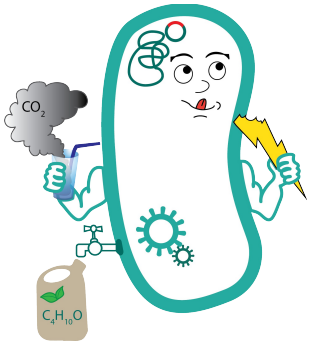
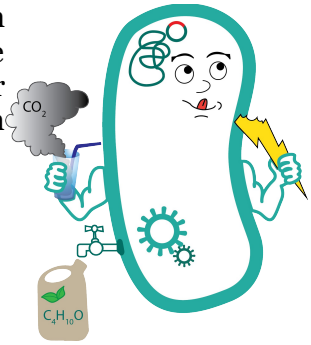
Plasmid isolation (Promega)

- Production of cleared lysate:
  - Pellet 1-10 ml of overnight culture for 5 minutes
  - Thoroughly resuspend pellet with 250 µl of Cell Resuspension Solution
  - Add 250 µl of Cell Lysis Solution to each sample; invert 4 times to mix
  - Add 10 µl of Alkaline Protease Solution; invert 4 times to mix. Incubate 5 minutes at room temperature
  - Add 350 µl of Neutralization Solution; invert 4 times to mix
  - Centrifuge at top speed for 10 minutes at room temperature
- Binding of plasmid DNA
  - Insert Spin Column into Collection Tube
  - Decant cleared lysate into Spin Column
  - Centrifuge at top speed for 1 minute at room temperature. Discard flowthrough, and reinsert Column into Collection Tube.
- Washing
  - Add 750 µl of Wash Solution (ethanol added). Centrifuge at top speed for 1 minute. Discard flowthrough and reinsert column into Collection Tube
  - Repeat step before with 250 µl of Wash solution
  - Centrifuge at top speed for 2 minutes at room temperature
- Elution
  - Transfer Spin Column to a sterile 1.5 ml microcentrifuge tube, being careful not to transfer any of the Column Wash Solution with the Spin Column. If the Spin Column has Column Wash Solution associated with it, centrifuge again for 1 minute at top speed, then transfer the Spin Column to a new, sterile 1.5 ml microcentrifuge tube
  - Add 100 µl of Nuclease-Free Water to the Spin Column. Centrifuge at top speed for 1 minute at room temperature
  - Discard column, and store DNA at -20 °C or below



Plasmid isolation (Thermo Scientific)

- Note:
  - All purification steps should be carried out at room temperature.
  - All centrifugation should be carried out in a table-top microcentrifuge at >12000 x g (10 000-14 000 rpm, depending on the rotor type).
- Resuspend the pelleted cells in 250 µl of the Resuspension Solution. Transfer the cell suspension to a microcentrifuge tube. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain.
  - Note Ensure RNase A has been added to the Resuspension Solution.
- Add 250 µl of the Lysis Solution and mix thoroughly by inverting the tube 4-6 times until the solution becomes viscous and slightly clear.
  - Note Do not vortex to avoid shearing of chromosomal DNA. Do not incubate for more than 5 minutes to avoid denaturation of supercoiled plasmid DNA.
- Add 350 µl of the Neutralization Solution and mix immediately and thoroughly by inverting the tube 4-6 times.
  - Note It is important to mix thoroughly and gently after the addition of the Neutralization Solution to avoid localized precipitation of bacterial cell debris. The neutralized bacterial lysate should become cloudly.
- Centrifuge for 5 minutes to pellet cell debris and chromosomal DNA.
- Transfer the supernatant to the supplied GeneJET spin coloumn by decanting or pipetting. Avoid disburbing or transferring the white precipitate.
- Centrifuge for 1 minute. Discard the flow-through and place the coloumn back into the same collection tube.
  - Note Do not add bleach to the flow-through.
- Add 500 µl of the Wash Solution (diluted with ethanol prior to first use) to the GeneJET spin column. Centrifuge for 30-60 seconds and discard the flow-through. Place the column back into the same collection tube.
- Repeat the wash procedure (step before) using 500 µl of the Wash Solution.
- Discard the flow-through and centrifuge for an additional 1 minute to remove residual Wash Solution. This step is essential to avoid residual ethanol in plasmid preps.
- Transfer the GeneJET spin column into a fresh 1.5 ml microcentrifuge tube (not included). Add 50 µl of the Elution Buffer to the center of GeneJET spin column membrane to elute the plasmide DNA. Take care not to contact the membrane with the pipette tip. Incubate for 2 minutes at room temperature and centrifuge for 2 minutes.
  - Note An additional elution step (optional) with Elution Buffer or water will recover residual DNA from the membrane and increase the overall yield by 10-20%. For elution of plasmids or cosmids >20 kb, prewarm Elution Buffer to 70 °C before applying to silica membrane.
- Discard the column and store the purified plasmid DNA at -20 °C.



Plasmid isolation (QIAGEN)

- Note: All centrifugation steps are carried out at 13,000 rpm (~17,900 x g) in a conventional able-top microcentrifuge.
- Pellet 1-5 ml bacterial overnight culture by centrifugation at >8000 rpm (6800 x g) for 3 min at room temperature (15-25 °C).
- Resuspend pelleted bacterial cells in 250 µl Buffer P1 and transfer to a microcentrifuge tube.
- Add 250 µl Buffer P2 and mix thoroughly by inverting the tube 4-6 times until the solution becomes clear. Do not allow the lysis reaction to proceed for more than 5 min. If using LyseBlue reagent, the solution will turn blue.
- Add 350 µl Buffer N3 and mix immediately and thoroughly by inverting the tube 4-6 times. If using LyseBlue reagent, the solution will turn colorless.
- Centrifuge for 10 min at 13,000 rpm (~17,900 x g) in a table-top microcentrifuge.
- Apply the supernatant from step 5 to the QIprep spin column by decanting or pipetting. Centrifuge for 30-60 s and discard the flow-through, or apply vacuum to the manifold to draw the solution through the QIAprep spin column and switch off the vacuum source.
- Recommended: Wash the QIAprep spin column by adding 500 µl Buffer PB. Centrifuge for 30-60 s and discard the flow-through, or apply vacuum to the manifold to draw the solution through the QIprep spin column and switch off the vacuum source.
- Wash the QIAprep spin column by adding 750 µl Buffer PE. Centrifuge for 30-60s and discard the flow-through, or apply vacuum to the manifold to draw the solution through the QIAprep spin column and switch off the vacuum source. Transfer the QIAprep spin column to the collection tube.
- Centrifuge for 1 min to remove residual wash buffer
- Place the QIAprep column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 50 µl Buffer EB (10 mM Tris*Cl, pH 8.5) or water to the center of the QIAprep spin column. Let it stand for 1 min, and centrifuge for 1 min.