# Single Stranded dut ung DNA Prep

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This method is for plasmids containing the phage F1 DNA replication origin that can be made single straded by infection of cells with helper phage.

Transform the plasmid to be mutagenized into strain CJ236 (dut- ung- ). Patch several transformants to YT amp +chloramphenicol (30 micrograms/ml; make fresh chloramphenicol stock at 3 mg/ml in ethanol) plates and grow >8 hr @37 degrees. Use a blob of these patched cells to start an overnight culture in YT amp + chloramphenicol. It is best to make 2-3 single stranded preps in parallel as the cells do not always grow.

Inoculate 0.5 ml O/N to 35 ml same media. Grow 3-4 hrs at 37 deg til A600 ~0.5. Add 0.5 ml 4 mg/ml uridine (50 mg/ml final). Grow till A600 ~ 1.0 ( ~ 45 min). Add 30 microliter K07 helper phage (our current phage stock is ~3 x 1011 pfu/ml). Incubate 1 hr at 37 degrees with gentle shaking.

Add kanomycin (0.7 ml of 3 mg/ml) to select for cells infected by phage and incubate 8 hrs to overnight (not longer than ~14 hr).

Spin cells 10,000 rpm for 10 min and remove sup. Re spin the supernatant for 10 min to remove any remaining E. coli. To 20 ml sup. add 5 ml 20% PEG 8000/2.5 M NaCl. Put on ice or at 4 degrees for at least 2 hr (can leave up to 36 hr. at 4o but not longer).

Spin out phage at 5,000 rpm for 10 min (SS34) and remove sup. A visible pellet should be seen, otherwise the phage prep did not work (note: PET plasmids give a very small pellet that requires a longer incubation with PEG to see). Re spin and remove remaining liquid taking care to remove all liquid. Resuspend by vigorous vortexing in 0.5 ml TE and transfer to 1.5 ml microfuge tube.

Extract phage suspension with equal volume phenol (no chloroform) by vortexing for 3 X 1 min with 3-5 min breaks in between vortexing (very vigorous vortexing is crucial for good phage yield). Vortex once more for good measure and spin in microfuge for 5 min. Remove aqueous phase (be careful to avoid interphase) and extract twice with an equal volume phenol/chloroform (2/1). Ethanol precipitate DNA and wash pellet with 70% ETOH and dry.

Resuspend DNA in 50-75 microliters TE, pH 7.5 and quantitate absorbance at A260. Expect 10 -20 micrograms DNA if the prep works well.

For more details, see: BioRad mutagene method handbook.