Dissection & RNA Isolation from Embryonic Chicken Retina (wet lab)

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Dissection & Total RNA Isolation from Embryonic Chicken Retina
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Objectives:

• Dissect retina & cornea from developing chicken embryos
• Extract total cellular RNA from tissues for downstream gene expression analysis

The past few weeks have been devoted to discussing structure, composition and analysis of the DNA genome. DNA genomes are expressed as RNA transcripts. There are many different types of RNAs expressed in cells that we will discuss during the course of the semester. Though messenger RNAs (mRNAs) only make up ~3% of total RNA, they are the most commonly studied transcripts due their protein coding nature. Over the next 6 weeks in lab, we will analyze mRNA gene expression in the developing chicken retina.

I. Dissection of Embryonic Chicken Eyes

My research program studies gene expression in the developing retina. We’re interested in epigenetic modifications to the genome that determine if retina-specific genes are transcribed or silenced. Today we will dissect whole retina and cornea from early and late chicken embryos in preparation for RNA extractions to be for downstream gene expression analysis. The developing retina in early day 8 embryos (E8) is immature and composed of undifferentiated cells. By late development (E18-E21) retinal neurons have differentiated into highly specialized retinal neurons expressing retina-specific genes. Though the genomic DNA sequence of these cells does not change during development, patterns of gene expression change dramatically during this developmental window.
**Dissection of Live Animals:** This lab activity involves the dissection of live embryos. The State of Virginia does not recognize that chicken embryos are live animals until they hatch from the egg, which is ridiculous and an ethical topic in its own right. However, if you feel uncomfortable participating in this activity please let the instructor know and you will be given an alternative assignment. Refraining from participation will not negatively impact your grade.

**Before beginning:**

a. Plan your experiment in your notebook. How many samples will your group make? What age embryos are you using?
   a. Every table should collect 2-3X E8 + E18 retina for individual RNA preps
   b. Each group at a table should select either E8 or E18 embryos to dissect
b. Things to gather for dissections: dissection tools, E8+E18 eggs, sterile 1.5 mL tubes; large plastic beaker with tissue disposal bag; small culture dishes filled with PBS

**Safety concerns:** Use care with sharp dissection tools, wear gloves and safety glasses when handling live embryos

**Embryo Dissection protocol**

1) Label 1.5 mL tubes for each tissue sample (i.e. “E8 retina”) and place in rack at room temp.
2) Open egg from broadside and remove or spill out embryo into the dissection tray. Immediately decapitate embryo with scissors to decapitate. Keep only the head of embryo and dispose of remaining waste in bucket.
3) Use scissors and forceps to cut away eyelids then to carefully remove the whole eye without piercing it. The eye is attached by connective tissue on the sides and the optic nerve in the back. The common mistake here is to pierce the eye globe. You will know this happened if the eye looses its firm round shape.

4) Place enucleated eyes into a small culture dish on ice with PBS for later dissection. Batch several eyes before moving on to further dissecting.

5) After collecting several eyes, cut the eyes in ½ along their equator. Grab the eye using forceps to stabilize and pierce the side with a scalpel along the equator of the eye. Keep hold of the eye with forceps and use scissors to cut away front half of the eye exposing the vitreous and retina. Save the front of the eye to cut out and collect cornea tissue.

6) After removing the front of the eye, remove the translucent gelatinous vitreous humor sitting on top of the retina using forceps.

7) Dissect out the pink retina tissue (sits on top of dark epithelial cells) from the eyecup using the forceps to pull loose. Transfer to a dish with PBS on ice to rinse and cut into several small pieces.
8) Transfer several small pieces from a single retina to a tube with 350 µL RLT+BME buffer and vortex vigorously. Store on ice for RNA extraction.

9) Go back to the front of the eye and cut away the cornea. Place into a prechilled mortar and grind into a fine powder with the pestle and scrape small amount into a labeled tube with 350 µL RLT+BME. **Note: Dr. Enke will grind corneas after you prep them.**

## II. Total RNA Extraction Using Silica Spin Columns

In this protocol we will use Qiagen RNeasy silica-based spin columns to isolate total RNA from embryonic chicken tissues. Each group will harvest RNA from 1) E8 chicken retina, 2) E18 chicken retina and 3) E18 chicken cornea.

Remember that **RNase** enzymes are everywhere. Your hands, hair, and breath are 3 major sources. Wear gloves throughout this protocol and avoid using your gloves to touch your skin or hair during the procedures. Your benches and pipettes should be washed down with a special detergent called RNAZap that will help get rid of RNases before you start the protocol. These are very basic and not great to get on your skin.

*** **Protocol Note:** For all spin steps & incubations use this downtime to look ahead and prepare for downstream steps. **Multitasking** is a hallmark of a good scientist. For instance, I wrote this lab protocol last night **while** I watched the Grammys.

**Disrupting and homogenizing retinal & cornea cells:** The goal of these steps is to break open the cells & organelles to release RNAs (**disrupting**) and to shear high molecular weight DNA to reduce viscosity of cell lysates (**homogenization**). Retinal tissue can be disrupted simply by dissolving them in RLT buffer + BME followed by vigorous vortexing for 15-20 seconds. This buffer contains a denaturing agent called guanadine thiocynate that temporarily inactivates RNases. Cornea tissue is more challenging to disrupt and requires snap freezing in a mortar on dry ice followed by grinding to a fine powder with a pestle before being dissolved in RLT buffer.

**Lab Bench Set Up:** clear all unneeded items, put on gloves/safety glasses, and wipe down your bench, pippetes and the outside of other objects you might be touching like pens +
pipettes using RNAzap wipes. From now on you want to try not to touch anything that is not RNase Free with your gloves. Change your gloves as often as needed.

**Reagents to gather:** ice, buffers (RLT+BME, 70% EtOH, RW1, RPE), DNaseI (keep on ice), filter pipette tips, pre PCR pipettes, bag with Qiashredder, RNeasy columns and 1.5 mL tubes

1. Vortex tissues samples from part I in RLT+BME buffer again for 15-20 sec to disrupt tissue

2. Spin down insoluble material for 1 min at top speed

3. Transfer only liquid supernatant to a Qiashredder tissue homogenizer column, and spin at full speed 2 min. If you transfer tissue chunks at this point you will clog the column.

4. While spinning, label an RNeasy spin column for each sample.

5. After the spin, add 350 µL of 70% ethanol to the column flow through, throw away the Qiashredder as long as there is no remaining liquid in it

6. Transfer flow through + ethanol to your labeled RNeasy spin columns and spin 1 min at 10K rpm. All RNAs >200nt will remain on the column while the rest of the cellular material will flow through the column. *Do not throw away the column*

7. *Save the column and dump out the flow through. Replace the column in the same collection tube to reuse. A common mistake here is throwing away the RNA fraction on the column.*

8. Add 350 µL of RW1 buffer spin 1 min at 10K rpm to wash away impurities from the column. Keep the column and dump the flow through. Reuse the same collection tube.

9. **Add 80 µL of DNasel mix to each column and incubate 15 min at room temp.** This on column DNasel treatment will eliminate any trace amounts of contaminating genomic DNA in our RNA samples. This is a critical step for downstream qRT-PCR analysis.

10. During the incubation, a new sterile 1.5 mL tubes for each sample for the final RNA sample. These tubes will be used for long term storage so be sure the label has all of the critical information:

   **Top of tube:** indicate organism, type of tissue, type of nucleic acid (e.g. “Gg E18 retina RNA” for RNA from a day 18 chicken embryo retina
   **Side of tube:** initials, date, concentration of nucleic acid in ng/µL (we will get this information after purification).
11. At the end of the 15 min incubation, add **350 µL of RW1 buffer**, spin 30 sec at 10K rpm, discard flow through.

12. Add **500 uL of RPE** buffer, spin 30 sec at 10K rpm, discard flow through but reuse collection tube.

13. Add a 2\textsuperscript{nd} addition of **500 uL of RPE** buffer, spin 30 sec at 10K rpm, discard flow through but reuse collection tube.

14. Place RNA column back in the collection tube and spin dry at full speed for 1 min. This will get rid of any trace alcohol from the column, which is inhibitory to DNA polymerase.

15. Steps 11-14 have thoroughly washed the RNA on the column, which is now ready for elution.

16. Place RNA column in a well-labeled 1.5 mL tube and add **50 uL nuclease-free (n.f.) H2O**, let sit for 1 minute, then spin with open caps 30 sec at 10K rpm to **elute the RNA**. *Make sure not to throw away the eluted RNA after this step.*

17. Throw away the column and store your RNA tube on ice. Store at -20°C or -80°C long term.