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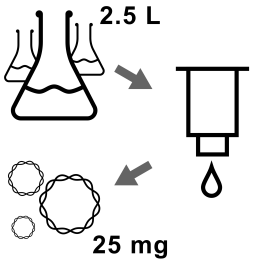
Production and purification of plasmid DNA with the Zymo Research ZymoPURE™ II Plasmid Gigaprep kit



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We use this protocol and it's working

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Disclaimer

Abstract

This protocol is for production and purification of highest yields of highly pure and endotoxin-free plasmid DNA with the Zymo Research ZymoPURE™ II Plasmid Gigaprep Kit. The total yield can be up to 25 mg of DNA, we typically achieve between 6 and 12 mg in ≥3 mL elution volume. The concentration and purity is sufficient for electroporation in Aiptasia zygotes.

Image Attribution

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Guidelines

This protocol assumes an ampicillin selection marker (gene for β-lactamase - *bla*) on the plasmid, if using a plasmid with a different marker, use the appropriate antibiotic in the recommended concentration.
For high-yield plasmid production, we use carbenicillin instead of ampicillin due to its higher stability (it still uses the same resistance gene). This prevents satellite colonies from forming on plate and higher selection for the plasmids in the overnight liquid culture.

We use the fast-growing *Escherichia coli* strain Mach1, other cloning strains such as DH5α, DH10B, TOP10 and XL-1 blue should produce similar yields (or possibly higher ones due to the different growth characteristics).

Terrific broth (TB) should result in a higher plasmid yield compared to Lysogeny broth (LB).

All steps are to be performed at room temperature (RT) unless otherwise noted.

UNLIKE THE Maxiprep protocol, the Gigaprep protocol strictly requires vacuum steps.

Materials

Equipment:

- Incubator at 37°C for *E. coli* plates
- Shaker for ≥2000-mL Erlenmeyer flasks at 37°C and capable of 250 rpm
- Water bath at 50°C
- OPTIONAL: Heat block at 37°C
- Tabletop centrifuge for 50-mL and 500-mL conical-bottom tubes capable of ≥3,500 ×g at RT
- Microcentrifuge capable of ≥16,000 ×g at RT
- House vacuum or vacuum pump for ≤400 mm Hg
- Vacuum manifold with Luer Lock connectors and ~1 L holding capacity, *e.g.*, Zymo Research - S7000
- Thermo Scientific™ NanoDrop™ (or comparable microvolume UV-Vis spectrophotometer), *alternatively fluorescence-based DNA-quantification kit and detection device such as a Qubit™ fluorometer or fluorescence microplate reader (not part of this protocol)*
- Standard DNA-electrophoresis equipment

Consumables and plastic/glassware:

- Sterile 1.5-mL microcentrifuge tubes
- Sterile, DNase-free 1.5-mL tubes with attached screwcaps and O-rings for long-term storage, *e.g.*, Fisherbrand - 02-707-353
- 50-mL conical-bottom tubes, *e.g.*, Corning - 352070
- 2000 baffled Erlenmeyer flasks with ventilated cap, *e.g.*, Greiner Bio-One - 679515

These flasks are made of polycarbonate and can be autoclaved and reused many times (including the lids). Glass flasks work as well, just close them with Parafilm or caps which allow for some air exchange. If the shaker can handle larger flasks, 3000-mL ones are also an option to increase aeration of the cultures.

- 500-mL conical-bottom polypropylene centrifuge bottles, *e.g.*, Corning - 431123

These bottles can be re-used and sterilized with ethanol or autoclaved (the cap is HDPE and might not tolerate too many cycles). Make sure the bottles fit the centrifuge buckets and do not use without adequate support adapters, e.g., Corning - 431124

Compatible centrifuge bottles with a flat bottom can also be used (and don't require adapters) but do not produce a nice pellet and result in more cell loss when decanting the supernatant.

- *5-L graduated beaker or pitcher, e.g., SP Bel-Art* - F28994-0000 (for preparing the TB medium)
- 33 mm or 45 mm-neck glass bottle able to withstand vacuum and scrupulously clean (for taking up the cleared lysate with the plasmid DNA!).

Reagents:

- 10 mM Tris-Cl, pH 8.5, *e.g.*, QIAGEN Elution Buffer EB - 19086

This can also be self-made in ultra-pure water (e.g., MilliQ or HPLC-grade ddH_2O) and autoclaved to denature any potential DNases.

- Carbenicillin disodium salt, *e.g.*, GoldBio - C-103
- Carbenicillin stock solution (1000×): Dissolve 100 mg/mL Carbenicillin disodium salt in ultra-pure water, sterile-filter with a 0.22-µm pore size. Make 1-mL aliquots in sterile 1.5-mL microcentrifuge tubes and store at −20°C.
- DNA-electrophoresis buffer, loading dye, DNA ladder, 0.8-1% agarose gel, DNA stain.

Zymo Research ZymoPURE™ II Plasmid Gigaprep Kit:
https://www.zymoresearch.com/collections/zymopure-plasmid-kits/products/zymopure-ii-plasmid-gigaprep-kit
- Kit for 10 preps - D4204
- Upon arrival, store buffer **ZymoPURE™ P1** (red color, contains RNaseA) at 4°C.
- Store other kit components at RT.

Additional reagents required before first use of the ZymoPURE™ II Plasmid Maxiprep Kit:
- Molecular-biology grade 100% ethanol (for initial preparation of ZymoPURE Wash 2 Buffer)

GROWTH MEDIA:

LB agar with carbenicillin: for **500 mL**:
- 1 % Trypton 5 g
- 0.5 % Yeast Extract 2.5 g
- 1 % NaCl 5 g
- 2% Agar 10 g

In a 1-L bottle, add 400 mL de-ionized water and mix with a stir bar until everything except the agar has dissolved, top off to 500 ,mL with de-ionized water. Autoclave for 20 min at 121°C. Place on stir plate to mix and cool to ~60°C. Add 500 µL of a 100 mg/mL carbenicillin stock solution, mix briefly and pour plates. Let plates cool down and dry overnight at room temperature. Store at 4°C in the sleeve the plates came in.

TB (Terrific Broth): for **3 L**:
- 1.2 % Tryptone 36 g
- 2.4 % Yeast Extract 72 g
- 0.4 % mM Glycerol 12 mL

In a 5-L beaker, bring to 2.7 L with de-ionized water and mix until all components have been fully dissolved.
Split into 4× 540 mL in 2000-mL baffled flasks and the remainder (also 540 mL) into a 1-L bottle, autoclave 20 min at 121°C.
After autoclaving add 60 ml of separately autoclaved potassium phosphate solution to each container.
Store at RT without added carbenicillin. Add carbenicillin directly to culture vessels before inoculation with bacteria (from the 1000× stock solution).

10x potassium phosphate solution: for **300 mL**:
- 0.17 M KH_2PO_4 6.93 g
- 0.72 M K_2HPO_4 37.62 g

Add de-ionized water to 300 mL, autoclave 20 min at 121°C.

Safety warnings

Use standard best practices for molecular-biological work to prevent contamination.
Before start, make yourself familiar with the whole protocol.
Use only undamaged and approved equipment for vacuum steps.
Always use proper balances for centrifugation.
ZymoPURE P2 buffer contains NaOH, ZymoPURE Wash 2 buffer contains isopropanol and ethanol, handle with care and wear PPE. Dispose of all liquid waste according to your Institution's chemical waste guidelines.

Before start

Preparation of reagents before first usage:
Prepare ZymoPURE™ **Wash 2** Buffer:

- Add 107 mL 100% ethanol to the 28 mL ZymoPURE™ **Wash 2** concentrate and mark addition on the bottle.

There are eight bottles of Wash 2 provided, use them one-by-one.
It is a good idea to label all bottles (also for the other buffers) to indicate to which kit they belong and when it was received to not confuse them with reagents from other Zymo Research kits. Also write the received date on the fields of the boxes.

Before every purification:

- Place buffer **ZymoPURE™ P3** at 4°C or on ice 1-2 h before starting with the purification.
- Warm buffers **ZymoPURE™ P2** and **ZymoPURE™ Binding Buffer** in a water bath to 37°C for 10-20 min and mix gently to dissolve any potential precipitates. DO NOT MICROWAVE!
- Heat ≥10 mL of 10 mM Tris-Cl, pH 8.5 to 50°C in a heat block or a water bath for elution.
- OPTIONAL: Use a 500-mL graduated cylinder to measure 400 mL de-ionized water, pour it into the 33- or 45-mm neck bottle which will be later used to collect the cleared lysate and mark the filling level on the outside of the bottle, use a serological pipette to remove 25 mL and mark again (375-mL mark), remove another 25 mL and add the 350-mL mark, this will make it easier to determine the volume of the cleared lysate before adding the ZymoPURE Binding buffer.

2 days before plasmid preparation

16h 5m

1 **Streak out bacterial strain harboring the plasmid from a −80°C glycerol stock on LB with 100 µg/mL carbenicillin for single colonies.** 5m

2 **Incubate plate overnight at 37°C.** 16h

1 day before plasmid preparation

22h 5m

3 **In the morning, use a single colony to inoculate a starter culture of 3 mL TB medium with 100 µg/mL carbenicillin in a culture tube and incubate under agitation at 37°C for 6-8 h.** 8h

4 **In the evening, use 400 µL of the starter culture for each 600 mL TB medium with 100 µg/mL carbenicillin for the main cultures.** 5m
Use at least 2000-mL baffled flasks for this culture.

5 **Incubate 12-18 h at 37°C on a shaker at 250 rpm.** 14h
The culture density before plasmid preparation is a critical parameter, so try to let the culture grow for 12-14 h and then start measuring the OD. This is also highly dependent on the specific E. coli *strain used.*

Plasmid preparation

4h 35m

6 **Check the OD_{600} of the culture.**

6.1 **Add 100 µL of each culture to a clean half-micro cuvette, add 900 µL TB and mix well by pipetting without introducing bubbles.** 5m

6.2 **Measure OD_{600} of each culture in a spectrophotometer blanked against plain TB.** 5m
The OD should be between 2.0 and 3.5 but ideally 2.5-3.0 for best yield. Overgrown cultures might result in lower than expected yield but we also have obtained acceptable results for ODs as high as 4.0.

7 **Distribute the cultures into four 500-mL conical-bottom bottles and pellet the cells at 3,500 ×g for 20 min.** 25m
Each bottle fits roughly 550 mL, the last 50 mL of each culture can be collected in 50-mL tubes and spun down while proceeding through the next two steps with the big batches. Make sure everything is properly balanced and do not use the 500-mL conical bottles without their adaptors.

The supernatant should be clear and in each tube should be a large pellet.

8 **Discard the supernatant immediately and proceed with the protocol.** 5m
There is no benefit in additional washing of the pellets.

9 **Add 150 mL of ZymoPURE P1 buffer (Red) to the bacterial cell pellet in the first bottle, resuspend completely by pipetting, transfer the whole suspension to the next bottle, resuspend and so on.** *Make sure to dissolve all cell clumps. If the remainder of the cultures was pelleted as well, then take 10-20 mL of the already resuspended cells in P1 buffer and use them to resuspend the smaller pellets one after the other and add it back to the big bottle. This reduces the 500-mL bottles to just one.* 15m

10 **Add 150 mL of ZymoPURE P2 buffer (Green) and immediately mix by inverting the tube 8-12 times. DO NOT VORTEX! Let sit at room temperature for 3-5 min.** 7m
Don't exceed 5 min, otherwise the DNA quality might suffer. Cells are completely lysed when the solution appears clear (hard to see through the centrifuge bottle), purple, and viscous.

11 **Add 150 mL of cold ZymoPURE P3 buffer (Yellow) and mix gently but thoroughly by inversion. DO NOT VORTEX!** *The sample will turn yellow when the neutralization is complete and a yellowish-green precipitate will form, there must not be any purple left. If the precipitate has formed a homogenous layer at the surface of the neutralized lysate then invert the bottle 3-4 times prior to loading the lysate into the ZymoPURE Giga Filter.* 5m

12 **OPTIONAL: To ease filtration in the next step, centrifuge the lysate for 15 min at ≥3500 ×g in a tabletop centrifuge at 20°C. Afterwards remove the top layer of precipitate with a pipette tip or inoculation loop and proceed with the next step.** 18m

13 **Place the ZymoPURE Giga Filter onto a clean 33 mm or 45 mm-neck glass bottle (with 350, 375 and 400-mL marks) and load the lysate into the ZymoPURE Giga Filter. Ensure the ZymoPURE Giga Filter is resting securely on top of the glass bottle and wait 10 minutes for the precipitate to float to the top.** 12m
If the optional centrifugation step was done and the lysate does not contain much more precipitate, proceed directly to the next step.

14 **Connect the ZymoPURE Giga Filter to a vacuum source and turn on the vacuum until all liquid has passed (approximately 375 mL of cleared lysate should be recovered). Save the cleared lysate!** 10m
The filter should attach tightly to the bottle top once the vacuum is applied, if not, press it carefully down.
It is possible that more (or in rare cases less) than 375 mL are recovered (see instructions for next step).

15 **Add 150 mL ZymoPURE Binding buffer to the cleared lysate from the previous step and mix thoroughly by inverting the capped bottle 10 times.** 3m

If the recovered lysate volume differs drastically from 375 mL, adjust the volume of Binding buffer in the next step to 0.4× the recovered volume of cleared lysate (e.g., 140 mL Binding buffer to 350 mL lysate or 160 mL Binding buffer to 400 mL lysate).
A good amount of DNA will cause the solution to get slightly cloudy after addition of the binding buffer.

16 **Attach the 600-mL reservoir to the top of the Zymo-Spin VI-PX column and place the assembly onto a vacuum manifold.** 3m
Make sure that all components are attached tightly and sit straight on the vacuum manifold, the assembly is quite high and there will be half a kilogram weight sitting on the single Luer connection.
Make sure that the vacuum manifold is clean and empty and that it has the capacity to take up ~800-1000 mL of flow-through, for smaller capacities, empty the manifold between steps as necessary.

17 **With the vacuum off, add the entire mixture from step 6 into the 600-ml reservoir/Zymo-Spin VI-PX column assembly, then turn on the vacuum until all of the liquid has passed completely through the column.** 5m

18 **Remove the 600-mL reservoir (keep the reservoir for the next steps) from the top of the Zymo-Spin VI-PX column, place the column into a 50-mL conical-bottom tube (you can screw the lid on) and then centrifuge the column at 500 ×g for 2 min.** 5m
This ensures that all DNA is bound to the column matrix before doing the wash steps when the vacuum is too weak to fully pass all liquid through.

19 **Re-attach the 600-mL reservoir to the Zymo-Spin VI-PX column and place the assembly back onto the vacuum manifold.**

20 **With the vacuum off, add 100 mL of ZymoPURE Wash 1 to the Zymo-Spin VI-PX column assembly, then turn on the vacuum until all of the liquid has passed completely through the column.** 3m

21 **With the vacuum off, add 100 mL of ZymoPURE Wash 2 to the Zymo-Spin V-PX column assembly, then turn on the vacuum until all of the liquid has passed completely through the column.** 3m

22 **With the vacuum off, again, add 100 mL of ZymoPURE Wash 2 to the Zymo-Spin VI-PX column assembly, then turn on the vacuum until all of the liquid has passed completely through the column and let the vacuum on for an additional 2 min.** 3m

23 **Remove and discard the 600-mL reservoir and place the Zymo-Spin VI-PX column in a 50-mL conical-bottom tube. Centrifuge at ≥3,400 ×g for 10 min to remove any residual wash buffer.** 2m
Make sure to properly balance the rotor and that there is enough clearance for the column in the tube.

24 **Place the Zymo-Spin VI-PX column in a new, clean 50-mL conical-bottom tube.** 1m

25 **Add 3-5 mL 50°C-warm 10 mM Tris-Cl, pH 8.5 directly to the center of the column matrix.** 2m
Zymo Research recommends to use 5 mL for expected yields of >5 mg; 3 mL can produce a higher concentration but a lower total yield.

26 **Incubate at RT for 10 min.** 10m

27 **Centrifuge at ≥3,400 ×g for 5 min.** 7m

28 **Save the tube with the first eluate and place the column into a new, clean 50-mL conical-bottom tube and add 5 mL 50°C-warm 10 mM Tris-Cl, pH 8.5 directly to the center of the column matrix.** 3m
The first eluate should have a DNA concentration in the µg/µL range, the second usually a 10-20 times lower concentration which is still comparable to a standard plasmid prep and can be used for concentration adjustment of the first eluate or for cloning and PCRs.

29 **Incubate for 10 min at RT.** 10m

30 **Centrifuge at ≥3,400 ×g for 5 min.** 7m

31 **Save the tube with the second eluate.** 2m
If unsure of the success of the elution, keep the spin column in a 50-mL conical-bottom tube somewhere safe until after the quantification, to have the possibility of another elution if the yield is unexpectedly low.
Otherwise, it's a good idea to keep at least one old column as a (clearly marked!) future balance for centrifugation.

32 **Take the 15-mL Reservoir-X and EndoZero III spin-column from the kit, ensure a tight connection of the two and place the assembly into a new, clean 50-mL conical-bottom tube.** 3m

33 **Add the entire first eluate into the 15 ml Reservoir-X/EndoZero III spin-column assembly and wait 2 min.** 3m

34 **Centrifuge at 3,400-5,000 ×g for 10 min.** 12m

35 **Save the tube with the first eluate and repeat the previous step one more time with a new 50-mL conical-bottom tube but the same 15 ml Reservoir-X/EndoZero III spin-column assembly and the second eluate.** 15m

36 **Run 0.5 µL of each eluate on a 0.8-1% agarose gel (depending on plasmid size) with ~1 µg of an appropriate DNA size ladder.** 40m
This should produce a thick band for the first and a weaker one for the second eluate. There might be multiple bands due to supercoiled confirmations. For an exact size analysis (really not necessary under normal circumstances), linearize the plasmid with a single-cutter restriction endonuclease.

37 **Quantify an appropriate dilution via NanoDrop or a fluorimetric method (e.g., Qubit).** 10m
*The NanoDrop doesn't have problems measuring high DNA concentrations directly (even far over 10 µg/µL), the much more sensitive Qubit kits require very high dilutions and precise pipetting to measure accurately. If the concentration is too low, see the protocol on **concentrating plasmid DNA with Amicon® Ultra Centrifugal Filter**.*

38 **Adjust concentration of the eluates and split them into separate 1.5-mL screw cap microcentrifuge tubes.** 15m

39 **Store the plasmid DNA at −20°C.** 1m
The concentrated DNA at pH 8.5 should be stable through freeze-thaw cycles but if worried about degradation prepare small-volume aliquots.

Protocol references

This protocol follows the user guide from Zymo Research and incorporates further optimizations recommended by in large parts by the technical support for high yields of highly concentrated plasmid DNA and some more tweaks. The manual can be found here:
https://files.zymoresearch.com/protocols/_d4204_zymopure_ii_plasmid_gigaprep.pdf