cDNA synthesis

Components needed:

SuperScript® III Reverse Transcriptase Kit (invitrogen)

dNTP mix 10mM (BioLine)

Random Primers (Invitrogen)

Nuclease Free Water

Method

- [ ] 1. Quantify RNA after DNase treatment -> normalise samples to = lowest [RNA] in 10ul
- [ ] 2. Turn on PCR machine and warm to 64°C for step 5
- [ ] 3. Add the following to a 200µl tube

Component	Amount (µl)
DNase treated RNA	10
dNTPs (10mM mix)	1
Random primers	1
Nucl. Free water	1

- [ ] 4. Vortex and briefly spin down to collect drops
- [ ] 5. Heat mixture to 64°C for 5 minutes
- [ ] 6. Incubate on ice for ~ 2mins
- [ ] 7. Add the following components to the same tube

Component	Amount (µl)
5 x first strand buffer	4
0.1M DTT	1
SuperScript enzyme	1

- [ ] 8. Mix gently by pipetting up and down
- [ ] 9. Incubate at 25°C for 5 minutes (random primers anneal)
- [ ] 10. Incubate at 50°C for 50 minutes (Elongation)
- [ ] 11. Incubate at 70°C for 15 minutes (Inactivation of reaction)

12. cDNA can now be used as a template for PCR amplification.