................................................

MHV IC NC_001846  11161 tggcttcaacgtttttgttctgtagcattatctcagcatttgtaaaatggactatgttta
LA16 #39          11160 ............................................................
NC2 #3            11160 ............................................................

MHV IC NC_001846  11221 tgtatgttactacccatatgttgggagtgacattgtgtgcactttgttttgtaagctttg
LA16 #39          11220 ............................................................
NC2 #3            11220 ............................................................

MHV IC NC_001846  11281 ctatgttgttgatcaagcataagcatttgtatttaactatgtatattatgcctgtgttat
LA16 #39          11280 ............................................................
NC2 #3            11280 ............................................................

MHV IC NC_001846  11341 gcacactgttttacaccaactatttggttgtgtacaaacagagttttagaggtctagctt
LA16 #39          11340 ............................................................
NC2 #3            11340 ............................................................

MHV IC NC_001846  11401 atgcttggctttcacactttgtccctgctgtagattatacatatatggatgaagttttat
LA16 #39          11400 ............................................................
NC2 #3            11400 ............................................................
```

```
MHV IC NC_001846  11461 atggtgttgtgttgctagtagctatggtgtttgttaccatgcgtagcataaaccacgacg
LA16 #39          11460 ............................................................
NC2 #3            11460 ............................................................

MHV IC NC_001846  11521 tcttttctattatgttcttggttggtagacttgtcagcctggtatccatgtggtattttg
LA16 #39          11520 ............................................................
NC2 #3            11520 ............................................................

MHV IC NC_001846  11581 gagccaatttagaggaagaggtactattgttcctcacatccctatttggcacgtacacat
LA16 #39          11580 ............................................................
NC2 #3            11580 ............................................................

MHV IC NC_001846  11641 ggactactatgttgtcattggctaccgctaaggttattgctaaatggttggctgtgaatg
LA16 #39          11640 ............................................................
NC2 #3            11640 ............................................................

MHV IC NC_001846  11701 tcttgtacttcacagacgtaccgcaaattaaattagttcttttgagctacttgtgtattg
LA16 #39          11700 ............................................................
NC2 #3            11700 ............................................................

MHV IC NC_001846  11761 gttatgtgtgttgttgttattggggaatcttgtcactccttaatagcatttttaggatgc
LA16 #39          11760 ............................................................
NC2 #3            11760 ............................................................

MHV IC NC_001846  11821 cattgggcgtctacaattataaaatctccgttcaggagttacgttatatgaatgctaatg
LA16 #39          11820 ............................................................
NC2 #3            11820 ............................................................

MHV IC NC_001846  11881 gcttgcgcccacctagaaatagttttgaggccctgatgcttaattttaagctgttgggaa
LA16 #39          11880 ............................................................
NC2 #3            11880 ............................................................

MHV IC NC_001846  11941 ttggtggtgtgccagtcattgaagtatctcaaattcaatcaagattgacggatgttaaat
LA16 #39          11940 ............................................................
NC2 #3            11940 ............................................................

MHV IC NC_001846  12001 gtgctaatgttgtgttgcttaattgcctccagcacttgcatattgcatctaattctaagt
LA16 #39          12000 ............................................................
NC2 #3            12000 ............................................................

MHV IC NC_001846  12061 tgtggcagtattgtagtactttgcacaatgaaatactggctacatctgatttgagcgtgg
LA16 #39          12060 ............................................................
NC2 #3            12060 ............................................................

MHV IC NC_001846  12121 ccttcgataagttggctcagctcttagttgttttatttgctaatccagcagcagtggata
LA16 #39          12120 ............................................................
NC2 #3            12120 ............................................................

MHV IC NC_001846  12181 gcaagtgccttgcaagtattgaagaagtgagcgatgattacgttcgcgacaatactgtct
LA16 #39          12180 ............................................................
NC2 #3            12180 ............................................................

MHV IC NC_001846  12241 tgcaagccttacagagtgaatttgttaatatggctagcttcgttgagtatgaacttgcta
LA16 #39          12240 ............................................................
NC2 #3            12240 ............................................................

MHV IC NC_001846  12301 agaagaatctagatgaggctaaggctagcggctctgccaatcaacagcagattaagcagc
LA16 #39          12300 ............................................................
NC2 #3            12300 ............................................................
```

```
MHV IC NC_001846  12361 tagagaaggcgtgtaatattgctaagtcagcatatgagcgcgacagagctgttgctcgta
LA16 #39          12360 ....................................................
NC2 #3            12360 ....................................................

MHV IC NC_001846  12421 agctggaacgtatggctgatttagctcttacaaacatgtataaagaagctagaattaatg
LA16 #39          12420 ....................................................
NC2 #3            12420 ....................................................

MHV IC NC_001846  12481 ataagaagagtaaggtagtgtctgcattgcaaaccatgctctttagtatggtgcgtaagc
LA16 #39          12480 ....................................................
NC2 #3            12480 ....................................................

MHV IC NC_001846  12541 tagataaccaagctcttaattctattttagataatgcagttaagggttgtgtacctttga
LA16 #39          12540 ....................................................
NC2 #3            12540 ....................................................

MHV IC NC_001846  12601 atgcaataccatcattgacttcgaacactctgactataatagtgccagataagcaggttt
LA16 #39          12600 ....................................................
NC2 #3            12600 ....................................................

MHV IC NC_001846  12661 ttgatcaggttgtggataatgtgtatgtcacctatgctgggaatgtatggcatatacagt
LA16 #39          12660 ....................................................
NC2 #3            12660 ....................................................

MHV IC NC_001846  12721 ttattcaagatgctgatggtgctgttaaacaattgaatgagatagatgttaattcaacct
LA16 #39          12720 ....................................................
NC2 #3            12720 ....................................................

MHV IC NC_001846  12781 ggcctctagtcattgctgcaaataggcataatgaagtgtctactgttgttttgcagaaca
LA16 #39          12780 ....................................................
NC2 #3            12780 ....................................................

MHV IC NC_001846  12841 atgagttgatgcctcagaagttgagaactcaggttgtcaatagtggctcagatatgaatt
LA16 #39          12840 ....................................................
NC2 #3            12840 ....................................................

MHV IC NC_001846  12901 gtaatactcctacccagtgttactataatactactggcacgggtaagattgtgtatgcta
LA16 #39          12900 ....................................................
NC2 #3            12900 ....................................................

MHV IC NC_001846  12961 tacttagtgactgtgatggtctcaagtacactaagatagtaaaagaagatggaaattgtg
LA16 #39          12960 ....................................................
NC2 #3            12960 ....................g.....................................

MHV IC NC_001846  13021 ttgttttggaattggatcctccctgtaagttttctgttcaggatgtgaagggccttaaaa
LA16 #39          13020 ....................................................
NC2 #3            13020 ....................................................

MHV IC NC_001846  13081 ttaagtacctttactttgtgaaggggtgtaatacactggctagaggctgggttgtaggca
LA16 #39          13080 ....................................................
NC2 #3            13080 ....................................................

MHV IC NC_001846  13141 ccttatcctcgacagtgagattgcaggcgggtacggcaactgagtatgcctccaactctg
LA16 #39          13140 ....................................................
NC2 #3            13140 ....................................................

MHV IC NC_001846  13201 caatactgtcgctgtgtgcgttttctgtagatcctaagaaaacgtacttggattatataa
LA16 #39          13200 ....................................................
NC2 #3            13200 ....................................................
```

```
MHV IC NC_001846  13261 aacagggtggagttcccgttactaattgtgttaagatgttatgtgaccatgctggcactg
LA16 #39          13260 ........................................a.................
NC2 #3            13260 ............................................................

MHV IC NC_001846  13321 gtatggccattactattaagccggaggcaaccactaatcaggattcttatggtggtgctt
LA16 #39          13320 ............................................................
NC2 #3            13320 ............................................................

MHV IC NC_001846  13381 ccgtttgtatatattgccgctcgcgtgttgaacatccagatgttgatggattgtgcaaat
LA16 #39          13380 ............................................................
NC2 #3            13380 ............................................................

MHV IC NC_001846  13441 tacgcggcaagtttgtccaagtgcccttaggcataaaagatcctgtgtcatatgtgttga
LA16 #39          13440 ............................................................
NC2 #3            13440 ............................................................

MHV IC NC_001846  13501 cgcatgatgtttgtcaggtttgtggcttttggcgagatggtagctgttcctgtgtaggca
LA16 #39          13500 ............................................................
NC2 #3            13500 ............................................................

MHV IC NC_001846  13561 caggctcccagtttcagtcaaaagacacgaacttttttaaacgggttcggggtacaagtgt
LA16 #39          13560 ........................................a.................
NC2 #3            13560 ........................................a.................

MHV IC NC_001846  13621 aaatgcccgtcttgtaccctgtgccagtggcttggacactgatgttcaattaagggcatt
LA16 #39          13620 ............................................................
NC2 #3            13620 ............................................................

MHV IC NC_001846  13681 tgacatttgtaatgctaatcgagctggcattggtttgtattataaagtgaattgctgccg
LA16 #39          13680 ............................................................
NC2 #3            13680 ............................................................

MHV IC NC_001846  13741 cttccagcgtgtagatgaggacggcaacaagttggataagttctttgttgttaaaagaac
LA16 #39          13740 ............................................................
NC2 #3            13740 ............................................................

MHV IC NC_001846  13801 taatttagaagtgtataataaggagaaagaatgctatgagttgacaaaagaatgcggtgt
LA16 #39          13800 ............................................................
NC2 #3            13800 ............................................................

MHV IC NC_001846  13861 tgtggctgaacacgagttcttcacatttgatgtggagggaagtcgggtaccacacatagt
LA16 #39          13860 ............................................................
NC2 #3            13860 ............................................................

MHV IC NC_001846  13921 ccgtaaagatctttcaaagtttactatgttagatctttgctatgcattgcgtcattttga
LA16 #39          13920 ............................................................
NC2 #3            13920 ............................................................

MHV IC NC_001846  13981 ccgcaatgattgttcaactcttaaggaaattctccttacatatgctgagtgtgaagagtc
LA16 #39          13980 ............................................................
NC2 #3            13980 ............................................................

MHV IC NC_001846  14041 ctacttccaaaagaaggactggtatgattttgttgagaatcctgatataattaatgtgta
LA16 #39          14040 ............................................................
NC2 #3            14040 ......g.....................................................

MHV IC NC_001846  14101 taaaaagcttggtcctatatttaatagagccctgcttaacactgccaagtttgcagacgc
LA16 #39          14100 ............................................................
NC2 #3            14100 ............................................................
```

```
MHV IC NC_001846  14161 attagtggaggcaggcttagtaggtgttttaacacttgataatcaagatttatatggtca
LA16 #39          14160 ..................................................
NC2 #3            14160 ..................................................

MHV IC NC_001846  14221 atggtatgactttggagattttgtcaagacagtacctggttgtggtgttgccgtggcaga
LA16 #39          14220 ..................................................
NC2 #3            14220 ..................................................

MHV IC NC_001846  14281 ctcttattattcatatatgatgccaatgctgactatgtgtcatgcgttggatagtgagtt
LA16 #39          14280 ..................................................
NC2 #3            14280 ..................................................

MHV IC NC_001846  14341 gtttgttaatggtacttatagggagtttgaccttgttcagtatgattttactgatttcaa
LA16 #39          14340 ..................................................
NC2 #3            14340 ..................................................

MHV IC NC_001846  14401 gctagagctcttcactaagtattttaagcattggagtatgacctaccacccgaacacctg
LA16 #39          14400 ..................................................
NC2 #3            14400 ..................................................

MHV IC NC_001846  14461 tgagtgcgaggatgacaggtgcattattcattgcgccaattttaatatacttttagtat
LA16 #39          14460 ..................................................
NC2 #3            14460 ..................................................

MHV IC NC_001846  14521 ggtcttacctaagacctgttttgggcctcttgttaggcagatatttgtggatggtgttcc
LA16 #39          14520 ..................................................
NC2 #3            14520 ..................................................

MHV IC NC_001846  14581 tttcgttgtgtcgatcggttaccattataaagaattaggtgttgttatgaatatggatgt
LA16 #39          14580 ..................................................
NC2 #3            14580 ..................................................

MHV IC NC_001846  14641 ggatacacatcgttatcgcttgtctcttaaggacttgcttttgtatgctgcagaccctgc
LA16 #39          14640 ..................................................
NC2 #3            14640 ..................................................

MHV IC NC_001846  14701 ccttcatgtggcgtctgctagtgcactgcttgatttgcgcacatgttgttttagcgttgc
LA16 #39          14700 ..................................................
NC2 #3            14700 ..................................................

MHV IC NC_001846  14761 agctattacaagtggcgtaaaatttcaaacagttaaacctggaaattttaatcaggattt
LA16 #39          14760 ..................................................
NC2 #3            14760 ..................................................

MHV IC NC_001846  14821 ttatgagtttattttgagtaaaggcctgcttaaagaggggagctccgttgatttgaagca
LA16 #39          14820 ..................................................
NC2 #3            14820 ..................................................

MHV IC NC_001846  14881 cttcttctttacgcaggatggtaatgctgctattactgattataattattacaagtataa
LA16 #39          14880 ..................................................
NC2 #3            14880 ..................................................

MHV IC NC_001846  14941 tctacccaccatggtggatattaagcagttgttgtttgttttagaagttgttaataagta
LA16 #39          14940 ..................................................
NC2 #3            14940 ..................................................

MHV IC NC_001846  15001 ttttgagatctatgagggtgggtgtatacccgcaacacaggtcattgttaataattatga
LA16 #39          15000 ..................................................
NC2 #3            15000 ..................................................
```

```
MHV IC NC_001846  15061 taagagtgctggctatccatttaataaatttggaaaggccaggctctattatgaggcatt
LA16 #39          15060 ............................................................
NC2 #3            15060 ............................................................

MHV IC NC_001846  15121 atcatttgaggagcaggatgaaatttatgcgtataccaaacgcaatgtcctgccgaccct
LA16 #39          15120 ............................................................
NC2 #3            15120 ............................................................

MHV IC NC_001846  15181 aactcaaatgaatcttaaatatgctattagtgctaagaatagggcccgcaccgttgctgg
LA16 #39          15180 ............................................................
NC2 #3            15180 ............................................................

MHV IC NC_001846  15241 tgtctctattctcagtactatgactggcagaatgtttcatcaaaagtgtctaaagagtat
LA16 #39          15240 ............................................................
NC2 #3            15240 ............................................................

MHV IC NC_001846  15301 agcagctactcgcggtgttcctgtagttataggcaccacgaagttctatggcggttggga
LA16 #39          15300 ............................................................
NC2 #3            15300 ............................................................

MHV IC NC_001846  15361 tgatatgttacgccgccttattaaagatgttgatagtcctgtactcatgggttgggacta
LA16 #39          15360 ............................................................
NC2 #3            15360 ............................................................

MHV IC NC_001846  15421 tcctaaatgtgatcgtgctatgccaaacatactgcgtattgttagtagtttggtgctagc
LA16 #39          15420 ............................................................
NC2 #3            15420 ............................................................

MHV IC NC_001846  15481 ccgtaaacatgattcgtgctgttcgcatacggatagattctatcgtcttgcgaacgagtg
LA16 #39          15480 ............................................................
NC2 #3            15480 ............................................................

MHV IC NC_001846  15541 cgcccaagttttgagtgaaattgttatgtgtggtggttgttattatgttaaaccaggtgg
LA16 #39          15540 ............................................................
NC2 #3            15540 ............................................................

MHV IC NC_001846  15601 cactagtagtggggatgcaaccactgcttttgctaattctgtgtttaacatttgtcaagc
LA16 #39          15600 ............................................................
NC2 #3            15600 ............................................................

MHV IC NC_001846  15661 tgtttccgccaatgtatgctcgcttatggcatgcaatggacacaaaattgaagatttgag
LA16 #39          15660 ............................................................
NC2 #3            15660 ............................................................

MHV IC NC_001846  15721 tatacgcgagttacaaaagcgcctatactctaatgtctatcgtgcggaccatgttgaccc
LA16 #39          15720 ............................................................
NC2 #3            15720 ............................................................

MHV IC NC_001846  15781 cgcatttgttagtgagtattatgagtttttaaataagcattttagtatgatgattttgag
LA16 #39          15780 ............................................................
NC2 #3            15780 ............................................................

MHV IC NC_001846  15841 tgatgatggtgttgtgtgttataattcagagtttgcgtccaagggttatattgctaatat
LA16 #39          15840 ............................................................
NC2 #3            15840 ............................................................

MHV IC NC_001846  15901 aagtgcctttcaacaggtattatattatcaaaataatgtgtttatgtctgaggccaaatg
LA16 #39          15900 ............................................................
NC2 #3            15900 ............................................................
```

```
MHV IC NC_001846  15961 ttgggtagaaacagacatcgaaaagggaccgcatgaattttgttctcaacatacaatgct
LA16 #39          15960 ............................................................
NC2 #3            15960 ............................................................

MHV IC NC_001846  16021 agtcaagatggatggtgatgaagtctaccttccataccctgatccttcgagaatcttagg
LA16 #39          16020 ............................................................
NC2 #3            16020 ............................................................

MHV IC NC_001846  16081 agcaggctgttttgttgatgatttattaaagactgatagcgttctcttgatagagcgttt
LA16 #39          16080 ............................................................
NC2 #3            16080 ............................................................

MHV IC NC_001846  16141 cgtaagtcttgcaattgatgcttatcctttagtataccatgagaacccagagtatcaaaa
LA16 #39          16140 ............................................................
NC2 #3            16140 ............................................................

MHV IC NC_001846  16201 tgtgttccgggtatatttagaatatataaagaagctgtacaatgatctcggtaatcagat
LA16 #39          16200 ............................................................
NC2 #3            16200 ............................................................

MHV IC NC_001846  16261 cctggacagctacagtgttattttaagtacttgtgatggtcaaaagtttactgatgagac
LA16 #39          16260 ............................................................
NC2 #3            16260 ............................................................

MHV IC NC_001846  16321 cttttacaagaacatgtatttaagaagtgcagtgctgcaaagcgttggtgcctgcgttgt
LA16 #39          16320 ............................................................
NC2 #3            16320 ............................................................

MHV IC NC_001846  16381 ctgtagttctcaaacatcattacgttgtggcagttgcatacgcaagcctttgctgtgttg
LA16 #39          16380 ............................................................
NC2 #3            16380 ............................................................

MHV IC NC_001846  16441 caaatgcgcctatgatcatgttatgtccactgatcataaatatgtcctgagtgtgtcacc
LA16 #39          16440 ............................................................
NC2 #3            16440 ............................................................

MHV IC NC_001846  16501 atatgtgtgtaattcaccgggatgtgatgtaaatgatgttaccaaattgtatttaggtgg
LA16 #39          16500 ............................................................
NC2 #3            16500 ............................................................

MHV IC NC_001846  16561 tatgtcatattattgtgaggaccataaaccacagtattcattcaaattggtgatgaatgg
LA16 #39          16560 ............................................................
NC2 #3            16560 ............................................................

MHV IC NC_001846  16621 tatggtttttggtttatataaacaatcttgtactggttcgccctacatagaggatttaa
LA16 #39          16620 ............................................................
NC2 #3            16620 ............................................................

MHV IC NC_001846  16681 taaaatagctagttgcaaatggacagaagtcgatgattatgtgctagctaatgaatgcac
LA16 #39          16680 ............................................................
NC2 #3            16680 ............................................................

MHV IC NC_001846  16741 cgaacgccttaaattgtttgccgcagaaacgcagaaggccacagaagaggcctttaagca
LA16 #39          16740 ............................................................
NC2 #3            16740 ............................................................

MHV IC NC_001846  16801 atgttatgcgtcagcaacgatccgtgagatcgtgagcgatcgggagttaattttatcttg
LA16 #39          16800 ............................................................
NC2 #3            16800 ............................................................
```

```
MHV IC NC_001846  16861 ggaaattggtaaagtgagaccaccacttaataaaaattatgtttttactggctaccattt
LA16 #39          16860 ...........................................................
NC2 #3            16860 ...........................................................

MHV IC NC_001846  16921 tactaataatggtaagacagttttaggtgagtatgtttttgataagagtgagttgactaa
LA16 #39          16920 ...........................................................
NC2 #3            16920 ...........................................................

MHV IC NC_001846  16981 tggtgtgtactatcgcgccacaaccacttataagttatctgtaggtgatgtgttcatttt
LA16 #39          16980 .........t.................................................
NC2 #3            16980 .........t.................................................

MHV IC NC_001846  17041 aacatcacacgcagtgtctagtttaagtgctcctacattagtaccgcaggagaattatac
LA16 #39          17040 ...........................................................
NC2 #3            17040 ...........................................................

MHV IC NC_001846  17101 tagcattcgttttgctagtgtttatagtgtgcctgagacgtttcagaataatgtgcctaa
LA16 #39          17100 ...........................................................
NC2 #3            17100 ...........................................................

MHV IC NC_001846  17161 ttatcagcacattggaatgaagcgctattgtactgtacagggaccgcctggtactggtaa
LA16 #39          17160 ...........................................................
NC2 #3            17160 ...........................................................

MHV IC NC_001846  17221 gtcccatctagccattgggctagctgtttattattgtacagcgcgcgtggtgtataccgc
LA16 #39          17220 ...........................................................
NC2 #3            17220 ...........................................................

MHV IC NC_001846  17281 tgctagccatgctgcagttgacgcgctgtgtgaaaaggcacataaatttttaaatattaa
LA16 #39          17280 ...........................................................
NC2 #3            17280 ...........................................................

MHV IC NC_001846  17341 tgactgcacgcgtattgttcctgcaaaggtgcgtgtagattgttatgataaatttaaggt
LA16 #39          17340 ...........................................................
NC2 #3            17340 ...........................................................

MHV IC NC_001846  17401 caatgacaccactcgcaagtatgtgtttactacaataaatgcattacctgagttggtgac
LA16 #39          17400 ...........................................................
NC2 #3            17400 ...........................................................

MHV IC NC_001846  17461 tgacattattgtcgttgatgaagttagtatgcttaccaactatgagctgtctgttattaa
LA16 #39          17460 ...........................................................
NC2 #3            17460 ...........................................................

MHV IC NC_001846  17521 cagtcgtgttagtgctaagcattatgtgtatattggagaccctgcgcagttacctgcacc
LA16 #39          17520 ............g..............................................
NC2 #3            17520 ............g..............................................

MHV IC NC_001846  17581 acgtgtgctactgaataagggaactctagaacctagatattttaattccgttaccaagct
LA16 #39          17580 ...........................................................
NC2 #3            17580 ...........................................................

MHV IC NC_001846  17641 aatgtgttgtttgggtccagatattttcttgggcacctgttatagatgccctaaggagat
LA16 #39          17640 ...........................................................
NC2 #3            17640 ...........................................................

MHV IC NC_001846  17701 tgtggatacggtgtcagccttggtttataataataagctgaaggctaaaaatgataatag
LA16 #39          17700 ...........................................................
NC2 #3            17700 ...........................................................
```

```
MHV IC NC_001846  17761 ctccatgtgctttaaggtttattataagggccagactacacatgagagttctagtgctgt
LA16 #39          17760 ............................................................
NC2 #3            17760 ............................................................

MHV IC NC_001846  17821 taatatgcagcaaatacatttaattagtaagtttttaaaggcaaaccccagttggagtaa
LA16 #39          17820 ............................................................
NC2 #3            17820 ............................................................

MHV IC NC_001846  17881 cgccgtatttattagtccttataatagtcagaactatgttgctaagagagtcttgggatt
LA16 #39          17880 ............................................................
NC2 #3            17880 ............................................................

MHV IC NC_001846  17941 acaaacccagacagtagactcagcgcagggttctgaatatgattttgttatttattcaca
LA16 #39          17940 ............................................................
NC2 #3            17940 ............................................................

MHV IC NC_001846  18001 gactgcggaaacagcgcattctgtcaatgtaaatagattcaatgttgctattacacgtgc
LA16 #39          18000 ............................................................
NC2 #3            18000 ............................................................

MHV IC NC_001846  18061 taagaagggtattctctgtgtcatgagtagtatgcaattatttgagtctcttaattttac
LA16 #39          18060 ..............t.............................................
NC2 #3            18060 ............................................................

MHV IC NC_001846  18121 tacactgacgttggataagattaacaatccacgattacagtgtactacaaatttgtttaa
LA16 #39          18120 ............................................................
NC2 #3            18120 ............................................................

MHV IC NC_001846  18181 ggattgtagcaggagctatgtaggatatcacccagcccatgcaccatccttttggcagt
LA16 #39          18180 ............................................................
NC2 #3            18180 ............................................................

MHV IC NC_001846  18241 tgatgacaaatataaggtaggcggtgatttagccgtttgccttaatgttgctgattctgc
LA16 #39          18240 ............................................................
NC2 #3            18240 ............................................................

MHV IC NC_001846  18301 tgtcacttattcgcggcttatatcactcatgggattcaagcttgacttgacccttgatgg
LA16 #39          18300 ............................................................
NC2 #3            18300 ............................................................

MHV IC NC_001846  18361 ttattgtaagctgtttataactagagatgaagctatcaaacgtgttagagcctgggttgg
LA16 #39          18360 ............................................................
NC2 #3            18360 ............................................................

MHV IC NC_001846  18421 cttcgatgcagaaggtgcccatgcgatacgtgatagcattgggacaaatttcccattaca
LA16 #39          18420 ............................................................
NC2 #3            18420 ............................................................

MHV IC NC_001846  18481 attaggcttttcgactggaattgattttgttgtcgaagccactggaatgtttgctgagag
LA16 #39          18480 ............................................................
NC2 #3            18480 ............................................................

MHV IC NC_001846  18541 agatggttatgtctttaaaaaggcagccgcacgagctcctcctggcgaacaatttaaaca
LA16 #39          18540 ............................................................
NC2 #3            18540 ............................................................

MHV IC NC_001846  18601 ccttatcccacttatgtcaagagggcagaaatgggatgtggttcgaattagaatagtaca
LA16 #39          18600 ............................................................
NC2 #3            18600 ............................................................
```

```
MHV IC NC_001846  18661 aatgttgtcagaccacctagcggatttggcagacagtgttgtacttgtgacgtgggctgc
LA16 #39          18660 ..................t.........................................
NC2 #3            18660 ..................t.........................................

MHV IC NC_001846  18721 cagctttgagctcacatgtttgcgatatttcgctaaagttggaagagaagttgtgtgtag
LA16 #39          18720 ...........................................................
NC2 #3            18720 ...........................................................

MHV IC NC_001846  18781 tgtctgcaccaagcgtgcgacatgttttaattctagaactggatactatggatgctggcg
LA16 #39          18780 ...........................................................
NC2 #3            18780 ...........................................................

MHV IC NC_001846  18841 acatagttattcctgtgattacctgtacaacccactaatagttgacattcaacagtgggg
LA16 #39          18840 ...........................................................
NC2 #3            18840 ...........................................................

MHV IC NC_001846  18901 atatacaggatctttaactagcaatcatgatcctatttgcagcgtgcataagggtgctca
LA16 #39          18900 ...........................................................
NC2 #3            18900 ...........................................................

MHV IC NC_001846  18961 tgttgcatcatctgatgctatcatgacccggtgtctagctgttcatgattgcttttgtaa
LA16 #39          18960 ...........................................................
NC2 #3            18960 ...........................................................

MHV IC NC_001846  19021 gtctgttaattggaatttagaataccccattatttcaaatgaggtcagtgttaatacctc
LA16 #39          19020 ...........................................................
NC2 #3            19020 ...........................................................

MHV IC NC_001846  19081 ctgcaggttattgcagcgcgtaatgtttagggctgcgatgctatgcaataggtatgatgt
LA16 #39          19080 ...........................................................
NC2 #3            19080 ...........................................................

MHV IC NC_001846  19141 gtgttatgacattggcaaccctaaaggtcttgcctgtgtcaaaggatatgattttaagtt
LA16 #39          19140 ...........................................................
NC2 #3            19140 ...........................................................

MHV IC NC_001846  19201 ttatgatgcctcccctgttgttaagtctgttaaacagtttgtttataaatacgaggcaca
LA16 #39          19200 ...........................................................
NC2 #3            19200 ...........................................................

MHV IC NC_001846  19261 taaagatcaatttttagatggtttgtgtatgttttggaactgcaatgtggataagtatcc
LA16 #39          19260 ...........................................................
NC2 #3            19260 ...........................................................

MHV IC NC_001846  19321 agcgaatgcagttgtgtgtaggtttgacacgcgtgtgttgaacaaattaaatctccctgg
LA16 #39          19320 ...........................................................
NC2 #3            19320 ...........................................................

MHV IC NC_001846  19381 ctgtaatggtggcagtttgtatgttaacaaacatgcattccacaccagtccctttacccg
LA16 #39          19380 ...........................................................
NC2 #3            19380 ...........................................................

MHV IC NC_001846  19441 ggctgccttcgagaatttgaagcctatgcctttcttttattattcagatacgccctgtgt
LA16 #39          19440 ...........................................................
NC2 #3            19440 ...........................................................

MHV IC NC_001846  19501 gtatatggaaggcatggaatctaagcaggtcgattatgtcccattgagaagcgctacatg
LA16 #39          19500 ...........................................................
NC2 #3            19500 ...........................................................
```

```
MHV IC NC_001846  19561 catcacaagatgcaatttaggtggcgctgtttgtttaaaacatgctgaggagtatcgtga
LA16 #39          19560 ............................................................
NC2 #3            19560 ............................................................

MHV IC NC_001846  19621 gtaccttgagtcttacaatacggcaaccacagcgggttttacttttgggtctataagac
LA16 #39          19620 ............................................................
NC2 #3            19620 ............................................................

MHV IC NC_001846  19681 ttttgatttttataacctttggaatactttactaggctccaaagtttagaaaatgtagt
LA16 #39          19680 ............................................................
NC2 #3            19680 ............................................................

MHV IC NC_001846  19741 gtataatttggtcaatgctggacactttgatggccgggcgggtgaactgccttgtgctgt
LA16 #39          19740 ............................................................
NC2 #3            19740 ............................................................

MHV IC NC_001846  19801 tataggtgagaaagtcattgccaagattcaaaatgaggatgtcgtggtctttaaaaataa
LA16 #39          19800 ............................................................
NC2 #3            19800 ............................................................

MHV IC NC_001846  19861 cacgccattccccactaatgtggctgtcgaattatttgctaagcgcagtattcggcccca
LA16 #39          19860 ............................................................
NC2 #3            19860 ............................................................

MHV IC NC_001846  19921 ccccgagcttaagctctttagaaatttgaatattgacgtgtgctggagtcacgtcctttg
LA16 #39          19920 ............................................................
NC2 #3            19920 ............................................................

MHV IC NC_001846  19981 ggattatgctaaggatagtgtgttttgcagttcgacgtataaggtctgcaaatacacaga
LA16 #39          19980 ............................................................
NC2 #3            19980 ............................................................

MHV IC NC_001846  20041 tttacagtgcattgaaagcttgaatgtacttttgatggtcgtgataatggtgctcttga
LA16 #39          20040 ............................................................
NC2 #3            20040 ............................................................

MHV IC NC_001846  20101 agcttttaagaagtgccggaatggcgtctacattaacacgacaaaaattaaaagtctgtc
LA16 #39          20100 ............................................................
NC2 #3            20100 ............................................................

MHV IC NC_001846  20161 gatgattaaaggcccacaacgtgccgatttgaatggcgtagttgtggagaaagttggaga
LA16 #39          20160 ............................................................
NC2 #3            20160 ............................................................

MHV IC NC_001846  20221 ttctgatgtggaattttggtttgctgtgcgtaaagacggtgacgatgttatcttcagccg
LA16 #39          20220 ............................................................
NC2 #3            20220 ............................................................

MHV IC NC_001846  20281 tacagggagccttgaaccgagccattaccggagcccacaaggtaatccgggtggtaatcg
LA16 #39          20280 ............................................................
NC2 #3            20280 ............................................................

MHV IC NC_001846  20341 cgtgggtgatctcagcggtaatgaagctctagcgcgtggcactatctttactcaaagcag
LA16 #39          20340 ............................................................
NC2 #3            20340 ............................................................

MHV IC NC_001846  20401 attattatcttctttcacacctcgatcagagatggagaaagattttatggatttagatga
LA16 #39          20400 ............................................................
NC2 #3            20400 ............................................................
```

```
MHV IC NC_001846  20461 tgatgtgttcattgcaaaatatagtttacaggactacgcgtttgaacacgttgtttatgg
LA16 #39          20460 ..........................................................
NC2 #3            20460 ..........................................................

MHV IC NC_001846  20521 tagttttaaccagaagattattggaggtttgcatttgcttattggcttagcccgtaggca
LA16 #39          20520 ..........................................................
NC2 #3            20520 ..........................................................

MHV IC NC_001846  20581 gcaaaaatccaatctggtaattcaagagttcgtgacatacgactctagcattcattcgta
LA16 #39          20580 ..........................................................
NC2 #3            20580 ..........................................................

MHV IC NC_001846  20641 ctttatcactgacgagaacagtggtagtagtaagagtgtgtgcactgttattgatttatt
LA16 #39          20640 ..........................................................
NC2 #3            20640 ..........................................................

MHV IC NC_001846  20701 gttagatgattttgtggacattgtaaagtccctgaatctaaagtgtgtgagtaaggttgt
LA16 #39          20700 ..........................................................
NC2 #3            20700 ..........................................................

MHV IC NC_001846  20761 taatgttaatgttgattttaaagatttccagtttatgttgtggtgcaatgaggagaaggt
LA16 #39          20760 ..........................................................
NC2 #3            20760 ..........................................................

MHV IC NC_001846  20821 catgactttctatcctcgtttgcaggctgctgctgactggaaacctggttatgttatgcc
LA16 #39          20820 ..........................................................
NC2 #3            20820 ..........................................................

MHV IC NC_001846  20881 tgtcttatataagtatttggaatcgcctctggaaagagtaaacctctggaattatggcaa
LA16 #39          20880 ..........................................................
NC2 #3            20880 ..........................................................

MHV IC NC_001846  20941 gccgattactttacctacaggatgtatgatgaatgttgctaagtatactcaattatgtca
LA16 #39          20940 ..........................................................
NC2 #3            20940 ..........................................................

MHV IC NC_001846  21001 atatttgagcactacaacattagcagttccggctaatatgcgtgtcttacaccttggtgc
LA16 #39          21000 ..........................................................
NC2 #3            21000 ..........................................................

MHV IC NC_001846  21061 cggttcggataagggtgttgcccctgggtctgcagttcttaggcagtggctaccagcggg
LA16 #39          21060 ..........................................................
NC2 #3            21060 ..........................................................

MHV IC NC_001846  21121 aagtattcttgtagataatgatgtgaatccatttgtgagtgacagtgtcgcctcatatta
LA16 #39          21120 ..........................................................
NC2 #3            21120 ..........................................................

MHV IC NC_001846  21181 tggaaattgtataaccttacccctttgattgtcagtgggatctgataatttctgatatgta
LA16 #39          21180 ..........................................................
NC2 #3            21180 ..........................................................

MHV IC NC_001846  21241 cgaccctcttactaagaacattggggagtacaacgtgagtaaagatggattctttactta
LA16 #39          21240 ..........................................................
NC2 #3            21240 ..........................................................

MHV IC NC_001846  21301 cctctgtcatttaattcgtgacaagttggctctgggtggcagtgttgccataaaaataac
LA16 #39          21300 ..........................................................
NC2 #3            21300 ..........................................................
```

```
MHV IC NC_001846  21361 agagttttcttggaacgctgagttatatagtttaatggggaagtttgcgttctggacaat
LA16 #39          21360 ............................................................
NC2 #3            21360 ............................................................

MHV IC NC_001846  21421 cttttgcaccaacgtaaacgcctcttcaagtgaaggatttttgattggcataaattggtt
LA16 #39          21420 ............................................................
NC2 #3            21420 ............................................................

MHV IC NC_001846  21481 gaataagacccgtaccgaaattgacggtaaaaccatgcatgccaattatctgttttggag
LA16 #39          21480 ............................................................
NC2 #3            21480 ............................................................

MHV IC NC_001846  21541 aaatagtacaatgtggaatggaggggcttacagtctctttgacatgagtaagttcccttt
LA16 #39          21540 ............................................................
NC2 #3            21540 ............................................................

MHV IC NC_001846  21601 gaaagcggctggtacggctgttgttagccttaaaccagaccaaataaatgacttagtcct
LA16 #39          21600 ............................................................
NC2 #3            21600 ............................................................

MHV IC NC_001846  21661 ctccttgattgagaagggcaagttattagtgcgtgatacacgcaaagaagtttttgttgg
LA16 #39          21660 ............................................................
NC2 #3            21660 ............................................................

MHV IC NC_001846  21721 cgatagcctagtaaatgtcaaataaatctatacttgtcgtggctgtgaaaatggcctttg
LA16 #39          21720 ............................................................
NC2 #3            21720 ............................................................

MHV IC NC_001846  21781 ctgacaagcctaatcatttcataaactttcccctggcccaatttagtggctttatgggta
LA16 #39          21780 ............................................................
NC2 #3            21780 ............................................................

MHV IC NC_001846  21841 agtatttaaagctacagtctcaacttgtggaaatgggtttagactgtaaattacagaagg
LA16 #39          21840 ............................................................
NC2 #3            21840 ............................................................

MHV IC NC_001846  21901 caccacatgttagtattaccctgcttgatattaaagcagaccaatacaaacaggtggaat
LA16 #39          21900 ............................................................
NC2 #3            21900 ............................................................

MHV IC NC_001846  21961 ttgcaatacaagaaataatagatgatctggcggcatatgagggagatattgtctttgaca
LA16 #39          21960 ............................................................
NC2 #3            21960 ............................................................

MHV IC NC_001846  22021 accctcacatgcttggcagatgccttgttcttgatgttagaggatttgaagagttgcatg
LA16 #39          22020 ............................................................
NC2 #3            22020 ............................................................

MHV IC NC_001846  22081 aagatattgttgaaattctccgcagaaggggttgcacggcagatcaatccagacactgga
LA16 #39          22080 ............................................................
NC2 #3            22080 ............................................................

MHV IC NC_001846  22141 ttccgcactgcactgtggcccaatttgacgaagaaagagaaacaaaaggaatgcaattct
LA16 #39          22140 ............................................................
NC2 #3            22140 ............................................................

MHV IC NC_001846  22201 atcataaagaacccttctacctcaagcataacaacctattaacggatgctgggcttgagc
LA16 #39          22200 ............................................................
NC2 #3            22200 ............................................................
```

```
MHV IC NC_001846  22261 tcgtgaagataggttcttccaaaatagatgggttttattgtagtgaactgagtgtttggt
LA16 #39          22260 ..........................................................
NC2 #3            22260 .g........................................................

MHV IC NC_001846  22321 gtggtgagaggctttgttataagcctccaacacccaaattcagtgatatatttggctatt
LA16 #39          22320 ..........................................................
NC2 #3            22320 ..........................................................

MHV IC NC_001846  22381 gctgcatagataaaatacgtggtgatttagaaataggagacctaccgcaggatgatgagg
LA16 #39          22380 ..........................................................
NC2 #3            22380 ..........................................................

MHV IC NC_001846  22441 aagcgtgggccgagctaagttaccactatcaaagaaacacctacttcttcagacatgtgc
LA16 #39          22440 ..........................................................
NC2 #3            22440 t.........................................................

MHV IC NC_001846  22501 acgataatagcatctattttcgtaccgtgtgtagaatgaagggttgtatgtgttgatttg
LA16 #39          22500 ..........................................................
NC2 #3            22500 ..........................................................

MHV IC NC_001846  22561 tttttacactattagtgtaataagcttattattttgttgaaaagggcaggatgtgcatag
LA16 #39          22560 ..........................................................
NC2 #3            22560 ..........................................................

MHV IC NC_001846  22621 ctatggctcctcgcacactgcttttgctgatttgatgtcagctggtgtttgggttcaatg
LA16 #39          22620 ..........................................................
NC2 #3            22620 ..........................................................

MHV IC NC_001846  22681 aacctcttaacatcgtttcacatttaaatgatgactggtttctatttggtgacagtcgtt
LA16 #39          22680 ..........................................................
NC2 #3            22680 ..........................................................

MHV IC NC_001846  22741 ctgactgtacctatgtagaaaataacggtcatcctaaattagattggcttgacctcgacc
LA16 #39          22740 ..........................................................
NC2 #3            22740 ..........................................................

MHV IC NC_001846  22801 caaagttgtgtaattcaggaaagatttccgcaaagagtggtaactctctctttaggagtt
LA16 #39          22800 ..........................................................
NC2 #3            22800 ..........................................................

MHV IC NC_001846  22861 ttcacttcactgatttttacaattatacgggtgagggagaccaaattgtattttatgaag
LA16 #39          22860 ..........................................................
NC2 #3            22860 ..........................................................

MHV IC NC_001846  22921 gagttaattttagtcccagccatggctttaaatgcctggctcatggagataataaaagat
LA16 #39          22920 ..........................................................
NC2 #3            22920 ..........................................................

MHV IC NC_001846  22981 ggatgggcaataaagctcgattttatgcccgagtgtatgagaagatggcccaatatagga
LA16 #39          22980 ..........................................................
NC2 #3            22980 ..........................................................

MHV IC NC_001846  23041 gcctatcgtttgttaatgtgtcttatgcctatggaggtaatgcaaagcccgcctccattt
LA16 #39          23040 ..........................................................
NC2 #3            23040 ..........................................................

MHV IC NC_001846  23101 gcaaagacaatactttaacactcaataaccccaccttcatatcgaaggagtctaattatg
LA16 #39          23100 ..........................................................
NC2 #3            23100 ............................g.............................
```

```
MHV IC NC_001846  23161 ttgattattactatgagagtgaggctaatttcacactagaaggttgtgatgaatttat-a
LA16 #39          23160 .......g....----------------------------------------.....t.
NC2 #3            23160 .......g.......----------------------------------.....t.

MHV IC NC_001846  23220 gtaccgctctgtggttttaatggccattccaagggcagctcttcggatgctgccaataaa
LA16 #39          23179 ............................................................
NC2 #3            23182 ............................................................

MHV IC NC_001846  23280 tattatactgactctcagagttactataatatggatattggtgtcttatatgggttcaat
LA16 #39          23239 ............................................................
NC2 #3            23242 ............................................................

MHV IC NC_001846  23340 tcgaccttggatgttggcaacactgctaaggatccgggtcttgatctcacttgcaggtat
LA16 #39          23299 ............................................................
NC2 #3            23302 ............................................................

MHV IC NC_001846  23400 cttgcattgactcctggtaattataaggctgtgtccttagaatatttgttaagcttaccc
LA16 #39          23359 ............................................................
NC2 #3            23362 ............................................................

MHV IC NC_001846  23460 tcaaaggctatttgcctccataagacaaagcgctttatgcctgtgcaggtagttgactca
LA16 #39          23419 ............................................................
NC2 #3            23422 ............................................................

MHV IC NC_001846  23520 aggtggagtagcatccgccagtcagacaatatgaccgctgcagcctgtcagctgccatat
LA16 #39          23479 ............................................................
NC2 #3            23482 ............................................................

MHV IC NC_001846  23580 tgtttctttcgcaacacatctgcgaattatagtggtggcacacatgatgcgcaccatggt
LA16 #39          23539 ............................................................
NC2 #3            23542 ............................................................

MHV IC NC_001846  23640 gattttcatttcaggcagttattgtctggtttgttatataatgtttcctgtattgcccag
LA16 #39          23599 ............................................................
NC2 #3            23602 ............................................................

MHV IC NC_001846  23700 cagggtgcatttctttataataatgttagttcctcttggccagcctatgggtacggtcat
LA16 #39          23659 ............................................................
NC2 #3            23662 ............................................................

MHV IC NC_001846  23760 tgtccaacggcagctaacattggttatatggcacctgtttgtatctatgaccctctcccg
LA16 #39          23719 ............................................................
NC2 #3            23722 ............................................................

MHV IC NC_001846  23820 gtcatactgctaggtgtgttattgggtatagctgtgttgattattgtgtttttgatgttt
LA16 #39          23779 ............................................................
NC2 #3            23782 ............................................................

MHV IC NC_001846  23880 tattttatgacggatagcggtgttagattgcatgaggcataatctaaacatgctgttcgt
LA16 #39          23839 ............................................................
NC2 #3            23842 ............................................................

MHV IC NC_001846  23940 gtttattctatttttgccctcttgtttagggtatattggtgattttagatgtatccagct
LA16 #39          23899 ............................................................
NC2 #3            23902 ............................................................

MHV IC NC_001846  24000 tgtgaattcaaacggtgctaatgttagtgctccaagcattagcactgagaccgttgaagt
LA16 #39          23959 ............................................................
NC2 #3            23962 ............................................................c..
```

```
MHV IC NC_001846  24060 ttcacaaggcctggggacatattatgtgttagatcgagtttatttaaatgccacattatt
LA16 #39          24019 ..........................................................
NC2 #3            24022 ..........................................................

MHV IC NC_001846  24120 gcttactggttactacccggtcgatggttctaagtttagaaacctcgctcttacgggaac
LA16 #39          24079 ..........................................................
NC2 #3            24082 ..........................................................

MHV IC NC_001846  24180 taactcagttagcttgtcgtggtttcaaccaccctatttaagtcagtttaatgatggcat
LA16 #39          24139 ..........................................................
NC2 #3            24142 ..........................................................

MHV IC NC_001846  24240 atttgcgaaggtgcagaaccttaagacaagtacgccatcaggtgcaactgcatattttcc
LA16 #39          24199 ..........................................................
NC2 #3            24202 ..........................................................

MHV IC NC_001846  24300 tactatagttataggtagtttgtttggctatacttcctataccgttgtaatagagccata
LA16 #39          24259 ..........................................................
NC2 #3            24262 ..........................................................

MHV IC NC_001846  24360 taatggtgttataatggcctcagtgtgccagtataccatttgtctgttaccttacactga
LA16 #39          24319 ..........................................a...............
NC2 #3            24322 ..........................................a...............

MHV IC NC_001846  24420 ttgtaagcctaacactaatggtaataagcttatagggttttggcacacggatgtaaaacc
LA16 #39          24379 ..........................................................
NC2 #3            24382 ..........................................................

MHV IC NC_001846  24480 cccaatttgtgtgttaaagcgaaatttcacgcttaatgttaatgctgatgcattttattt
LA16 #39          24439 ..........................................................
NC2 #3            24442 ..........................................................

MHV IC NC_001846  24540 tcatttttaccaacatggtggtacttttttatgcgtactatgcggataaaccctccgctac
LA16 #39          24499 ..........................................................
NC2 #3            24502 ..........................................................

MHV IC NC_001846  24600 tacgtttttgtttagtgtatatattggcgatattttaacacagtattatgtgttaccttt
LA16 #39          24559 ..........................................................
NC2 #3            24562 ..........................................................

MHV IC NC_001846  24660 catctgcaacccaacagctggtagcacttttgctccgcgctattgggttacacctttggt
LA16 #39          24619 ..........................................................
NC2 #3            24622 ..........................................................

MHV IC NC_001846  24720 taagcgccaatatttgtttaatttcaaccagaagggtgtcattactagtgctgttgattg
LA16 #39          24679 ..........................................................
NC2 #3            24682 ..........................................................

MHV IC NC_001846  24780 tgctagtagttataccagtgaaataaaatgtaagacccagagcatgttacctagcactgg
LA16 #39          24739 ..........................................................
NC2 #3            24742 ..........................................................

MHV IC NC_001846  24840 tgtctatgagttatccggttatacggtccaaccagttggagttgtataccggcgtgttgc
LA16 #39          24799 ..........................................................
NC2 #3            24802 ..........................................................

MHV IC NC_001846  24900 taacctcccagcttgtaatatagaggagtggcttactgctaggtcagtcccctccctct
LA16 #39          24859 ..........................................................
NC2 #3            24862 ..........................................................
```

```
MHV IC NC_001846  24960 caactgggagcgtaagacttttcagaattgtaattttaatttaagcagcctgttacgtta
LA16 #39          24919 ............................................................
NC2 #3            24922 ............................................................

MHV IC NC_001846  25020 tgttcaggctgagagtttgttttgtaataatatcgatgcttccaaagtgtatggcaggtg
LA16 #39          24979 ............................................................
NC2 #3            24982 ............................................................

MHV IC NC_001846  25080 ctttggtagtatttcagttgataagtttgctgtaccccgaagtaggcaagttgatttaca
LA16 #39          25039 ............................................................
NC2 #3            25042 ............................................................

MHV IC NC_001846  25140 gcttggtaactctggatttctgcagactgctaattataagattgatacagctgccacttc
LA16 #39          25099 ............................................................
NC2 #3            25102 ............................................................

MHV IC NC_001846  25200 gtgtcagctgcattacaccttgcctaagaataatgtcaccataaacaaccataacccctc
LA16 #39          25159 ............................................................
NC2 #3            25162 ............................................................

MHV IC NC_001846  25260 gtcttggaataggaggtatggctttaatgatgctggcgtctttggcaaaaaccaacatga
LA16 #39          25219 ............................................................
NC2 #3            25222 ............................................................

MHV IC NC_001846  25320 cgttgtttacgctcagcaatgttttactgtaagatctagttattgcccgtgtgctcaacc
LA16 #39          25279 ............................................................
NC2 #3            25282 ............................................................

MHV IC NC_001846  25380 ggacatagttagcccttgcactactcagactaagcctaagtctgcttttgttaatgtggg
LA16 #39          25339 ............................................................
NC2 #3            25342 ............................................................

MHV IC NC_001846  25440 tgaccattgtgaaggcttaggtgttttagaagataattgtggcaatgctgatccacataa
LA16 #39          25399 ............................................................
NC2 #3            25402 ............................................................

MHV IC NC_001846  25500 gggttgtatctgtgccaacaattcatttattggatggtcacatgatacctgccttgttaa
LA16 #39          25459 ............................................................
NC2 #3            25462 ............................................................

MHV IC NC_001846  25560 tgatcgctgccaaatttttgctaatatattgttaaatggcattaatagtggtaccacatg
LA16 #39          25519 ............................................................
NC2 #3            25522 ............................................................

MHV IC NC_001846  25620 ttccacagatttgcagttgcctaatactgaagtggttactggcatttgtgtcaaatatga
LA16 #39          25579 ............................................................
NC2 #3            25582 ............................................................

MHV IC NC_001846  25680 cctctacggtattactggacaaggtgtttttaaagaggttaaggctgactattataatag
LA16 #39          25639 ............................................................
NC2 #3            25642 ............................................................

MHV IC NC_001846  25740 ctggcaaaccttctgtatgatgttaatggtaatttgaatggttttcgtgatcttaccac
LA16 #39          25699 ............................................................
NC2 #3            25702 ............................................................

MHV IC NC_001846  25800 taacaagacttatacgataaggagctgttatagtggccgtgtttctgctgcatttcataa
LA16 #39          25759 ............................................................
NC2 #3            25762 ............................................................
```

```
MHV IC NC_001846  25860 agatgcacccgaaccggctctgctctatcgtaatataaattgtagctatgtttttagcaa
LA16 #39          25819 ....................aa....................................
NC2 #3            25822 ....................aa....................................

MHV IC NC_001846  25920 taatatttcccgtgaggagaacccacttaattactttgatagttatttgggttgtgttgt
LA16 #39          25879 ............................................................
NC2 #3            25882 ............................................................

MHV IC NC_001846  25980 taatgctgataaccgcacggatgaggcgcttcctaattgtgatctccgtatgggtgctgg
LA16 #39          25939 ............................................................
NC2 #3            25942 ............................................................

MHV IC NC_001846  26040 cttatgcgttgattattcaaaatcacgcagggctgaccgatcagtttctactggctatcg
LA16 #39          25999 ................................c...........................
NC2 #3            26002 ................................c...........................

MHV IC NC_001846  26100 gttaactacatttgagccatacactccgatgttagttaatgatagtgtccaatccgttga
LA16 #39          26059 ............................................................
NC2 #3            26062 ............................................................

MHV IC NC_001846  26160 tggattatatgagatgcaaataccaaccaattttactattgggcaccatgaggagttcat
LA16 #39          26119 ............................................................
NC2 #3            26122 ............................................................

MHV IC NC_001846  26220 tcaaactagatctccaaaggtgactatagattgtgctgcatttgtctgtggtgataacac
LA16 #39          26179 ............................................................
NC2 #3            26182 ............................................................

MHV IC NC_001846  26280 tgcatgcaggcagcagttggttgagtatggctctttctgtgttaatgttaatgccattct
LA16 #39          26239 ............................................................
NC2 #3            26242 ............................................................

MHV IC NC_001846  26340 taatgaggttaataacctcttggataatatgcaactacaagttgctagtgcattaatgca
LA16 #39          26299 ............................................................
NC2 #3            26302 ............................................................

MHV IC NC_001846  26400 gggtgttactataagctcgagactgccagacggcatctcaggccctatagatgacattaa
LA16 #39          26359 ............................................................
NC2 #3            26362 ............................................................

MHV IC NC_001846  26460 ttttagtcctctacttggatgcataggttcaacatgtgctgaagacggcaatggacctag
LA16 #39          26419 ............................................................
NC2 #3            26422 ............................................................

MHV IC NC_001846  26520 tgcaatccgagggcgttctgctatagaggatttgttatttgacaaggtcaaattatctga
LA16 #39          26479 ............................................................
NC2 #3            26482 ............................................................

MHV IC NC_001846  26580 tgttggctttgtcgaggcttataataattgcaccggtggtcaagaagttcgtgacctcct
LA16 #39          26539 ............................................................
NC2 #3            26542 ............................................................

MHV IC NC_001846  26640 ttgtgtacaatcttttaatggcatcaaagtattacctcctgtgttgtcagagagtcagat
LA16 #39          26599 ............................................................
NC2 #3            26602 ............................................................

MHV IC NC_001846  26700 ctctggctacacaaccggtgctactgcggcagctatgttcccaccgtggtcagcagctgc
LA16 #39          26659 ............................................................
NC2 #3            26662 ............................................................
```

```
MHV IC NC_001846  26760 cggtgtgccatttagtttaagtgttcaatatagaattaatggtttaggtgtcactatgaa
LA16 #39          26719 ..........................................................
NC2 #3            26722 ..........................................................

MHV IC NC_001846  26820 tgtgcttagtgagaaccaaaagatgattgctagtgcttttaacaatgcgctgggtgctat
LA16 #39          26779 ..........................................................
NC2 #3            26782 ..........................................................

MHV IC NC_001846  26880 ccaggatgggtttgatgcaaccaattctgctttaggtaagatccagtccgttgttaatgc
LA16 #39          26839 ..........................................................
NC2 #3            26842 ..........................................................

MHV IC NC_001846  26940 aaatgctgaagcactcaataacttactaaatcaactttctaacaggtttggtgctattag
LA16 #39          26899 ..........................................................
NC2 #3            26902 ..........................................................

MHV IC NC_001846  27000 tgcttctttacaagaaattctaactcggcttgaggctgtagaagcaaaagcccagataga
LA16 #39          26959 ..........................................................
NC2 #3            26962 ..........................................................

MHV IC NC_001846  27060 tcgtcttattaatggcaggttaactgcacttaatgcgtatatatccaagcaacttagtga
LA16 #39          27019 ..........................................................
NC2 #3            27022 ..........................................................

MHV IC NC_001846  27120 tagtacgcttattaaagttagtgctgctcaggccatagaaaaggtcaatgagtgcgttaa
LA16 #39          27079 ..........................................................
NC2 #3            27082 ..........................................................

MHV IC NC_001846  27180 gagccaaaccacgcgtattaatttctgtggcaatggtaatcatatattatctcttgtcca
LA16 #39          27139 ..........................................................
NC2 #3            27142 ..........................................................

MHV IC NC_001846  27240 gaatgcgccttatggcttatattttatacacttcagctatgtgccaatatcctttacaac
LA16 #39          27199 ..........................................................
NC2 #3            27202 ..........................................................

MHV IC NC_001846  27300 cgcaaatgtgagtcctggactttgcatttctggtgatagaggattagcacctaaagctgg
LA16 #39          27259 ..........................................................
NC2 #3            27262 ..........................................................

MHV IC NC_001846  27360 atattttgttcaagatgatggagaatggaagttcacaggcagttcatattactaccctga
LA16 #39          27319 ..........................................................
NC2 #3            27322 ..........................................................

MHV IC NC_001846  27420 acccattacagataaaaacagtgtcattatgagtagttgcgcagtaaactacacaaaggc
LA16 #39          27379 ..........................................................
NC2 #3            27382 ..........................................................

MHV IC NC_001846  27480 acctgaagttttcttgaacacttcaatacctaatccacccgactttaaggaggagttaga
LA16 #39          27439 ..........................................................
NC2 #3            27442 ..........................................................

MHV IC NC_001846  27540 taaatggtttaagaatcagacgtctattgcgcctgatttatctctcgatttcgagaagtt
LA16 #39          27499 ..........................................................
NC2 #3            27502 ..........................................................

MHV IC NC_001846  27600 aaatgttactttgctggacctgacgtatgagatgaacaggattcaggatgcaattaagaa
LA16 #39          27559 ..........................................................
NC2 #3            27562 ..........................................................
```

```
MHV IC NC_001846  27660 gttaaatgagagctacatcaacctcaaggaagttggcacatatgaaatgtatgtgaaatg
LA16 #39          27619 ............................................................
NC2 #3            27622 ............................................................

MHV IC NC_001846  27720 gccttggtatgtttggttgctaattggattagctggtgtagctgtttgtgtgttgttatt
LA16 #39          27679 ............................................................
NC2 #3            27682 ............................................................

MHV IC NC_001846  27780 ctttatatgttgctgcacaggttgtggctcatgttgttttaagaagtgtggaaattgttg
LA16 #39          27739 ............................................................
NC2 #3            27742 ............................................................

MHV IC NC_001846  27840 tgatgagtatggaggacaccaggacagtattgtgatacataatatttcctctcatgagga
LA16 #39          27799 ............................................................
NC2 #3            27802 ............................................................

MHV IC NC_001846  27900 ttgactatcacagcctctcctggaaagacagaaaatctaaacaatttatagcattctcat
LA16 #39          27859 ............................................................
NC2 #3            27862 ............................................................

MHV IC NC_001846  27960 tgctacctggccccgtaagaggcagtcatagctatggccgtgttggtcctaaggctacat
LA16 #39          27919 ........................................t...................
NC2 #3            27922 ........................................t...................

MHV IC NC_001846  28020 tggctgctgtctttattggtccatttattgtagcatgtatgctaggcattggcctagttt
LA16 #39          27979 ............................................................
NC2 #3            27982 ............................................................

MHV IC NC_001846  28080 atttattgcaattgcaagttcaaatttttcatgttaaggataccatacgtgtgactggca
LA16 #39          28039 ............................................................
NC2 #3            28042 ............................................................

MHV IC NC_001846  28140 agccagccactgtgtcttatactacaagtacaccagtaacaccgagcgcgacgacgctcg
LA16 #39          28099 ............................................................
NC2 #3            28102 ............................................................

MHV IC NC_001846  28200 atggtactacgtatactttaattagacccactagctcttatacaagagtttatcttggta
LA16 #39          28159 ............................................................
NC2 #3            28162 ............................................................

MHV IC NC_001846  28260 ctccaagaggttttgattatagtacatttgggcctaagaccctagattatgttactaatc
LA16 #39          28219 ............................................................
NC2 #3            28222 ............................................................

MHV IC NC_001846  28320 taaacctcatcttaattctggtcgtccatatacttttaaggcattgtccaggcatatgag
LA16 #39          28279 ............................................................
NC2 #3            28282 ............................................................

MHV IC NC_001846  28380 accaacagccacatggatttggcatgtgagtgatgcatggttacgccgcacgcgggactt
LA16 #39          28339 ............................................................
NC2 #3            28342 ............................................................

MHV IC NC_001846  28440 tggtgtcattcgcctagaagatttttgttttcaatttaattatagccaaccccgagttgg
LA16 #39          28399 ............................................................
NC2 #3            28402 ............................................................

MHV IC NC_001846  28500 ttattgtagagttccttttaaaggcttggtgtagcaaccagggtaaatttgcagcgcagtt
LA16 #39          28459 ............................................................
NC2 #3            28462 ............................................................
```

```
MHV IC NC_001846  28560 taccctaaaaagttgcgaaaaaccaggtcacgaaaaatttattactagcttcacggccta
LA16 #39          28519 ............................................................
NC2 #3            28522 ............................................................

MHV IC NC_001846  28620 cggcagaactgtccaacaggccgttagcaagttagtagaagaagctgttgattttattct
LA16 #39          28579 ............................................................
NC2 #3            28582 ............................................................

MHV IC NC_001846  28680 ttttagggccacgcagctcgaaagaaatgtttaatttattccttacagacacagtatggt
LA16 #39          28639 ............................................................
NC2 #3            28642 ............................................................

MHV IC NC_001846  28740 atgtggggcagattatttttatattcgcagtgtgtttgatggtcaccataattgtggttg
LA16 #39          28699 ............................................................
NC2 #3            28702 ............................................................

MHV IC NC_001846  28800 ccttccttgcgtctatcaaactttgtattcaactttgcggtttatgtaatactttggtgc
LA16 #39          28759 ............................................................
NC2 #3            28762 ............................................................

MHV IC NC_001846  28860 tgtccccttctatttatttgtatgataggagtaagcagctttataagtattataatgaag
LA16 #39          28819 ............................................................
NC2 #3            28822 ............................................................

MHV IC NC_001846  28920 aaatgagactgcccctattagaggtggatgatatctaatctaaacattatgagtagtact
LA16 #39          28879 ........................................c...................
NC2 #3            28882 ........................................c...................

MHV IC NC_001846  28980 actcaggccccagagcccgtctatcaatggacggccgacgaggcagttcaattccttaag
LA16 #39          28939 ............................................................
NC2 #3            28942 ............................................................

MHV IC NC_001846  29040 gaatggaacttctcgttgggcattatactactctttattactatcatactacagttcggt
LA16 #39          28999 ............................................................
NC2 #3            29002 ............................................................

MHV IC NC_001846  29100 tacacgagccgtagcatgtttatttatgttgtgaaaatgataatcttgtggttaatgtgg
LA16 #39          29059 ............................................................
NC2 #3            29062 ............................................................

MHV IC NC_001846  29160 ccactgactattgttttgtgtattttcaattgcgtgtatgcgctaaataatgtgtatctt
LA16 #39          29119 ............................................................
NC2 #3            29122 ............................................................

MHV IC NC_001846  29220 ggattttctatagtgtttactatagtgtccattgtaatctggattatgtattttgttaat
LA16 #39          29179 ............................................................
NC2 #3            29182 ............................................................

MHV IC NC_001846  29280 agcataaggttgtttatcaggactggtagctggtggagcttcaaccccgaaacaaacaac
LA16 #39          29239 ............................................................
NC2 #3            29242 ............................................................

MHV IC NC_001846  29340 cttatgtgtatagatatgaaaggtaccgtgtatgttagacccattattgaggattaccat
LA16 #39          29299 ............................................................
NC2 #3            29302 ............................................................

MHV IC NC_001846  29400 acactaacagccactattattcgtggccacctctacatgcaaggtgttaagctaggcacc
LA16 #39          29359 ............................................................
NC2 #3            29362 ............................................................
```

```
MHV IC NC_001846  29460 ggtttctctttgtctgacttgcccgcttatgttacagttgctaaggtgtcacacctttgc
LA16 #39          29419 ............................................................
NC2 #3            29422 ............................................................

MHV IC NC_001846  29520 acttataagcgcgcattcttagacaaggtagacggtgttagcggttttgctgtttatgtg
LA16 #39          29479 ............................................................
NC2 #3            29482 ............................................................

MHV IC NC_001846  29580 aagtccaaggtcggaaattaccgactgccctcaaacaaaccgagtggcgcggacaccgca
LA16 #39          29539 ............................................................
NC2 #3            29542 ............................................................

MHV IC NC_001846  29640 ttgttgagaatctaatctaaactttaaggatgtcttttgttcctgggcaagaaaatgccg
LA16 #39          29599 ..........c.................................................
NC2 #3            29602 ..........c.................................................

MHV IC NC_001846  29700 gtggcagaagctcctctgtaaaccgcgctggtaatggaatcctcaagaagaccacttggg
LA16 #39          29659 ............................................................
NC2 #3            29662 ............................................................

MHV IC NC_001846  29760 ctgaccaaaccgagcgtggaccaaataatcaaaatagaggcagaaggaatcagccaaagc
LA16 #39          29719 ............................................................
NC2 #3            29722 ............................................................

MHV IC NC_001846  29820 agactgcaactactcaacccaactccgggagtgtggttccccattactcctggttttctg
LA16 #39          29779 ............................................................
NC2 #3            29782 ............................................................

MHV IC NC_001846  29880 gcattacccagttccaaaagggaaaggagtttcagtttgcagaaggacaaggagtgccta
LA16 #39          29839 ............................................................
NC2 #3            29842 ............................................................

MHV IC NC_001846  29940 ttgccaatggaatccccgcttcagagcaaaagggatattggtatagacacaaccgccgtt
LA16 #39          29899 ............................................................
NC2 #3            29902 ............................................................

MHV IC NC_001846  30000 cttttaaaacacctgatgggcagcagaagcaattactgcccagatggtatttttactatc
LA16 #39          29959 ............................................................
NC2 #3            29962 ............................................................

MHV IC NC_001846  30060 ttggcacagggccccatgctggagccagttatggagacagcattgaaggtgtcttctggg
LA16 #39          30019 ..................................................a.........
NC2 #3            30022 ..................................................a.........

MHV IC NC_001846  30120 ttgcaaacagccaagcggacaccaatacccgctctgatattgtcgaaagggacccaagca
LA16 #39          30079 ............................................................
NC2 #3            30082 ............................................................

MHV IC NC_001846  30180 gtcatgaggctattcctactaggtttgcgcccggcacggtattgcctcagggcttttatg
LA16 #39          30139 ............................................................
NC2 #3            30142 ............................................................

MHV IC NC_001846  30240 ttgaaggctctggaaggtctgcacctgctagccgatctggttcgcggtcacaatcccgtg
LA16 #39          30199 ............................................................
NC2 #3            30202 ..............................................................g.

MHV IC NC_001846  30300 ggccaaataatcgcgctagaagcagttccaaccagcgccagcctgcctctactgtaaaac
LA16 #39          30259 ............................................................
NC2 #3            30262 ............................................................
```

```
MHV IC NC_001846  30360 ctgatatggccgaagaaattgctgctcttgttttggctaagctcggtaaagatgccggcc
LA16 #39          30319 ....................................................
NC2 #3            30322 ....................................................

MHV IC NC_001846  30420 agcccaagcaagtaacgaagcaaagtgccaaagaagtcaggcagaaaattttaaacaagc
LA16 #39          30379 ....................................................
NC2 #3            30382 ....................................................

MHV IC NC_001846  30480 ctcgccaaaagaggactccaaacaagcagtgcccagtgcagcagtgttttggaaagagag
LA16 #39          30439 ....................................................
NC2 #3            30442 ....................................................

MHV IC NC_001846  30540 gccccaatcagaattttggaggctctgaaatgttaaaacttggaactagtgatccacagt
LA16 #39          30499 ....................................................
NC2 #3            30502 .t..................................................

MHV IC NC_001846  30600 tccccattcttgcagagttggctccaacagttggtgccttcttctttggatctaaattag
LA16 #39          30559 ....................................................
NC2 #3            30562 ....................................................

MHV IC NC_001846  30660 aattggtcaaaaagaattctggtggtgctgatgaacccaccaaagatgtgtatgagctgc
LA16 #39          30619 ....................................................
NC2 #3            30622 ....................................................

MHV IC NC_001846  30720 aatattcaggtgcagttagatttgatagtactctacctggttttgagactatcatgaaag
LA16 #39          30679 ....................................................
NC2 #3            30682 ....................................................

MHV IC NC_001846  30780 tgttgaatgagaatttgaatgcctaccagaaggatggtggtgcagatgtggtgagcccaa
LA16 #39          30739 ....................................................
NC2 #3            30742 ....................................................

MHV IC NC_001846  30840 agccccaaagaaaagggcgtagacaggctcaggaaaagaaagatgaagtagataatgtaa
LA16 #39          30799 ....................................................
NC2 #3            30802 ....................................................

MHV IC NC_001846  30900 gcgttgcaaagcccaaaagctctgtgcagcgaaatgtaagtagagaattaaccccagagg
LA16 #39          30859 ....................................................
NC2 #3            30862 ....................................................

MHV IC NC_001846  30960 atagaagtctgttggctcagatccttgatgatggcgtagtgccagatgggttagaagatg
LA16 #39          30919 ....................................................
NC2 #3            30922 ....................................................

MHV IC NC_001846  31020 actctaatgtgtaaagagaatgaatcctatgtcggcgctcggtggtaacccctcgcgaga
LA16 #39          30979 ....................................................
NC2 #3            30982 ....................................................

MHV IC NC_001846  31080 aagtcgggataggacactctctatcagaatggatgtcttgctgtcataacagatagagaa
LA16 #39          31039 ....................................................
NC2 #3            31042 ....................................................

MHV IC NC_001846  31140 ggttgtggcagaccctgtatcaattagttgaaagagattgcaaaatagagaatgtgtgag
LA16 #39          31099 ....................................................
NC2 #3            31102 ....................................................

MHV IC NC_001846  31200 agaagttagcaaggtcctacgtctaaccataagaacggcgataggcgccccctgggaaga
LA16 #39          31159 ....................................................
NC2 #3            31162 ....................................................
```

```
MHV IC NC_001846  31260 gctcacatcagggtactattcctgcaatgccctagtaaatgaatgaagttgatcatggcc
LA16 #39          31219 ..........................................................
NC2 #3            31222 ..........................................................

MHV IC NC_001846  31320 aattggaaga-atcacaaaaaaaaaaaaaaaaaaaaaaa----------------------
LA16 #39          31279 ..........t............................aaaaaaaaaaaaaaaaaaaaa
NC2 #3            31282 ..........t............................aaaaaaaaaaaaaaaaaaaaa

MHV IC NC_001846        ---------------------------------
LA16 #39          31339 aaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa-
NC2 #3            31342 aaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa
```

REFERENCE LIST

1. Taubenberger, J. K. & Morens, D. M. 1918 Influenza: the Mother of All Pandemics. *Emerg. Infect. Dis.* **12,** 15–22 (2006).

2. WHO | Number of deaths due to HIV. *WHO* (2017).

3. WHO | Middle East respiratory syndrome coronavirus (MERS-CoV) – Saudi Arabia. *WHO* (2016).

4. WHO | Summary of probable SARS cases with onset of illness from 1 November 2002 to 31 July 2003. *WHO* (2015).

5. Bradburne, A. F., Bynoe, M. L. & Tyrrell, D. A. Effects of a "new" human respiratory virus in volunteers. *Br. Med. J.* **3,** 767–9 (1967).

6. Hamre, D. & Procknow, J. J. A new virus isolated from the human respiratory tract. *Proc. Soc. Exp. Biol. Med.* **121,** 190–3 (1966).

7. Monto, A. S. Medical reviews. Coronaviruses. *Yale J. Biol. Med.* **47,** 234–51 (1974).

8. Chan, J. F. W. *et al.* Middle East respiratory syndrome coronavirus: another zoonotic betacoronavirus causing SARS-like disease. *Clin. Microbiol. Rev.* **28,** 465–522 (2015).

9. Yang, D. & Leibowitz, J. L. The structure and functions of coronavirus genomic 3' and 5' ends. *Virus Res.* **206,** 120–33 (2015).

10. Pasternak, A. O., Spaan, W. J. M. & Snijder, E. J. Nidovirus transcription: how to make sense...? *J. Gen. Virol.* **87,** 1403–21 (2006).

11. de Wit, E., van Doremalen, N., Falzarano, D. & Munster, V. J. SARS and MERS: recent insights into emerging coronaviruses. *Nat. Rev. Microbiol.* **14,** 523–534 (2016).

12. Fehr, A. R. & Perlman, S. Coronaviruses: an overview of their replication and pathogenesis. *Methods Mol. Biol.* **1282,** 1–23 (2015).

13. Perlman, S. & Netland, J. Coronaviruses post-SARS: update on replication and pathogenesis. *Nat. Rev. Microbiol.* **7,** 439–50 (2009).

14. Zhu, X., Liu, Q., Du, L., Lu, L. & Jiang, S. Receptor-binding domain as a target for developing SARS vaccines. *J. Thorac. Dis.* **5 Suppl 2,** S142-8 (2013).

15. Calisher, C. H., Childs, J. E., Field, H. E., Holmes, K. V & Schountz, T. Bats: important reservoir hosts of emerging viruses. *Clin. Microbiol. Rev.* **19,** 531–45 (2006).

16. Eckerle, L. D. *et al.* Infidelity of SARS-CoV Nsp14-exonuclease mutant virus replication

is revealed by complete genome sequencing. *PLoS Pathog.* **6,** e1000896 (2010).

17. Snijder, E. J. *et al.* Unique and conserved features of genome and proteome of SARS-coronavirus, an early split-off from the coronavirus group 2 lineage. *J. Mol. Biol.* **331,** 991–1004 (2003).

18. Menachery, V. D., Graham, R. L. & Baric, R. S. Jumping species-a mechanism for coronavirus persistence and survival. *Curr. Opin. Virol.* **23,** 1–7 (2017).

19. Fong, I. W. Emerging Animal Coronaviruses: First SARS and Now MERS. in *Emerging Zoonoses* 63–80 (Springer International Publishing, 2017). doi:10.1007/978-3-319-50890-0_4

20. Anthony, S. J. *et al.* A strategy to estimate unknown viral diversity in mammals. *MBio* **4,** e00598-13 (2013).

21. Luis, A. D. *et al.* A comparison of bats and rodents as reservoirs of zoonotic viruses: are bats special? *Proc. R. Soc. B Biol. Sci.* **280,** 20122753–20122753 (2013).

22. Wynne, J. W., Wang, L.-F., Cui, J., Field, H. & Holmes, E. Bats and Viruses: Friend or Foe? *PLoS Pathog.* **9,** e1003651 (2013).

23. Mandl, J. N. *et al.* Reservoir Host Immune Responses to Emerging Zoonotic Viruses. *Cell* **160,** 20–35 (2015).

24. Fong, I. W. *Emerging Zoonoses*. (Springer International Publishing, 2017). doi:10.1007/978-3-319-50890-0

25. Reshi, M. L., Su, Y.-C. & Hong, J.-R. RNA Viruses: ROS-Mediated Cell Death. *Int. J. Cell Biol.* **2014,** 1–16 (2014).

26. Zhang, G. *et al.* Comparative Analysis of Bat Genomes Provides Insight into the Evolution of Flight and Immunity. *Science (80-.).* **339,** (2013).

27. Zhou, P. *et al.* Contraction of the type I IFN locus and unusual constitutive expression of IFN-α in bats. *Proc. Natl. Acad. Sci. U. S. A.* **113,** 2696–701 (2016).

28. Zhong, N. S. *et al.* Epidemiology and cause of severe acute respiratory syndrome (SARS) in Guangdong, People's Republic of China, in February, 2003. *Lancet (London, England)* **362,** 1353–8 (2003).

29. Zaki, A. M., van Boheemen, S., Bestebroer, T. M., Osterhaus, A. D. M. E. & Fouchier, R. A. M. Isolation of a Novel Coronavirus from a Man with Pneumonia in Saudi Arabia. *N. Engl. J. Med.* **367,** 1814–1820 (2012).

30. Mouse Hepatitis Virus.

31. Boot, R. & Bisgaard, M. Reclassification of 30 *Pasteurellaceae* strains isolated from rodents. *Lab. Anim.* **29,** 314–319 (1995).

32. Barthold, S. W. & Smith, A. L. Viremic dissemination of mouse hepatitis virus-JHM following intranasal inoculation of mice. *Arch. Virol.* **122,** 35–44 (1992).

33. Baker, D. G. Natural pathogens of laboratory mice, rats, and rabbits and their effects on research. *Clin. Microbiol. Rev.* **11,** 231–66 (1998).

34. University of Missouri-Comparative Medicine Program and IDEXX-BioReaeasrch. Mouse Hepatitis Virus (MHV). (2013). Available at: http://dora.missouri.edu/mouse/mouse-hepatitis-virus-mhv/. (Accessed: 17th August 2017)

35. Coley, S. E. *et al.* Recombinant mouse hepatitis virus strain A59 from cloned, full-length cDNA replicates to high titers in vitro and is fully pathogenic in vivo. *J. Virol.* **79,** 3097–106 (2005).

36. Edgar, R. S. & Lielausis, I. Temperature-sensitive Mutants of Bacteriophage T4D: Their Isolation and Genetic Characterization.H*Genetics* **49,** 649–62 (1964).

37. Edgar, R. S., Denhardt, G. H. & Epstein, R. H. A Comparative Genetic Study of Conditional Lethal Mutations of Bacteriophage T4D. *Genetics* **49,** 635–48 (1964).

38. Complementation and Recombination - Biology LibreTexts. (2016). Available at: https://bio.libretexts.org/TextMaps/Map%3A_Working_with_Molecular_Genetics_(Hardison)/Unit_I%3A_Genes%2C_Nucleic_Acids%2C_Genomes_and_Chromosomes/1%3A_Fundamental_Properties_of_Genes/Complementation_and_Recombination. (Accessed: 28th August 2017)

39. Koolen, M. J., Osterhaus, A. D., Van Steenis, G., Horzinek, M. C. & Van der Zeijst, B. A. Temperature-sensitive mutants of mouse hepatitis virus strain A59: isolation, characterization and neuropathogenic properties. *Virology* **125,** 393–402 (1983).

40. Mielech, A. M. *et al.* Murine coronavirus ubiquitin-like domain is important for papain-like protease stability and viral pathogenesis. *J. Virol.* **89,** 4907–17 (2015).

41. Schaad, M. C. *et al.* Genetics of mouse hepatitis virus transcription: identification of cistrons which may function in positive and negative strand RNA synthesis. *Virology* **177,** 634–45 (1990).

42. Dulbecco, R. & Vogt, M. Some problems of animal virology as studied by the plaque technique. *Cold Spring Harb. Symp. Quant. Biol.* **18,** (1953).

43. Hirano, N., Murakami, T., Fujiwara, K. & Matsumoto, M. Utility of mouse cell line DBT

for propagation and assay of mouse hepatitis virus. *Jpn. J. Exp. Med.* **48,** 71–5 (1978).

44. Padmanabhan, A. Genomic Deep Sequencing of LA16 and NC2. *Kansas State University Integrated Genomics Facility* (2017).

45. Donaldson, E. F., Graham, R. L., Sims, A. C., Denison, M. R. & Baric, R. S. Analysis of murine hepatitis virus strain A59 temperature-sensitive mutant TS-LA6 suggests that nsp10 plays a critical role in polyprotein processing. *J. Virol.* **81,** 7086–98 (2007).

46. Smith, E. C. *et al.* Mutations in coronavirus nonstructural protein 10 decrease virus replication fidelity. *J. Virol.* **89,** 6418–26 (2015).

VITA

The author, Amani T. Eddins, was born in Dekalb, IL on August 10, 1992 to Trevell and Brenda Eddins. She attended Hampton University in Hampton, Virginia where she earned her Bachelor's of Science, *cum laude*, in Cellular and Molecular Biology in May 2014. After graduation Amani participated in a post-baccalaureate program at the University of Chicago in Chicago, IL. While attending the University of Chicago, Amani worked in the virology lab of Glenn Randall, PhD. where she participated in Hepatitis C and Dengue Virus research. It was there under the mentorship of Dr. Randall and a Loyola University alumni Dr. Anna Shulla that urged Amani to pursue an advanced degree in Microbiology and Immunology.

In August of 2015, Amani matriculated into the Loyola University Chicago Graduate Program and joined the lab of Susan Baker, PhD in the department of Microbiology and Immunology. Amani's thesis work investigated the use of temperature-sensitive phenotyping of coronaviruses. After completion of her graduate studies, Amani will seek to obtain a degree in nursing to be involved with clinical vaccine studies involving human patients.