Invitrogen Superscript II Reverse Transcriptase
1st strand cDNA

- Primer

  Pd(N)6 oligos (e.g. from Invitrogen): stock comes 3 □g/□l

  dilute 1/30 in DEPC water to get 100 ng/□l working concentration

  20 □l working concentration are enough for a 100 □l RT

The following protocol is written for a 100 □l RT 1st strand cDNA reaction

- In screw-cap tube

  20 □l random primers

  30 □l RNA (e.g. directly from Amersham QuickPrep Micro mRNA Purification Kit)

  4 □l dNTPs (25 mM each)

  66 □l DEPC H2O

  □ 65 °C for 5 min, then quickly chill on ice

- collect drops by briefly spinning (6000 rpm quickspin)

  add:

  40 □l First-Strand Buffer (comes with the kit)

  20 □l 0,1 M DTT

  10 □ l RNAse Inhibitor*

  □ mix contents gently, 25°C for 2 min

- add 10□ (200u/□l) Superscript Transcriptase mix by gently pipetting up&down

  □ 25°C for 10 min

  □ 42°C for 50 min

  □ 70°C for 15 min

* e.g. Invitrogen RNAaseOUT or Amersham RNAguard