Howard Judelson's modification of colony disruption assay

I. In my experience, 1 mm diameter colonies are more than enough for pUC-based plasmids. I have obtained good results using both fresh colonies and week-old colonies (stored at lC).

2. Number colonies on the back of the plate.

3. Fill the wells of a microtiter plate with 40 µl of cracking buffer: 50 mM NaOH, 0.5% SDS, 5 mM EDTA.

4. Touch a flat toothpick to the colony to lift off the bacteria. 99% of the cells should lift off easily if you }ust raise the toothpick straight up.

4. Stick the toothpick into the well containing cracking buffer and swirl to remove cells. I find it best to do things in batches: just stick (without swirling) the p'ick into the well, do the same with 10 to 20 more colonies, and then go back to the beginning and swirl the picks to dislodge the cells. This waiting approach seems to help dislodge the cells, but don't wait too long or the toothpick might absorb a lot of the liquid!

5. Cover the plate with tape (wide packing tape is the best) and rub the tape to e:nsure a good seal. Then place the plate at 55C to 65C for 45 to 60 minutes. Then place the bacterial culture plates back at 37C to make the colonies visible to- help you pick desired colonies later.

6. Prepare a 0.7% agarose gel. I normally set up combs 4 to 6 cm apart, depending on the application; you don't have to run the ge-ls very far to resolve differences in size. DO NOT COVER THE GEL WITH BUFFER.

7. Load your samples (with undigested plasmid standards in some lanes if desired) into the wells, and fill unfilled wells with buffer. If you want, you can add a little 1oading buffer with dye (bromophenolblue) to each sample. I normally load sampies straight, without dye, and add a little dye to the edge well in each comb. Loading buffer is not requ~red since the gel has not been flooded with buffer. I do this way since the samples tend to float out of the wells as you remove the pipet tip if the gel has been submerged.

8. Add buffer just until it reaches the top edge of the gel, do not submerge! Run until the dye has run 0.5 to 1 cm, then submerge the gel. Run the gel until the dye has gone about 4 to 6 cm. You can run the gel fast; I typically run it at 6 volt/cm (150 V in TBE with a 25 cm apparatus). If you want to go even faster, it runs well also at 12 volt/cm in l/3 X TBE (300V).

9. Stain gel as usual to visualize bands. Sometimes good destaining is needed to see faint bands. Every lane will have the same chromosomal DNA band, some RNA, and the plasmid band.