

96-well DNA Extraction

Method:

1. Add 50 μ l of Lysis Buffer to each well.
2. Dampen some paper towels and place in the bottom of a tupperware container.
3. Place the plates on the paper towels and seal the lid of the tupperware container.
4. Incubate the plates (with lids on) in this sealed humid container in a 60° C incubator for 2-3 hours.
5. Add 150 μ l of Precipitation Buffer to each well; leave at RT for 30 min to allow the DNA to precipitate.
6. Decant the supernatant—be extremely cautious with this step because it is easy to lose DNA pellets if you don't invert the plate very slowly. In addition, don't let the paper towels touch the wells, otherwise the DNA will be sucked onto the paper towels! As an alternative which is safer, you can transfer the contents of each well into individual eppendorf tubes and continue the prep from there.
7. Wash the precipitated DNA with 150 μ l of Wash Buffer 3 times. Decant.
8. Let the pellets dry by leaving the plate on the bench (with the lid off or the eppy tubes open).
9. Resuspend the DNA with 30 μ l of Resuspension Buffer. Incubate o/n in the 37° C incubator (with the lid on or the tubes closed to prevent evaporation).

** The expected yield of DNA from each plate well is 10 μ g. The DNA from one plate will be sufficient to perform 1 southern blot (10 μ g per gel well) or several PCR reactions. With the duplicate plates we provide, you will have enough DNA to analyze both the 5' and 3' ends of your clones.**

10. If you need to run a PCR reaction, you may use 1-2 μ l of DNA from step 9. If you need to perform a Southern blot, continue to step 11.
11. Make up 10 μ l per sample of digest cocktail containing:
 - restriction enzyme
 - restriction enzyme buffer
 - BSA (if required by enzyme)
 - spermidine (to a final concentration of 1 mM; stored in -80° C freezer)
12. Add that 10 μ l to the 30 μ l DNA from step 9 for a final volume of 40 μ l.
13. Digest o/n @ 37° C (with the lid on or the tubes closed).
14. Electrophorese the digested DNA on an agarose gel. Do not exceed 80V.