Purification of Plasmid DNA by precipitation with PEG 10000

1. Pour 250 ml of bacterial culture into a centrifuge tube suitable for Kubota AG-2506 rotor or equivalent; centrifuge the bacteria at 8,000rpm for 6 min at 4^0C in a Kubota 6500 centrifuge or corresponding centrifuge.
2. Drain off the medium, leaving the bacterial pellet as dry as possible.
3. Resuspend the bacterial pellet with 5 ml of Solution I and add 50 µl (100 mg/ml) of DNase-free RNaseA, then transfer the bacterial suspension into 50-ml Nalgene high speed centrifuge tube. (Solution I: 50 mM Tris-C1, pH8.0; 10 mM EDTA, pH8.0)
4. Add 10 ml of freshly prepared Solution II & gently mix, then incubate at RT for 4 min. (Solution II: 0.2N NaOH, 1% SDS)
5. Add 200 µl of Alkaline Protease Solution (Promega, Catalog# A1441) & gently invert the tube up and down for several times to mix protease solution, and then incubate at RT for 4 min.
6. Add 7.5 ml of ice-cold Solution III & gently mix, then incubate on ice for 5 to 10 min. (Solution III: 60 ml 5M K-Acetate, 11.5 ml glacial acetic acid, 28.5 ml water)
7. Centrifuge at 15,000rpm for 20-30 min at 4^0C in a Kubota AG-508R rotor.
8. Transfer the supernatant to a fresh 50-ml Nalgene high speed centrifuge tube. Add 12.5 ml of isopropanol, mix well.
9. Recover plasmid DNA by centrifugation at 12,000rpm for 5-10 min at 4^0C in a Kubota AG-508R rotor. Rinse the pellet with 70% EtOH & remove the residual EtOH as much as possible (This step can be skipped).
10. Resuspend the pellet with 0.5 ml of autoclaved 0.1X TE buffer.
11. Remove undissolved material by centrifugation at 12,000rpm for 10 min at 4^0C in a microfuge.
12. Transfer supernatant to 1.5 ml eppendorf tube and add 2 µl (100 mg/ml) of DNase-free RNaseA and incubate at RT for 5 min.
13. Extract DNA solution once with equal volume of phenol/chloroform, and twice with equal volume of chloroform. (it is extremely important to remove residual phenol.)
14. Add equal volume of 1.6 M NaC1 containing 13% (W/V) polyethylene glycol 10,000 (PEG 10,000) or PEG 8,000 to the aqueous solution purified from step 13. Mix well and incubate on ice for 10-30 min, then recover the plasmid DNA by centrifugation at 12,000rpm for 3 min at RT in a microfuge.
15. Drain off supernatant and remove residual PEG solution as completely as possible.
16. Dissolve the pellet in 500µl of autoclaved 0.1X TE. Extract DNA with phenol/chloroform till interface is clean and finally extract once with chloroform.
17. Add 1/5 volume of 10M ammonia-acetate to the aqueous solution and two volumes of absolute EtOH, mix well and incubate the tube on ice for 5-10 min.
18. Recover the plasmid DNA by centrifugation at 12,000rpm for 3-5 min in a microfuge. Wash the DNA once with 70% EtOH, remove the residual EtOH as much as possible, and dry DNA pellet inside the hood or bio-safety cabinet under blowing condition.
19. Dissolve the DNA with appropriate volume of autoclaved 0.1X TE and determine the DNA concentration by spectrophotometry.
20. Check DNA quality/integrity according to the protocol as follow:

Protocol

1. 每個待測 DNA 取 500ng 於最終體積為 10 至 12 µl 的 1X loading buffer 中。
2. 將上之 DNA 樣本置於 0.5% TBE agarose gel (含 0.1µg/ml 的 EtBr) 孔內 (騰達行 SeaKem LE agarose; 迷你水平電泳槽)。
3. 以 50 伏特電泳兩小時 (0.5X TBE 緩衝液含 0.1µg/ml 的 EtBr) 此可觀察 plasmid DNA 是否有斷裂現象(nick)或受染色體 DNA 污染。
4. 關閉電源，將同樣且等量的 DNA 樣本 (同 1) 置於旁邊空孔內。
5. 再以 50 伏特繼續電泳半小時 此可觀察 plasmid DNA 是否有殘留 RNA。

範例

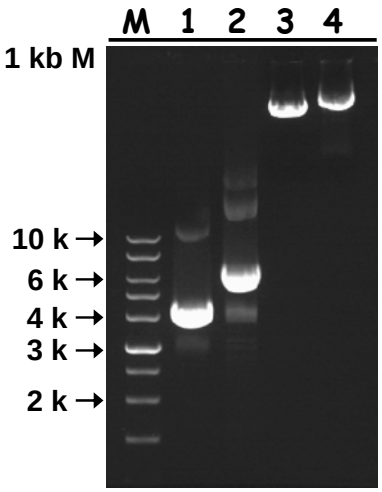

Figure legend: 500ng of TRC shRNA plasmid and pLKO_AS3w.hyg cDNA expression vector (AS series plasmids) with the sizes of 7085 bp (lane 1) and 9634 bp (lane 2) respectively, were subjected to electrophoresis (0.5% agarose gel containing 0.1µg/ml of EtBr, run at 50 volts in 0.5X TBE running buffer containing 0.1µg/ml of EtBr) for 2 hours, and then the equal amounts of these two plasmid DNAs are loaded into lane 3 and 4, and ran for another 30-min. Please note that the sizes of the major band of lane 1 and lane 2 are smaller than 7085 bp and 9634 bp, indicating that those DNAs are supercoiled form DNAs in nature. In addition, there are no other signals detected in lane 3 and lane 4 except major band (DNA), suggesting that there are no RNA contaminations in DNA preparation.

Note:

1. Gently extract plasmid DNA with phenol/chloroform by inverting the tube up and down rather than vigorously vortex.
2. The use of alkaline protease and autoclaved 0.1 X TE ensures the integrity of plasmid DNA throughout the purification procedure.