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In Vitro Transcription Lab

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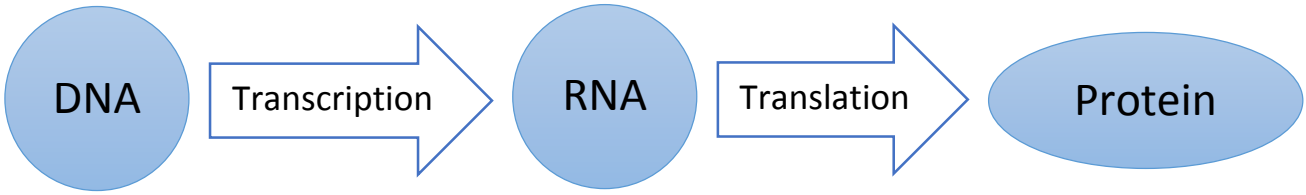
In Vitro Transcription

After completion of this exercise the student should be able to do the following:

1. Distinguish between yields after electrophoresis.
2. Describe which varied substance contributed to the largest yield.
3. Describe the importance of not contaminating the sample.

Introduction

Transcription is a crucial part of gene expression happening in both prokaryotes and eukaryotes. Francis Crick's central dogma is still regarded as a capstone in regards to the methodology of gene expression.



Transcription is the vital step from DNA to RNA that will be focused on today.

In vitro transcription is transcription that happens outside its natural context, or in a test tube. The DNA template used in this experiment is to produce phe-tRNA and not mRNA, however, the transcription process is the same. Today most of the template preparation has been performed for you so that you can focus on the transcription itself. After an initial composition of ingredients, the mixture will have to incubate for an hour. At this time EDTA is used to quench the reaction. This is important because the $MgCl_2$ is necessary for transcription to proceed. EDTA arrests the $MgCl_2$, significantly decreasing the amount available to continue transcription, thus stopping or "quenching" the reaction. Once this is completed, the samples will undergo gel electrophoresis for analysis.

It is **very** important when working with RNA to avoid sample contamination. RNases are enzymes that exist solely to break down RNA, or in this case, your hard work. Cells contain RNases that break down all of the RNA within the cell upon death. However a

cell tends to "pop" during apoptosis, releasing its RNases into the surrounding environment. This means it is of utmost importance to not sneeze or cough in the sample. Keep all hair away from the RNA and wear gloves at all times to reduce contamination risks. It is also suggested that lab partners should not speak to each other when working with the RNA, in order to further reduce risks of contamination. Contaminated samples yield poor results.

Procedure

Clean the lab bench with RNase spray.

Each member of the lab group will pick one sample to make of those listed below. As a group you will make all four, however each person will be responsible for one vial.

To each vial add:

- 5 µl 400mM Tris
- 5 µl 0.1% Triton X-100
- 2.5 µl 100 mM Spermadine
- 2.5 µl 100 mM DTT
- 10 µl 20 mM NTPs

Supplement these components with those listed for each respective vial below (in µL). Label each vial with the corresponding number in the table.

Add the linear plasmid and the T7 polymerase last after thawing the polymerase on ice.

	1	**2**	**3**	**4**
450 mM $MgCl_2$	2	4	2	3
Linear Plasmid	2	2	4	4
T7 polymerase	1.2	1.2	1.2	1.2
ddH_20	20	18	18	17

Use extreme caution when adding the T7 polymerase. This is the lowest value your pipette can reach.

Centrifuge the vials for 5-10 seconds and then place in a 37°C water bath or incubator for one hour. Begin preparing a gel for electrophoresis and a 65°C water bath during the incubation time.

Wearing gloves, carefully remove the comb from the polyacrylamide gel. Be sure not to rip or tear the gel, as it is very thin. Be sure to remove the piece of white tape at the bottom of the gel cassette as well. The necessary current will not circulate if the tape is not removed. Place the gel in the vertical gel electrophoresis chamber and fill the chamber with buffer so that it covers the electrodes. If buffer is lacking, electricity will not be conducted and the samples will not travel. Use a micropipette to clean out each well. Draw up buffer from the rear portion of the electrophoresis chamber and pump into the well. It should look similar to an oil-slick. Pump up and down on the micropipette plunger until this dissipates. After this, inspect every well. There will be minute differences in each and the most uniformly rectangle should be chosen for sample use.

Prepare another water bath or incubator at 65°C. After the one hour incubation period add 50 µL of TBE loading dye to each vial and place the vials in the 65°C water bath for 5 minutes to eliminate any secondary structures.

Load all vials into the centrifuge so that they are all oriented in the same direction. This allows you to know where the pellet is, even if you can't see it. You will be drawing from the supernatant, so avoiding the pellet is crucial.

Centrifuge for 5 minutes at maximum speed. This will separate all of the proteins that for our sake are unnecessary and unwanted for the electrophoresis.

Remove each vial from the centrifuge and draw 20 µL from the supernatant and load into the first well. Repeat this with each vial and note which vial the samples came from.

Run the gel for about 30 minutes at maximum voltage or until your instructor deems it finished.

Remove the gel from the electrophoresis chamber and reinsert the comb at the top. The tines should be in each well as they were when the gel was removed from the packaging. Use the comb as a lever to pry open the cassette. Caution should be exercised in this for the gels are incredibly thin.

Stain the gels with Ethidium Bromide (EtBr) cards using the same procedure from previous labs.

Warning: EtBr is known to be a carcinogen. Always use gloves when handling anything that has come into contact with the EtBr.

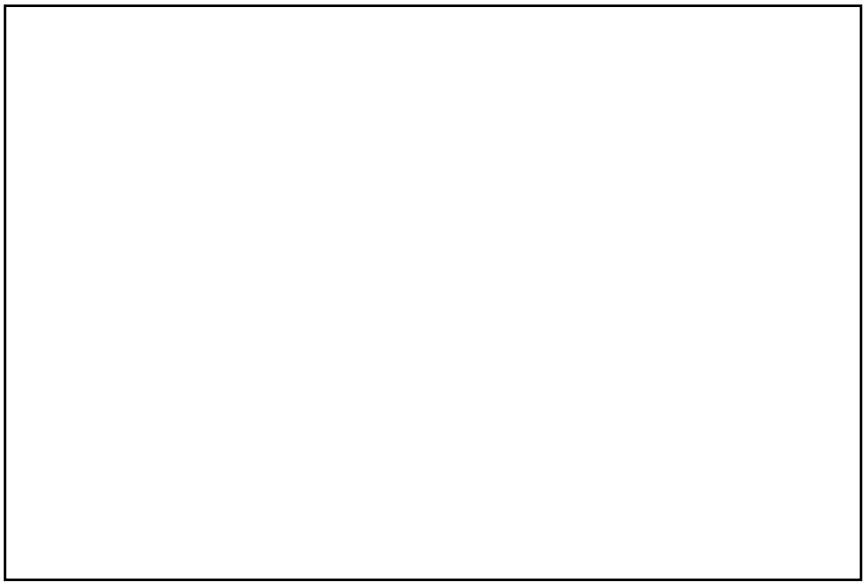
Place the gel on a UV transilluminator and roughly sketch your results in the results section.

RESULTS: IN VITRO TRANSCRIPTION

NAME:_____

SECTION:_____

1. Sketch your gel electrophoresis results here and number each lane with its corresponding vial number.



2. What indicates the amount of RNA synthesized?

3. Place an asterisk next to the lane that produced the most RNA.

4. Which lane exhibited evidence of the most transcription activity? Describe what was different about the contents of this vial and your best guess as to why it performed better than the others

5. Indicate any sources of error and/or contamination. Then list three sources of possible contamination for RNA transcription in any lab.

6. NTPs stands for Nucleotide Triphosphates. What NTPs made up the concoction you used to fuel the transcription of RNA?