***In vitro* transcription**

For each DNA to be transcribed add the following to a 0.5ml tube:

- 600 ng DNA
- 6 µl 5x Buffer
- 0.6 µl 25mM rNTPs (0.5 mM final)
- 3 µl DTT (10 mM final)
- npH_2O (enough to bring up to 30 µl)

Remove 9.66 µl of the mix, put in separate 0.5 ml tube (no polymerase control)
To remaining mixture add 1 µl of either T7 or T3 RNA polymerase depending on vector

Incubate all tubes: 30 min at 37ºC (transcription)
10 min at 70ºC (heat inactivation of RNA polymerase)

Add 1 µl of 1:1000 dilution of RNase A to reaction (to degrade single stranded RNA)

- split reaction into two more 0.5 ml tubes
- add 1 µl of RNase H to one of the tubes (degrading RNA bound to DNA, control)

Incubate all tubes: 30 min at 37ºC

Add 10 µl of 2x SDS loading dye to each tube.

Load all samples onto 0.8% agarose gel with NO Ethidium Bromide!

Run gel at 60 volts for approx 2 hrs (dye front will run faster then DNA)

Stain 0.8% gel with EtBr in water (4 µl in 100ml H_2O or 1x TAE) for an hour - overnight