James Madison University

From the SelectedWorks of Ray Enke Ph.D.

June, 2016

1st Strand cDNA synthesis from Total RNA (wet lab)

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Available at: https://works.bepress.com/raymond_enke/73/
Objective:

- Construct a cDNA library representative of total cellular messenger RNAs

1st strand cDNA synthesis from chicken total RNA

*Gloves required; clean bench with RNase Zap; use pre-PCR pipettes & filter tips

In order to measure gene-specific expression of mRNAs, we will 1st construct a more stable complementary DNA (cDNA) library of all cellular messages. To do this we will use the Bio-Rad iScript 1st strand synthesis kit to make cDNA copies of mRNAs from our total RNA extracted from embryonic chick retina & cornea. *Please use careful pipetting, these reagents are very expensive!

Reverse transcriptase (RT) is an enzyme encoded by retroviruses (such as HIV and Cauliflower Mosaic Virus) and retroviruses in our own genomes. It has the unique activity of synthesizing DNA from an RNA template. This enzyme activity has been co-opted by the biotech industry as a way to convert unstable RNAs to a more stable DNA copy for subsequent study. Today you will convert chicken mRNAs to cDNA copies using RT. We will use these cDNAs in subsequent labs to assay the abundance of specific cDNAs during retinal development.

Use the following embryonic chicken RNAs to set up cDNA reactions in a labeled PCR strip tube (group #, date, cDNA). Please note there are 2 recipes for groups working with E18 cornea and groups working with E8 retina. All RNAs are set to 26.7 ng/µL in strip tubes labeled “chick RNA”. Add 400 ng of each total RNA (15 µL) to a new strip tube labeled “cDNA, group#, date” on the side and #1-8 on the top. Here’s the RNA you’re pipetting into each cDNA tube:
Samples #1-3 & 5-7 will contain RT enzyme and a 5X enzyme buffer containing dNTPs and a poly dT primer to start the RT reaction.

Here’s the **RT reaction master mix:**

<table>
<thead>
<tr>
<th>per reaction</th>
<th>ingredient</th>
<th>X7</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 uL</td>
<td>5X buffer</td>
<td>28 uL</td>
</tr>
<tr>
<td>1 uL</td>
<td>RT</td>
<td>7 uL</td>
</tr>
</tbody>
</table>

Add 5 uL of RT mix to 15 uL RNA in tubes #1-3 & #5-7 and keep on ice.

Samples #4 & 8 will be a no RT control. These reactions will have all of the RNA template, primers, buffer, dNTPs without the RT enzyme. This will serve as an important control for subsequent cDNA quantitative PCR to ensure that trace amounts of contaminating DNA are not being amplified by your cDNA-specific primers.

Here’s the **no RT mix:**

<table>
<thead>
<tr>
<th>per reaction</th>
<th>ingredient</th>
<th>X3</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 uL</td>
<td>5X buffer</td>
<td>12 uL</td>
</tr>
<tr>
<td>1 uL</td>
<td>H2O</td>
<td>3 uL</td>
</tr>
</tbody>
</table>

Add 5 uL of no RT mix to 15 uL RNA in tubes #4 & 8 and keep on ice.

Using the PCR thermal cycler, run the reaction through the following temperatures ("iScript cDNA" program):

1. 5 min at 25C (primer annealing)
2. 30 min at 42C (RT synthesis)
3. 5 min at 85C (heat denaturation of RT enzyme)
4. Hold at 4C (cDNA storage)

cDNAs can be stored long term at -20C. The synthesized cDNAs are a collection of sequences complimentary to all of the mRNAs present in the total RNA extracted from each tissue. In subsequent labs we will use 2 uL of each cDNA reaction for quantitative gene-specific PCR (qPCR) of interesting candidate genes.