Standard Operation Procedure (S.O.P.) for Plasmid Purification using MINIprep Kit

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This S.O.P. will direct you in the procedure of plasmid purification through the QIAGEN QIAprep Spin Miniprep Kit (cat. No. 27104). The afore mentioned method provides a high yield of ultrapure super coiled plasmid DNA useful for transfection, in vitro transcription and translation, enzymatic modifications and digestion, sequencing, ligation and transformation, among others. This kit allows purification of high-copy plasmids up to 20 µg from a 1–5 mL overnight culture. Special considerations must be taken in account when purifying low-copy vectors and cosmids, large plasmids (>10 kb), and DNA prepared using other methods.

Different factors could affect plasmid purification, including

- Plasmid size: larger plasmids reduce elution efficiency.
- Host strain: certain strains inhibit enzyme activities and yield lower quality DNA
- Culture volume: larger culture volumes will lead to reduced purity.
- Environment salt levels
- pH levels, especially in the elution step.

Important:

- ***Bacterial cell culture should be no larger than 5 ml.***
- ***Do not shake the buffers vigorously.***
- ***Check RNase A and LyseBlue was added to Buffer P1 (a box in the cap should be checked). Buffer P1 has a life span of 1 year after RNase A addition.***
- ***Check Buffer P2 and N3 for salt precipitation. If that is the case, preheat the buffers at 37 °C.***
- ***The N3 and PB buffers contain guanidine HCl, which can form highly reactive compounds with bleach.***
- ***Check ethanol (96–100%) was added to Buffer PE (a box in the cap should be checked).***
- ***Close the buffer bottles immediately after use to avoid acidification by CO_2.***
- ***The QIAprep 2.0 Spin Column is made up of two parts: the column (blue) and the recollection tube (transparent).***
- ***Elution of DNA can be carried by water or Buffer EB (Tris·Cl, pH 8.5). Avoid using Tris–EDTA (TE) buffers if the purpose of the purification is not long-term storage. If the DNA will be further employed in enzymatic reactions is preferrable to elute in water. If water is use, store at –20 °C (preferably at –80 °C) if no immediate use is expected.***
- ***All tips and microtubes must be autoclaved.***
- ***All volumes are given on a per culture tube basis.***

Materials:

- 70% Alcohol
- Waste Container with Detergent (1% Alconox, stored at room temperature)
- Waste Container with Concentrated Bleach (2% Sodium Hypochlorite)
- Inoculation Loops
- 5 mL Micropipette and Tips (in case there is no 5 mL micropipette, you can use 5 mL serological pipets)
- Micropipettes and Tips for 100 and 1000 µL

- 14 mL Round-Bottom Culture Tube (Falcon 352059 or 352018)
- 1.5 mL Microtubes
- Miller LB Media with the Appropriate Antibiotics and Chemicals (1% Tryptone, 0.5% Yeast Extract, 1% NaCl)
- Buffer P1 (stored at 4 °C)
- Buffer P2 (stored at room temperature)
- Buffer N3 (stored at room temperature)
- Buffer PB (stored at room temperature)
- Buffer PE (stored at room temperature)
- Buffer EB or Nuclease-free Water (stored at room temperature)
- QIAprep 2.0 Spin Column (column + recollection tube)
- Incubator with Orbital Shaker at 37 °C and 225 r.p.m.
- Centrifuge for 14 mL Culture Tubes (preferably a refrigerated centrifuge)
- Microcentrifuge for 1.5 mL Microtubes
- Vortex

Procedure:

<u>*Day Before*</u>:

a) The working area should be sanitized using 70% ethanol prior to placing any materials on the bench.

b) Prepare the waste container with 2% bleach for decontamination. ***Any material that is in contact with intact bacteria must be decontaminated in this container for at least 30 minutes***.

c) Determine how many culture tubes will be purified and label the tubes properly. In each culture tube (14 mL tube), add 5 mL of LB media containing the appropriate antibiotic and chemicals and inoculate the media with the target bacteria from an agar plate or glycerol stock using an inoculation loop. *If you are using agar plates, grab the inoculation loop touching it the less possible and submerge the inoculation loop into the media, being careful not to touch the tube walls. Agitate the media with the tip for few seconds and cut the inoculation loop with a scissor, leaving one third of the loop inside of the culture tube*. ***If the source of the bacteria is cold, use cold media during inoculation. If the source is at 37 °C, pre-warm the media to the same temperature.***

d) Incubate the culture for ~12–16 hours (preferably 16 hours) in an orbital shaker incubator at 37 °C, 225 r.p.m. The samples should look turbid, indicating bacterial growth.

e) Carefully dispose the bleach solution into the sink and discard the materials in a biological waste (trash can with red bag).

f) The working area and any material should be sanitized using 70% ethanol at the end.

<u>*Purification Process*</u>:

a) The working area should be sanitized using 70% ethanol prior to placing any materials on the bench.

b) Prepare the waste containers for decontamination: one with bleach and the other one with alconox. ***Any material that is in contact with intact bacteria must be decontaminated in the container with bleach***.

c) Organize and label the appropriate amount of 1.5 mL microtubes to be used during the

purification process. Each culture tube must have two microtubes: one for bacterial lysis and another for the plasmid elution (***Step q***). Be sure to have the QIAprep 2.0 Spin Columns available but do not remove them from the bag until you reach ***Step j***.

d) *Optional*: If you use agar plates for inoculating the media, r

e) emove the inoculation loop from the culture tube and throw it into the waste container with bleach.

f) Centrifuge the culture tube containing the bacteria (<5 mL) at 3,000 x *g* (preferably at 4°C) for 15 minutes to harvest the bacterial cells. Use this time to place a medium piece aluminum foil on the bench and cover it with a layer of paper towel before centrifuge is done.
   - In case you are purifying one sample or an odd number of samples, fill a second centrifugation tube with 100 ml of water to balance rotor.

g) Remove the supernatant by carefully inverting the culture tube into the waste container with bleach and wait until almost all culture media has been transferred into the waste container. Place the culture tube upside down on the paper towel to get rid of final drops and allow it to dry.

h) Resuspend bacterial pellet by adding 250 µL of **Buffer P1** to culture tube and apply vortex until bacterial pellet is completely resuspended. Use micropipette to transfer the resuspended bacteria into a 1.5 mL microtube.

i) Add 250 µL of **Buffer P2** to the microtube and slowly invert it 4–6 times. The solution should turn blue if LyseBlue is present. Mixing should result in homogenously colored suspension. ***This step cannot last longer than 5 minutes***.
   - **DO NOT VORTEX.** This can result in shearing of genomic DNA.

j) Neutralize with 350 µL of **Buffer N3** to the microtube and slowly invert it 4–6 times. The suspension should produce a cloudy white precipitation, keep carefully inverting until solution has no trace of blue color.

k) Centrifuge the microtube at the fastest speed (minimum: 15,000 x *g*, maximum: 17,900 x *g*) for 10 minutes. A white pellet will be formed at the walls and bottom of the microtube.

l) During the centrifuge procedure, assemble the QIAprep 2.0 Spin Columns and verify that all microtubes and columns are properly labeled.

m) Carefully and avoiding touching the white pellet, pipet the supernatant into the column of the QIAprep 2.0 Spin Column. Centrifuge the Spin Column at the fastest speed for 1 minute.

n) Discard the liquid found in the recollection tube by disassembling the Spin Column and ***inverting the recollection tube into the waste container with alconox***. Reassemble the column with the same recollection tube.

o) <u>Optional</u>*: Perform this step when endonucleases need to be efficiently removed, especially when using endA+ bacterial strains or large culture volume, as is the case for low-copy vector*.
   - Add 500 µL of **Buffer PB** to the column and centrifuge the SPIN Column at the highest speed for 1 minute.
   - Discard the liquid found in the recollection tube into the waste container with alconox and reassemble the column with the same recollection tube.

p) Add 750 µL of **Buffer PE** to the column and centrifuge the Spin Column at the highest speed for 1 minute.

q) Discard the liquid found in the recollection tube into the waste container with alconox and reassemble the column with the same recollection tube.

r) Centrifuge the Spin Column again at the highest possible revolution for 1 minute.

s) Discard the recollection tube and assemble the column with a 1.5 ml microtube that is properly labeled, add 50 µL of **water** or **Buffer EB** into the center of the column floor and let it rest for 4 minutes.
- The decision between **water** and **Buffer EB** will depend on the subsequent use of the plasmid DNA. If you expect to perform subsequent experiments with the plasmid, it is recommendable to use **water** as the **Buffer EB** contains salts which could affect other experiments such as restriction enzyme digestion.
- If the sole purpose of the purification is long-term storage, it is recommendable to use Buffer EB or TE buffer, which provide better stabilization of DNA. Label the microtube to notify that EB or TE is used

t) Place microtubes with open caps in centrifuge and make sure the caps are pointing inwards, towards the center of the rotor. Centrifuge the assembly at a moderate speed until the caps properly adjust to the rotor (when it stops making a click sound), then increase to the highest possible revolution and centrifuge for 1 minute.

u) Discard the column and store the DNA at 4 °C if it will be used in short time (<2 weeks).
- If long-term storage is required, place the stock at −20 °C (preferably at –80 °C).
- ***Avoid freeze and thaw cycles because degrades DNA***.

v) Decontaminate all the equipment that was used with 70% ethanol. Carefully discard the bleach solution in the waste container into the proper waste container and discard the materials in a biological waste (trash can with red bag).

w) Be sure all equipment are shut down: centrifuges and incubator. Return any material and equipment to its proper place. The pipettes must be returned to their maximal volume.