Protocol for Producing cDNA from RNA

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library(tinytex)
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## Warning: package 'tinytex' was built under R version 4.1.3
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Background

For each RNA sample, it is recommended to produce cDNA in duplicate or triplicate to guard against potential contamination or pipetting errors.

Supplies Needed

- RNA samples (keep on ice until using)
- Sigma water
- oligo dT primer
- Thermal cycler
- MMLV buffer
- dNTP
- MMLV reverse transcriptase

Protocol

Step 1: Determine your dilution

When preparing your sample, aim for $100ng$ of RNA in 18 μl of H_2O. If encountering errors, can use the specific RNA concentration for the sample. However generally, using the average RNA concentration for all samples prepped is close enough.

Ex: If qubit scores average 100 $\frac{ng}{\mu l}$, within each tube you will add 1 μl of RNA to 17 μl of sigma H_2O.

If qubit scores average 50 $\frac{ng}{\mu l}$, within each tube you will add 2 μl of RNA to 16 μl of sigma H_2O

Or, to describe it as a formula,

$$RNA(\mu l) = \frac{100ng}{qubitscore(\frac{ng}{\mu l})}$$

$$sigmaH_2O(\mu l) = 18\mu l - \frac{100(ng)}{qubitscore(\frac{ng}{\mu l})} -$$

Step 2: Dilute, Add Primer, Incubate

- ☐ Label 0.5ml tubes with RNA sample ID (ex. 001) and a duplicate indicator (ex. a, b, c)
- ☐ Add sigma water to each 0.5ml tube (quantity determined as above - if RNA is at 50 $\frac{ng}{\mu l}$, add 16 μl sigma water)
- ☐ Add a quantity of RNA sample to its corresponding 0.5ml tubes (quantity determined as above, if RNA is at 50 $\frac{ng}{\mu l}$, add 2μl sample)
- ☐ Add 1 μl of oligo dT primer to each 0.5ml tube
- ☐ Incubate at 70°C for 5 minutes

Step 3: Create cDNA

Your master mix will consist of the following components, numbered by the number of sample vials you are working with (plus a few extra so you don't run out of master mix).

- 5 μl MMLV buffer
- 1.25 μl dNTP
- 0.5 μl MMLV reverse transcriptase

Ex: If you had 18 samples and wanted to prepare master mix for 20, your master mix would be 100 μl MMLV buffer, 25 μl dNTP, and 10 μl MMLV

- ☐ Calculate the amounts required for your master mix
- ☐ Create your master mix in a tube
- ☐ Add 6.75 μl of master mix to each sample vial
- ☐ Incubate at 42°C for 1 hour
- ☐ Incubate at 95°C for 3 minutes
- ☐ Store frozen at -20°C or below

Method Narrative

We took 11 extracted gill-tissue RNA samples from each of treatments A (chronic 18C) and B (variable cycling) and 10 extracted RNA samples from the control group C ()

Detailed Narrative

Batch 1

Date: 05/31/22

Lab work: Aidan Coyle and Sarah Tanja

Number of samples completed: 18

Duplicates per sample: 2

Summary: Completed 18 samples in duplicate,and labeled the duplicates a & b Not all samples had qubit scores, so we used the qubit scores generated by Chris Mantegna as a subsample. They ranged from 4.8 $\frac{ng}{\mu l}$ to 200 $\frac{ng}{\mu l}$ and averaged 52 $\frac{ng}{\mu l}$ of RNA. To get 100ng of RNA, we added 2μl of RNA extract to 16μl of sigma water.

Due to an error, added 2μl of oligo dT primer instead of 1μl!

Batch 2

Date: 06/01/22

Lab work: Sarah Tanja

Number of samples completed: 14

Duplicates per sample: 2

Incubated for 10min instead of 5 at 70°C :(

Master Mix: 5μl buffer * 30 = 150μl 1.25μl dNTP * 28 = 37.5μl 0.5μl MMLV *28 = 15μl

Equipment

All incubation was performed with a Peltier Thermal Cycler (PTC-200)

Samples were stored in at -20°C