NU-BIRD
Northwestern University Biology Investigations in Reproduction and Development

Mayo Module:
Gene Expression and Analysis in the Reproductive Axis

NU-BIRD is a collaborative effort between Northwestern University and Evanston Township High School
### Mayo Module:
**Schedule for Saturday February 22, 2014**  
**Pancoe Pavilion, Evanston Campus**

<table>
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<tr>
<th>Time &amp; Location</th>
<th>Event</th>
<th>Facilitator</th>
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<tr>
<td><strong>8:15 – 8:30 am</strong>&lt;br&gt;Pancoe Building</td>
<td>Arrive at the entrance to the Pancoe Building on the Northwestern Campus (see map, follow signs)</td>
<td>Ms Sarah Stein – call or text if you get lost 773-706-2292</td>
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<tr>
<td><strong>8:30 – 9:00 am</strong>&lt;br&gt;Pancoe 3103</td>
<td>Light breakfast</td>
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<tr>
<td><strong>9:00 – 10:00 am</strong>&lt;br&gt;Pancoe 3103</td>
<td>Mouse Reproduction and Genetics Lecture</td>
<td>Kelly Mayo, PhD</td>
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<tr>
<td><strong>10:00 – 11:50 am</strong>&lt;br&gt;Pancoe 1221</td>
<td>Laboratory Stations (students in 4 groups)</td>
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<tr>
<td>1. DNA Isolation and PCR</td>
<td>Pam Monahan, PhD</td>
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<td>2. DNA Gel Electrophoresis</td>
<td>Rexxi Prasasya and Nisan Hubbard</td>
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<td>3. Immunohistochemistry</td>
<td>Dallas Vanorny</td>
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<td>4. Microscopy and Immunofluorescence</td>
<td>Abha Chalpe, PhD</td>
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<td><strong>11:50 – 12:40 pm</strong>&lt;br&gt;Pancoe 3103</td>
<td>Lunch</td>
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<td><strong>12:40 – 2:30 pm</strong>&lt;br&gt;Pancoe 1221</td>
<td>Continue laboratory station rotations</td>
<td>Dr. Mayo and lab members</td>
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<tr>
<td><strong>2:30-3:15 pm</strong>&lt;br&gt;Pancoe 4321</td>
<td>Examine Planaria from Week 1 with Dr. Petersen</td>
<td>Dr. Petersen</td>
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| **3:15 – 3:30 pm**<br>Pancoe 3103 | Departure  
• Pick-up personal items | Ms Stein  
Dr. Murdoch |
Mayo Module: Guiding Questions and Learning Outcomes

Guiding Question:
How can we analyze genes within complex genomes and study their expression within cells and tissues?

<table>
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<tr>
<th>Module Question</th>
<th>Laboratory Questions</th>
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| What does gene expression in ovarian tissue tell us about the development and function of the mouse ovary? | • How can we detect individual genes?  
• How can we detect proteins in cells and tissue?  
• What are the advantages and limitations of the mouse as an experimental model system? |

Learning Outcomes:

Understand the goals of reproductive biology research and appreciate the structure and function of the ovary.

Explain the process of gene expression in general, and state how this applies to the ovary.

Explain DNA and gene structure and function, and how DNA can be isolated and analyzed.

Explain protein structure and function, and how to detect proteins within cells and tissues.

Understand the advantages and limitations of the mouse as a model for mammalian investigations relevant to human health.
Mayo Module:
Lecture and General Vocabulary

Ovary – the egg-producing reproductive organ found in female organisms

Oocyte – the female germ cell, also known as an egg

Follicle – in the ovary, follicles are the basic functional unit, and consist of a group of somatic cells that surround the oocyte

Corpus Luteum – the structure that develops from the somatic cells post-ovulation (through the process of luteinization) that produces progestins necessary for initiation and maintenance of pregnancy

Granulosa Cell – the somatic cell of the follicle that directly surrounds the oocyte and serves as a nurse cell for its maturation; granulosa cells proliferate as the follicle grows, and they produce estrogens

Thecal Cell – outer somatic cells of the follicle that surround the granulosa cells and the produce androgen substrates for estrogen production in the granulosa cell

Gametogenesis – one of two major functions of the follicle and the process by which precursor cells undergo division and differentiation to form the mature haploid gamete, in the ovary the mature ovum

Steroidogenesis – one of the two major functions of the follicle, the process of producing steroid hormones, in the female ovary predominantly estrogen and progesterone

Menstrual Cycle – the recurring cycle of physiological changes that occur in reproductive age humans and other primates that is hormonally regulated and results in ovulation and preparation for the events of fertilization of the ovum and implantation of an embryo

Estrous cycle – the related recurring reproductive cycle of many placental mammals, including the mouse

Hormone – a chemical messenger that carries information from one cell to another via the bloodstream. Major types include the small proteins (peptides) and the cholesterol-derivatives (steroids)

Receptor – a protein within a cell or on the cell membrane that binds a chemical ligand such as a hormone and initiates an appropriate cellular response
Mayo Module: DNA Isolation, PCR, & Gel Electrophoresis Vocabulary

Gene – the fundamental unit of heredity; a length of DNA that encodes a protein or a structural or functional RNA

DNA – deoxyribonucleic acid; the macromolecule that encodes genetic information inside the cell nucleus

Mutation – changes to the DNA sequence of the organism; mutations can be neutral, or can result in a change in gene function

Genotype – the genetic constitution of an individual, usually with reference to a specific characteristic

Phenotype – any observed quality of an organism, which is influenced by both genotype and environment

Transgene – an experimentally generated gene used to make a transgenic animal or plant

Transgenic mouse – a mouse that has a piece of foreign DNA incorporated into its genome as a result of experimental manipulation; this may confer new characteristics, or phenotypes, to the mouse

Gene Knockout – an experimentally targeted mutation or deletion designed to disrupt the expression and or function of the product of the gene

Knockout mouse – a mouse in which a specific gene has been disrupted through experimental manipulation. This might be a global disruption, or a conditional knockout, in which the gene is disrupted in select tissues or at select times

Recombination – the process by which genetic material is broken and re-joined to other genetic material. Site specific recombination can be used as a tool to disrupt or modify specific genes in mice. In this case, recombination occurs at select experimentally inserted sites in the DNA called LoxP sites. The enzyme that carries out the recombination is from a bacteriophage and is called the CRE recombinase. A gene containing two loxP sites designed to allow for recombination and gene disruption is referred to as a floxed allele of the gene

DNA Polymerase – an enzyme that copies or repairs DNA using a DNA template and a primer

Taq Polymerase – a very heat-stable polymerase isolated from the thermophile bacteria, Thermus aquaticus; it’s thermostability allows PCR to be an automated process

Primer – a DNA oligonucleotide that serves as a starting point for DNA polymerase to copy a DNA template
Mayo Module:
Immunohistochemistry & Fluorescence Microscopy Vocabulary

PCR – polymerase chain reaction, a technique for amplifying DNA, making it easier to isolate and analyze; Kary Mullis was awarded the Nobel prize in chemistry in 1993 for discovering this technique

Gel electrophoresis – a biochemical technique in which macromolecules (generally protein or DNA) are separated based on their size as they migrate through a gel (generally acrylamide or agarose) in an electric field

Agarose – a polysaccharide polymer isolated from seaweed, forms a liquid at high temperature, but a gel at low temperature, and thus can be used to form a mold for electrophoresis

Histology – the study of cells and tissues at the microscopic level, branch of anatomy dealing with tissue structure

Sectioning/Sections – using a microtome or cryostat to make thin pieces of tissue (sections) that are placed on microscope slides for further analysis

Staining – the act of using one or more vital dyes to decorate constituents of cells or tissues (such as proteins or nucleic acids) so they can be more readily visualized using microscopy

Antibody (primary, secondary) – a protein produced by the immune system that recognizes and binds to another protein (the antigen) to help the body fight infections. A primary antibody recognizes the antigen, a secondary antibody is one that recognizes the primary antibody (generally they are of different species). These are widely used in biology as a tool to detect specific proteins of interest.

Antigen – a foreign substance capable of generating an immune response (in our case, a protein)

Immunohistochemistry – the combined use of histology and antibodies to detect and localize a protein antigen within a tissue section

PAP Pen – a special marking pen used to draw a film-like barrier around tissue sections that is impermeable to water; it keeps reagents localized to the tissue section

Microscope – an instrument for viewing objects to small to be observed with the naked eye

Fluorescence microscopy – a type of light microscopy in which the specimen is irradiated at wavelengths that excite a fluorophore, causing it to emit light through fluorescence

Immunofluorescence – an antibody is tagged with a fluorochrome, meaning that it (and the antigen it binds to) can be localized by fluorescence microscopy

DAPI – a highly specific and sensitive fluorescing DNA stain, used to localize cell nuclei

Objective Lens – the lens within the microscope that gathers the lights coming from the specimen and focuses these light rays to form the image

Eyepiece or Ocular Lens – the lens within the microscope closest to the eye, which magnifies the image coming from the objective lens; total magnification is obtained by multiplying the magnification provided by the objective lens with that provided by the ocular lens
The Female Reproductive Axis and the Ovary
How can we detect individual genes within genomes?

**Station 1: DNA Isolation & PCR**

*Isolation of DNA from cells*

*Enzymatic amplification of DNA by PCR*

*Concentration of DNA by alcohol precipitation*

*A thermocycler for automated PCR*

Questions to Think About:
- What is the structure of DNA?
- How can DNA be physically isolated from cells?
- Why does DNA need to be copied to be studied?
- How does the polymerase chain reaction (PCR) work?
- What does DNA isolation & PCR tell us about the structure and function of genes in the ovary?
How can we detect individual genes within genomes?

**Purpose:** To isolate DNA and amplify specific genes, as a first step in the process of studying gene expression

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**Experiment 1:**

- Select a numbered sample microfuge tube containing mouse tail cell extract. The different numbers correspond to different genotypes, as Pam will explain.

- Mix the mouse tail cell extract with isopropyl alcohol to precipitate the DNA.

- Centrifuge your sample for 3 minutes in the microfuge in the cold box to pellet DNA. Decant (pour off) the supernatant, leaving the DNA pellet to dry.

- Examine the PCR thermocycler instrument to understand how it operates.

- When the DNA pellet is dry, add 25 μl of water to the dried mouse DNA, vortex to mix, and add 25 μl of the corresponding master PCR mix into this tube, then vortex to mix again.

- Place the tube into the PCR thermocycler and program the machine to begin the PCR amplification.

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**Questions to Think About:**

- What is the structure of DNA?
- How can DNA be physically isolated from cells?
- Why does DNA need to be copied to be studied?
- How does the polymerase chain reaction (PCR) work?
- What does DNA isolation & PCR tell us about the structure and function of genes in the ovary?
How can we detect individual genes within genomes?

Station 2: Gel Electrophoresis

The process of preparing, loading, and running an agarose gel

Questions to Think About:
• How does gel electrophoresis work?
• What properties of DNA allow it to be separated?
• How can DNA and genes be visualized?
• What does gel electrophoresis tell us about the structure and function of genes in the ovary?

Student Notes or Questions:


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Northwestern University Biology Investigations in Reproduction and Development

# How can we detect individual genes within genomes?

**Purpose:** To detect the amplified genes and determine the size of the amplified region of DNA

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**Experiment 2:**

- Select a numbered sample tube corresponding to the one you used in station 1. This sample has been through the entire PCR amplification process.

- Add dye to your tube, and load your sample onto the pre-made agarose gel (Rexxi and Nisan will demonstrate the loading technique).

- Connect the agarose gel to the power supply, select the appropriate current, and begin the gel electrophoresis, which will take about 20 minutes.

- When the gel is done, visualize the DNA by placing the gel onto the UV light box in the darkroom and take a picture of the gel.

- Determine based on the size to what mouse genotype your numbered DNA sample corresponds (Pam will give you a key to help determine this).

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**Safety Considerations:**

Do not handle the gel or buffer without gloves (Rexxi and Nisan will do this)  

Do not look at the UV light unless the plexiglass shield is in place

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**Questions to Think About:**

- How does gel electrophoresis work?
- What properties of DNA allow it to be separated?
- How can DNA and genes be visualized?
- What does gel electrophoresis tell us about the structure and function of genes in the ovary?

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**Student Notes or Questions:**

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How can we detect proteins within cells and tissue?

Station 3: Immunohistochemistry

**Questions to Think About:**
- What is the purpose of using both primary and secondary antibodies?
- How can DNA and proteins be co-localized in tissues?
- What does immunohistochemistry tell us about the structure and function of proteins in the ovary?

**Student Notes or Questions:**

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How can we detect proteins within cells and tissue?

**Purpose:** To detect proteins in ovarian tissues by binding labeled antibodies to the proteins

**Experiment 3:**
- Each pair of students should select one slide from each group (there will be two different antibody groups) that have been pre-incubated in primary and secondary antibody.

- Place your slide into a slide chamber with wash buffer, let sit for 5 minutes.

- Transfer your slide to a new slide chamber with fresh wash buffer, let sit 5 minutes. Repeat wash step as above a third time.

- While your slides are washing, observe and practice isolating sections using a PAP pen.

- Blot the edge of the slide on a paper towel to dry.

- Place slide with the tissue sections facing up. Cover the sections with DAPI mounting medium and add a coverslip (Dallas will demonstrate this).

- Use nail polish to make a seal around the outside edge of the coverslip.

**Safety Considerations:**
- Take care with the glass coverslips, they are very fragile
- When working with xylenes, wear gloves and keep all materials in a hood

**Questions to Think About:**
- What is the purpose of using both primary and secondary antibodies?
- How can DNA and proteins be co-localized in tissues?
- What does immunohistochemistry tell us about the structure and function of proteins in the ovary?

**Student Notes or Questions:**
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- ________________________________________________
- ________________________________________________
- ________________________________________________
How can we detect proteins within cells and tissue?

**Station 4: Fluorescence Microscopy**

**Questions to Think About:**
- How can proteins be visualized in tissues?
- How can DNA be co-localized in tissues?
- How does a microscope work?
- What does fluorescence microscopy tell us about the structure and function of proteins in the ovary?

**Student Notes or Questions:**
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**An epi-fluorescence microscope**

**Detection of cell nuclei labeled with DAPI using a fluorescence microscope**
How can we detect proteins within cells and tissue?

**Purpose:** To determine the location of proteins within ovarian tissue

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**Experiment 4:**

- Observe the fluorescence microscope, its working parts, and how images are captured digitally to the computer (Abha will demonstrate this).

- Look at an H&E stained slide of a mouse ovary in the microscope. Identify a follicle, and observe the oocyte, granulosa, and thecal cells of the ovary.

- Look at your IHC experimental slide using the DAPI filter to detect nuclei and using the FITC (Green) or TRITC (red) filters to detect the proteins of interest (Abha will first demonstrate this for you).

- Try to identify the cell types within the ovary in which each of the 2 proteins of interest in the IHC experiment are expressed.

- Capture to the computer a picture of your IHC experiment. Abha will save these images and provide them to you at a later time.

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**Safety Considerations:**

Use caution when focusing the microscope using the course adjustment, so as to not break the coverslip or slide with the objective lens of the microscope.

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**Detection of a granulosa cell marker (inhibin, left) and oocyte marker (Stat3, right) in the mouse ovary using immunofluorescence**

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**Questions to Think About:**

- How can proteins be visualized in tissues?

- How can DNA be co-localized in tissues?

- How does a microscope work?

- What does fluorescence microscopy tell us about the structure and function of proteins in the ovary?

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**Student Notes or Questions:**

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