Procedure for Mobility Gel Electrophoresis

Prepare Linearized Plasmid DNA

1. Purified pUC18 plasmid is incubated with Sma1 enzyme and react 4 buffer:
   - 1 unit of Sma1 cleaves 1ug of DNA, however it is recommended (Quiagen Handbook) that for long plasmids (pUC18 ~ 2600 bps) a 10 x excess be used or 10 Units Sma1 / 1ug DNA
   - [Sma1 Stock] = 10 units/uL
   - [pUC18] (purified) ~ 10 ug/ 25 uL

   Requirements :
   - No more than 10 ug of DNA can be loaded onto the Sma1 purification columns
   - Sma1 is added to solution last- keeping on ice as long as possible
   - Mix samples well
   - React 4 buffer needs to be 10% of final sample volume (10uL of react → V_{final} = 100 uL)
   - Fill to V_{final} with DI H_2O (100 uL – 25 uL DNA – 10 uL React 4 – 10 uL Sma1 = 55 uL H_2O)
2. Heat 1 hour at 30^{o}C (activating enzyme) followed by 10 min at 65^{o}C (deactivating enzyme)
3. Follow with the gel extraction kit (it doubles as an enzymatic clean-up kit)
4. Check conc. Of DNA by abs at λ = 260 nm (ε = 6600 $M^{-1}cm^{-1}$)

Check Linearization by Running a Test Gel

Gel Conditions

1. .75% agarose gel
2. TBE buffer (pH = 8.3)
3. 50 uM DNA
4. 10 mM Sodium Phosphate buffer
5. 10-500 uM complex (varies depending on complex)
6. 20 uL samples will fit in the wells produced by the 8 lane comb – fill to 20 uL with DI H_2O
7. 4 uL of loading buffer are added just before loading the samples into the gel
8. Run ~90 min at 95V
9. EtBr stain AFTER running gel- for up to 24 hrs (soak in H_2O with 0.5 ug/mL EtBr)
10. image the gel the same way imaged for photocleavage gels