PLASMID MINISCREEN PROTOCOL (BOIL PREP)

This method can also be used to prepare miniprep DNA which can be transcribed and translated. However it is much longer than the alkaline lysis/ammonium acetate method and gives similar quality DNA.

1. Pick colonies to be screened into 2 ml broth containing appropriate antibiotic. Grow with vigorous shaking at 37C until saturated, typically 8 hrs. Once grown to saturation, cells can sit around in cold room for days to weeks before screening, but be sure to label them clearly so you will remember exactly which set of miniscreens they are!
2. Chill cells (and while they are chilling put pressure cooker to high heat with enough water to cover the bottom 1/3 of tubes in a rack), transfer to microfuge tube (save residual cells in tube to be used as starter for large prep of positives after screening) and spin in microfuge for 20 seconds.
3. Suck off supernate, resuspend cells by vortex in 200 ul buffer A, add 10 ul buffer B, mix well and leave on ice for 10 min.
4. Add 20 ul buffer C, mix well and
5. Boil exactly one min. We recommend using the pressure cooker brought to a vigorous boil with enough water to be able to cover the bottom 1/3 of the tubes. Remove the top of the cooker, and place the double rack containing samples only in the lower one -- the upper rack is to keep the lower rack's tubes from popping -- into the boiling bath, watching timer. Using rubber gloves reach in, take out the set of two racks and as quickly as you can, transfer the tubes to an ice bath to quick chill. Leave them on ice at least 10 mins then
6. Spin in microfuge 15 mins. Discard the viscous pellet by sucking it out carefully with a Pasteur pipet, being careful not to lose supernate.
7. Add equal volume of 7.5M NH4OAC. EtOH precipitate supernatant. Resuspend in 20uL TE.
8. Add 200 ul TE saturated, re-distilled, pH equilibrated phenol and vortex vigorously. Spin in microfuge 15 secs.

   NOTE: **PHENOL IS AN ORGANIC ACID AND WILL CAUSE SEVERE BURNS; YOU MUST WEAR A LAB COAT, EYE PROTECTION, AND GLOVES. READ THE MSDS SHEET BEFORE HANDLING. YOU SHOULD ALSO WORK IN THE FUMEHOOD.**
9. Remove and discard the lower phase with a Pasteur pipet carefully so as not to lose aqueous phase as long as you wipe the outside of the Pasteur pipet tip, and are careful to allow flow only in one direction, i.e. sucking out of the tube, and if you wipe off the Pasteur pipet with a fresh kimwipe, you can reuse the same Pasteur pipet to remove all of the organic phases.
10. Re-extract aqueous phase with chloroform and discard organic (lower) phase, by transferring the upper phase to a new tube.

    NOTE: **CHLOROFORM IS POISONOUS. WEAR LAB COAT, EYE**

PROTECTION AND GLOVES AND WORK IN THE FUME HOOD. READ THE MSDS SHEET BEFORE HANDLING.

11. Add 20 ul 5 M NH4OAc, vortex, and add 500 ul (2« vol) 100% ethanol. Mix well and spin down ethanol precipitate, suck off supernate with a pulled Pasteur pipet and wash with 70% ethanol (500 ul), repeat spin, suck off carefully and completely and air dry at 37C.

    NOTE: **ETHANOL IS HIGHLY FLAMMABLE; USE EXTREME CAUTION WHEN HANDLING LARGE QUANTITIES NEAR A FLAME.**

12. Dissolve pellets in 20 ul TE (no RNase), vortex, spin down for 1 sec in microfuge and they are ready to map either by restriction endonucleases or transcription-translation and immunoprecipitation.

Buffer A 50 mM Tris pH 8 / 20 mM EDTA

Buffer B 4 mg/ml lysozyme in 50 mM Tris pH 8 (prepared fresh daily)

Buffer C 5% Triton X-100 / 40 mM EDTA / 50 mM Tris pH 8