Preparing Crude DNA Extract for PCR

** For larger quantities of cells increase all amounts by appropriate fold difference**

1) Collect 200ul of actively growing cells.
2) Transfer to 0.5 or 1.7ml epp. tube.
3) Spin down 15K for 30 sec.
4) Aspirate supernatant and resuspend in 60ul 0.2x PBS.
5) Incubate at 95° C for 15 min.
6) Add 6 ul Protease K at 10mg/ml.
7) 56° C for 1 hour.
8) 95° C for 30 mins.
9) Use 5 ul for PCR.

In vitro Transcription/ Translation Protocol

1) Thaw out and mix DNA/ nuclease free water.
   (have a luc control reaction that is half the size of the sample reactions)
2) Thaw out Master mix quickly in your hand and then put on ice, add to DNA mix.
3) Add S35 to the mix and incubate at 30C for 2 hours.
4) While incubating pour an appropriate conc. SDS-Acrylamide gel.
5) For first time Transcript/Translate run 5 ul of product on gel, further times can run 2-3ul. Denature for 5 mins in 20 ul SDS loading buffer.
6) After running gel either immediately dry the gel or follow the Autofluor protocol outlined below.
7) Expose gel to IP plate over night.

Autofluor/SDS-PAGE Gel Fixation Protocol

1) After electrophoresis, fix gel in 5% glacial acetic acid, 5% 2-propanol, 90% dH2O (approx. 20 ml) for 15-20 mins.
2) Decant and wash under continuously flowing tap water for 15 mins.
3) Cover with Autofluor/ tin foil and rock @ RT for 30 min / 1 of gel thickness.
4) Dry immediately for 30 mins at 80C.