3' RACE (RAPID AMPLIFICATION OF cDNA ENDS)

First strand synthesis

The first stage in this process is the production of a population of cDNA's, each with a known 'anchor primer' sequence incoporated at the 3' end, which reflect the original population of RNA. You will need 5 μg total RNA to start with. This protocol is best done in 0.2 ml PCR tubes with heating stages carried out in the PCR machine.

1. Make up cDNA synthesis reaction mix and place on ice:

5 μl	5 X RT buffer
2 μl	0.1 M DTT
1 μl	10 mM dNTP mix
0.25 μl	RNAase inhibitor

2. Take 5μg of total RNA and make up to 15.25 μl with DEPC H_2O. Add 0.5 μl of oligo dT anchor primer (10μM). Heat at 70°C for 10' and then place immediately on ice.

3. Add RNA/primer mix to RT mix.

4. Add 1 μl Superscript II and place in PCR machine.

5. Cycle – 18°C 5'
   42°C 90'
   50°C 10'
   70°C 10'

PCR reaction.

The second stage is a simple PCR step to amplify specific sequences from the pool of cDNA's using a gene specific primer and an anchor primer, which anneals at the 3' end incorporated in the first stage.

1. Set up PCR reaction as follows:

2 μl	cDNA template (from stage 1)

5 μl	10 X PCR buffer
4 μl	25 mM $MgCl_2$
1 μl	10 mM dNTP's
1 μl	Primer 1 (10μM) (gene specific)
1 μl	Primer 2 (10μM) (ANCHOR)
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.