<u>Transcription and RNA degradation laboratory</u>

Summer Science Institute II for rising 11th and 12th graders

Goal: Understand how RNA is made and how fragile it is.

Things to do:

1. Understand what RNA is.
2. Making RNA from DNA in a test tube.
3. Degrade RNA with an enzyme or a drop of your sweat.
4. Analyze the RNA in gel electrophoresis

Warm-up questions:

1. Have you heard about RNA? Where?
2. Will RNA change your gene? How?

Experimental protocol:

<u>Before you start</u>:

Wear gloves during the WHOLE process to avoid RNase contamination.

Avoid touching the pipet tips and inside of tube lid with ANYTHING except reagents.

<u>Step 1: check what you have</u>:

1. A tube of DNA template solution (in your ice bucket)

2. A tube of master mix containing 10X transcription buffer and NTP (in your ice bucket) **(25uL each)**

3. A tube of RNase-free water (on your bench) **(100uL each)**

5. A small bag of RNase-free disposable microcentrifuge tube (on your bench) **(4 tubes each)**

6. A set of pipette and a box of tips. (P-200, P-20, yellow tips) **(1 box of RNase free tips)**

<u>(The things you need to find on the front or side bench)</u>

1. T7 RNA polymerase (in the cooler on the front bench)

2. P-10 pipette and tips (on the front bench)

3. Agarose powder, 1X TBE buffer, and Cybersafe dye (on the side bench)

4. Balance, glass flasks, microwave, heat gloves (on the side bench)

5. RNase A (in the cooler on the front bench)

6. Purple gel loading dye (on the side bench) and RNA ladder (in the ice bucket on the front bench)

Step 2: Make the reaction mix

Do this in one tube on ice:

NTP + 10X Reaction Buffer	20 µl
Nuclease-free water	11 µl
Template DNA	5 µl
Total reaction mix	36 ul

Step 3: Prepare a negative control

Take 9 µl of the mixture from step2, put it into a clean microcentrifuge tube on ice. Add 1 µl RNase-free water. Mark this tube with your group number and a tube number 0. (For example, if you are in group 1, mark it as 1-0)

Step 4: Add RNA polymerase to the transcription

Add 3 µl of T4 RNA polymerase into the rest of reaction mix. Set the P20 pipet to 20 µl, pipet the solution in the tube up and down for 5-10 time. Mark this tube with your group number and a tube number 1. (For example, if you are in group 1, mark it as 1-1.)

Step 5: Transcription incubation

Put both of your tubes into the 37 °C water bath or thermocycler set at 37 deg C. Wait for 1 hour. (Set a timer or write down the time you start waiting)

Step 6: Make agarose gel (Do this while you are waiting for 1 hour incubation)

6-1. Set up your agarose gel mold tray

6-2. Measure approximately .7 grams of Agarose 1 powder and 50mL of 1X TBE buffer. Combine both in an Erlenmeyer Flask and swirl to mix.

6-3. Place in microwave for 1 minute

6-4. USE A HEAT GLOVE to take flask out and insert 7uL of gel dye and swirl to mix

6-5. Pour solution in agarose gel mold and place combs in immediately after

6-6. Wait about 20 minutes for gel to harden (set a timer or write down the time you start waiting)

Step 7: Get some RNA for digestion

7-1. When the 1 hour timer is up, take all our tubes from the water bath.

7-2. Take 9 µl of the solution from tube 1 (1-1, 2-1, etc depending on your group number), put it into a new tube. Mark this tube with your group number and a tube number 2.

7-3. Take 9 µl of the solution from tube 1, put it into another new tube. Mark this tube with your group number and a tube number 3.

Step 8: Digest the RNA you made

8-1. In tube 2, add 1 µl of RNase A (in the front bench cooler)

8-2. In tube 3, add 1 µl of your sweat, saliva, or tap water (you may try to get the sweat from skin. For saliva or tap water, fill a microcentrifuge tube with either, then us pipette to take 1 µl.

8-3. Put all the four tubes back to the water bath. Wait for 30 minutes. (Set a timer or write down the time you start waiting)

<u>Step 9: Set up gel electrophoresis system</u>

9-1. Remove the comb by pulling it up carefully. Remove the stopper at both end of the gel.

9-2. Fill the electrophoresis tank with 1X TBE buffer. The buffer top needs to cover the top of the gel.

<u>Step 10: RNA gel electrophoresis system</u>

10-1. When the 30 minutes digestion is done, add 2 ul of the blue-purple color gel loading buffer in each of the four tubes.

10-2. In your agarose gel, load the ladder in the first well from the left, then load the whole content of your four tubes into each one of the other wells.

10-3. Run gel at 100V 400AV for 25-30min (maybe let a student show their gel at the 5 min mark.)

<u>Step 11: Check the result</u>

Check your result on the UV box. In which lane(s) do you see the RNA band?