II. cDNA synthesis for PCR

*If RNA yield is below 30 ug, use all of it

Using the AMBION and FERMENTAS KIT

Quantify –We need 0.1 - 1μg RNA for cDNA.

a) If using our BioRad Spectrophotometer:

1. Add 498 uL of UP (ultra pure) water into a microcentrifuge tube
2. Add 2 uL of RNA into the same tube
3. In another microcentrifuge tube, add 498 uL of UP water and 2 uL of RNAse free water (This is your blank)
4. Make sure that you mix well or vortex tubes to disperse RNA
5. Clean the cuvette and add the blank solution
6. Do the same for RNA and print the report
    a. If RNA is below 30 ug = problem
7. Store RNA in the -80 C freezer

b) We can walk to the Science Building and use the nanodrop

Materials –

1. Prepare an ice bath, or take a frozen rack out of the freezer
2. Random hexamer
3. UP Water
4. 5x rxn Buffer (keep on ice)
5. RNAse Inhibitor (keep on ice)
6. dNTP 10 mM
7. Reverse transcriptase (keep on ice)

Methods –

1. Do all procedures under UVP using DNA/RNA free tips, pipettes, and microcentrifuge tubes (also using RNAse free spray on gloves)
2. Use the Excel program to determine the ratio at which you use RNA to water
    a. With > 30 ug of RNA → 10 uL RNA with 2 uL Water (switch if <30 ug)
3. Add RNA and Water
4. Add 1 uL of Random Hexamer (used to prime synthesis)
5. Skip the following step (these steps are however stated in the directions)
    a. Mix, spin
    b. 70°C for 5 minutes
    c. Chill on ice
    d. Spin down
6. Add the next in order
    a. 4 uL <u>5x rxn buffer</u>
    b. 1 uL RNAse inhibitor
    c. 2 uL dNTP 10mM
7. Skip this step (stated in directions)
    a. 25°C for 5 minutes
8. Add 2 uL of Reverse Transcriptase (The total volume now is 20 uL)
9. Spin down

Protocol on Minicycler

1. Place sample and control in center (if the bonnet misplaced, reset tubes or add tubes in the corners)
2. Place heating block over it
3. Run program → FERM-RT
    a. 25°C for 10 min
    b. 37°C for 60 min
    c. 70°C for 10 min
    d. 4°C store
4. Take samples out and place at 4°C

PCR

1. add 1 µl cDNA into new tubes
2. 1 µl Primer (F)
3. 1 µl Primer (R)
4. 10 µl Mastermix
5. 7 µl water

Total volume 20 µl

6. vortex and spin
7. put in mini cycler

Program actin

- Once reagents are used store on ice
- Always try the house keeping gene first
- make sure you write down the program you are using and the PCR machine