Developing and Optimizing RNA Isolation Methods from Avian Tissues

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Introduction

- Investigating tissue-specific gene expression first requires RNA isolation.
- We optimized and evaluated multiple methods of RNA extraction from blood and gonadal tissues.
- Our study species is the house sparrow (Passer domesticus), a widespread songbird.

Materials and Methods

- We used a Trizol-based phenol-chloroform protocol
- We compared the following:
  - Tissue: blood vs gonad
  - Storage: flash frozen vs. RNALater
  - Homogenization: Tissue Tearor vs. bashing beads

Main Issues Encountered

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Method</th>
<th>Issue</th>
<th>Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood</td>
<td>Flash Frozen</td>
<td>Bloodcicle hard to remove from tube before thawing</td>
<td>Work quickly to avoid thawing</td>
</tr>
<tr>
<td>Blood</td>
<td>RNALater</td>
<td>Thawed blood too viscous to pipette out completely</td>
<td>Avoid thawing</td>
</tr>
<tr>
<td>Gonad</td>
<td>Tissue Tearor</td>
<td>Gonad tissue stuck inside Tissue Tearor</td>
<td>Clean Tissue Tearor between samples</td>
</tr>
<tr>
<td>Gonad</td>
<td>Bashing Beads</td>
<td>Bashing beads did not break up tissue samples sufficiently</td>
<td>Use Tissue Tearor</td>
</tr>
</tbody>
</table>

Conclusions

- Blood that was flash frozen produced higher RNA quantity and quality (A260/280 closer to 2.0) than blood stored in RNA Later.
- Flash frozen blood may be a more reliable storage method than RNALater.
- Gonadal tissue homogenized with Tissue Tearor produced higher RNA quantity and quality than with bashing beads.
- Future research will use qPCR to measure differential gene expression in relation to variation in phenotypic behaviors.