

cDNA Synthesis for qPCR

Setup the following in a 1.5ml Eppendorf tube:

- 1µl random hexamer oligo (100ng/µl, Invitrogen 48190-011)
- 1µl dNTPs (10mM each)
- 3µg RNA
- DEPC H₂O to 13µl

- Incubate at 65°C for 5'
- Incubate on ice for at least 1'
- Spin down briefly
- Add 4µl 5X First Strand Buffer (Invitrogen Superscript III kit 18080-044), 1µl 0.1M DTT, 1µl RNasin
- Add 1µl Superscript III (Invitrogen 18080-044) to +RT reactions
- Add 1µl DEPC H₂O to -RT reaction.
- Incubate 5' @ RT (for random primers)
- Incubate 1hr @ 50°C
- Stop reaction by incubating 15' @ 70°C
- Spin down and store at -20°C until ready to use for RT-PCR