Miniprep for Isolating Transcription-quality Plasmid DNA

Generally, the cleaner the template DNA, the greater the yield of the transcription reaction. The following miniprep protocol yields high quality transcription template. This protocol is derived from a published procedure (Molecular Cloning, A Laboratory Manual), but differs in that the phenol/chloroform extraction is done after linearization of the plasmid with restriction enzyme(s) and proteinase K treatment (Step 9). In this way, any possible ribonuclease contamination from the restriction enzyme is eliminated without an additional proteinase K or phenol/chloroform extraction step. If you have difficulty getting good restriction digestion of your plasmid prep, it may be necessary to include a phenol/chloroform extraction before the ethanol precipitation at Step 5.

Required Solutions:	**Solution I**: 50 mM Glucose 10 mM EDTA, pH 8 25 mM Tris-HCl, pH 8 Autoclave for 15 min. Store at 4°C in small aliquots.	**Solution II**: 0.2 N NaOH 1% SDS Make fresh.	**Solution III** (100 ml): 60 ml 5 M potassium acetate 11.5 ml glacial acetic acid 28.5 ml water (distilled deionized) Store at room temperature.
1. Pellet cells	Centrifuge a 1.5 ml bacterial culture (grown overnight) for about 30 seconds; pour off supernatant, re-spin briefly (about 5 seconds), and remove residual supernatant via aspiration.		
2. Resuspend pellet in 110 µl Solution I, vortex	Add 110 µl of Solution I and vortex vigorously to resuspend the pellet. Check for complete resuspension of pellet by inverting the tube and confirming that the solution is homogenous.		
3. Add 220 µl Solution II, incubate 1 min on ice	Add 220 µl of Solution II and invert the tube several times to mix. Incubate the tube on ice for at least 1 min.		
4. Add 165 µl Solution III, incubate 5 min on ice, centrifuge 5 min	Add 165 µl of Solution III and vortex medium-fast for 10 seconds. Incubate the tube on ice for 5 min. Centrifuge for 5 min at maximum speed. This spin should be done at 4°C if possible. Most of the proteins, genomic DNA, and other cellular components will pellet during this spin.		
5. Add supernatant to a fresh tube with 1 ml ethanol	Add the supernatant to a fresh tube containing 1 ml of 100% ethanol and invert several times to mix. Incubate the mixture for 5 min on ice to		

incubate 5 min on ice, centrifuge 5 min	precipitate the plasmid DNA and some of the RNA. Centrifuge for 5 min at maximum speed at 4°C if possible. This will pellet the plasmid DNA. Pour off the supernatant, centrifuge briefly, and aspirate off any residual supernatant.
6. Resuspend in ~50 µl TE containing RNase, incubate 5 min at 37°C	Resuspend the DNA pellet in ~50 µl TE (10 mM Tris HCl, pH 8 and 1 mM EDTA). Add 0.5 U or 1 µg RNase A or use 1 µl of Ambion□s RNase Cocktail. Vortex vigorously, incubate about 5 minutes at 37□42°C, and re-vortex to thoroughly solubilize the pellet.
7. Digest with appropriate restriction enzyme	Digest with an enzyme that will linearize the plasmid so that the polymerase promoter site will be upstream of the sequence you want to transcribe. The volume of the restriction digest should be about 2□3 times the volume of plasmid DNA used. Follow the recommendations of the restriction enzyme supplier for buffer composition, units of enzyme to use, and incubation conditions.
8. Treat with Proteinase K and SDS	Add SDS to a final concentration of 0.5% (usually a 10-20% SDS stock solution is used). Add 50□100 µg/ml Proteinase K (final concentration). Mix well by inversion, and incubate at 50°C for at least 30 min.
9. Phenol/chloroform extract and ethanol precipitate	Add an equal volume of phenol/chloroform or phenol/chloroform/IAA, vortex vigorously, centrifuge ~1 min at room temp. Remove the aqueous (top) phase to fresh tube, add 1/10th volume of 5 M ammonium acetate (RNase-free), add 2 volumes ethanol, incubate at least 15 min at □20°C.
10. Pellet DNA	Pellet the DNA by centrifuging at top speed for 15 min. After the spin, discard the supernatant, re-spin briefly and remove any residual supernatant. Resuspend the DNA in 10□20 µl nuclease-free water per 1.5 ml culture. Vortex until the pellet has completely dissolved.
11. Gel analysis	Assess the DNA by running an aliquot on an agarose gel in the presence of ethidium bromide. Estimate the concentration of the DNA by comparison to a known quantity of similar-sized DNA run on the same gel.