# RT-PCR

Controls: Water control with no RNA
RT reaction with no RNA
RT reaction with no RT
RT reaction with non DNAase treated RNA

## DNAase treatment of RNA

Following protocol for Gibco Deoxyribonuclease I, **Amplification grade**
Most reagents are supplied with the enzyme (except oligo dT and dNTPs). Use 2 μg of total RNA. Concentration determined by $OD_{260}$.

1. Set up reaction as follows:

   1.5 μl 10x DNAase I reaction buffer
   1.5 μl DNAase (1u/μl)
   RNA plus DEPC treated water to 12 μl

   Incubate tubes for 15' at room temperature.

2. Inactivate DNAase by addition of 1.5 μl 25mM EDTA. Heat 10' at 65°C.

3. Run 3 μl on a gel to check for RNA integrity and absence of DNA

## First strand cDNA synthesis

Re-quantify DNAase treated RNA by measuring $OD_{260}$. Use equal amounts of RNA in the RT reaction (usually 1-2 μg).

1. Mix DNAase treated RNA from above with DEPC $H_2O$ to a final volume of 15 μl. Add 0.5 μl 10μM primer.

2. Heat mixture to 70°C 10'. Snap cool on ice and collect contents of the tube by brief centrifugation.

3. Make up RT mix (enough for 5 reactions – 4 controls plus sample):

   | | |
   |---|---|
   | 25 μl | 5 x RT buffer |
   | 10 μl | 0.1 M DTT |
   | 5 μl | 10 mM dNTP mix |
   | 1.25 μl | RNAsin |
   | 5μl | Superscript II |

   Mix gently. Aliquot 9.5 μl to each RNA sample in 0.2ml PCR tubes.

4. Place in PCR machine and cycle – 18°C 5′
42°C 90′
50°C 10′
70°C 10′

For no RT control, make RT mix with $H_2O$ instead of enzyme. Use as above.

## PCR reaction

1. Set up PCR reaction as follows:

| | |
|---|---|
| 2 μl | RT reaction (from above) |
| 5 μl | 10 X PCR buffer |
| 4 μl | 25 mM $MgCl_2$ |
| 1 μl | 10 mM dNTP's |
| 1 μl | Primer 1 (10μM) |
| 1 μl | Primer 2 (10μM) |
| 0.5 μl | Taq DNA Polymerase |
| 35.5 μl | $dH_2O$ |
| 50 μl | TOTAL |

2. Cycle as follows:

| | |
|---|---|
| 94°C (2′) | X 1 |
| 94°C (30s), 55°C (30s), 72°C (2′) | X 34 |
| 72°C (5′) | X 1 |
| cool to 4°C. | |

25 μl of the final PCR product should be run out on a 1% agarose gel, viewed and blotted for band verification by hybridization. The remaining 25 μl of the PCR reaction can then be used, if positives are forthcoming, to clone and sequence the band of interest.