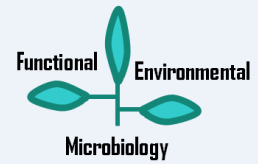


cDNA synthesis



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.