Sep 04, 2025

Plasmid DNA concentration for electroporation into Aiptasia zygotes via Amicon® Ultra Centrifugal Filters

In 1 collection

DOI
https://dx.doi.org/10.17504/protocols.io.n92ld6b77g5b/v1

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OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.n92ld6b77g5b/v1

Protocol Citation: Christian CR Renicke 2025. Plasmid DNA concentration for electroporation into Aiptasia zygotes via Amicon® Ultra Centrifugal Filters. **protocols.io** https://dx.doi.org/10.17504/protocols.io.n92ld6b77g5b/v1

Manuscript citation:
Renicke et al., 2015

Protocol status: Working
We use this protocol and it's working

Created: July 02, 2025

Last Modified: September 04, 2025

Protocol Integer ID: 221459

Keywords: DNA concentration, Aiptasia electroporation, plasmid dna concentration for electroporation, plasmid dna concentration, plasmid dna, using amicon ultra, centrifugal filter, electroporation, dna, aiptasia zygote

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Abstract

This protocol describes how to concentrate 500 µL or more of dilute plasmid DNA using Amicon Ultra 0.5 50 kDa MWCO centrifugal filters to a final volume of ≥15 µL.

Guidelines

This is the only method which was compatible with electroporation of plasmids into Aiptasia zygotes (in contrast to ethanol precipitation or vacuum concentration via SpeedVac) and could be used to obtain DNA with a sufficient concentration (>1.5 µg/µL) when a Maxi- or Gigaprep did not produce sufficient yields to be directly used.

Plasmids are usually above 1,000 kDa, but supercoiled plasmids might behave like much smaller molecules, so the Amicon Ultra 0.5 filter unit with a 50 kDa molecular-weight cut-off (MWCO) was chosen to be on the safe side. Lower cut-offs should work as well but the minimally retained liquid volume will be higher, the 100 kDa MWCO filter should also work and allow even lower volumes and/or shorter spin times.
We have concentrated up to 2 mL of ~40-100 ng/µL plasmid solutions and did not observe any problems with overloading. Our recovery was usually around 70-80%, which is far lower than the advertised <90%, so there might be some room for optimization.
The screw cap tube for storage is to ensure proper sealing and prevent any loss of material during storage.

Materials

Devices:

- Microcentrifuge at RT set to 16 000 ×g
- 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)*

Consumables:

- Sterile, DNase-free 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
- Amicon® Ultra 0.5 Centrifugal Filter, 50 kDa MWCO, EMD Millipore - UFC5050
- 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.

Safety warnings

Do not spin the tube with the inverted filter unit higher than the 2,000 ×g, the unit and/or the centrifuge might get damaged.
Always use proper balances for centrifugation. Always attach the rotor cover when using the filter units.

Before start

- Thaw plasmid prep to concentrate, adjust volume to 500 µL with 10 mM Tris-Cl, pH 8.5 if it is less than 500 µL.
- Place filter unit in one of the supplied 2-mL tubes.

Plasmid DNA concentration

51m

1 **Add 500 µL of the plasmid DNA in 10 mM Tris-Cl, pH 8.5.** 1m
If a larger volume of very dilute DNA needs to be concentrated, repeat the loading and following spin step until the whole volume has passed through the filter.

2 **Close lid and spin at 14,000 ×g for 10 min at RT. Discard flow-through.** 12m

3 **Add 500 µL 10 mM Tris-Cl, pH 8.5 to the filter unit.** 1m
This washing step is to remove any soluble impurities and traces of glycerol from the membrane.

4 **Close lid and spin at 14,000 ×g for 20 min at RT..** 22m
The longer centrifugation time is to reduce the volume down to ~15 µL.

5 **Place the filter unit inverted into the second supplied 2-mL tube.** 1m

6 **Spin at 2,000 ×g for 2 min at RT.** 3m

7 **Transfer the recovered, concentrated plasmid DNA into a 1.5-mL screw cap microcentrifuge tube.** 1m

8 **Quantify an appropriate dilution via NanoDrop or a fluorimetric method (e.g., Qubit).** 5m
The NanoDrop doesn't have problems with measuring high DNA concentrations directly (even far over 10 µg/µL), the much more sensitive Qubit kits require very high dilutions to measure.

9 **Dilute to the required concentration with 10 mM Tris-Cl, pH 8.5 and store at −20°C.** 5m
The highly concentrated DNA in pH 8.5 should be stable through freeze-thaw cycles but if worried about degradation prepare small-volume aliquots.

Protocol references

This protocol mostly follows the recommendations from EMD Millipore which can be found here: https://www.sigmaaldrich.com/deepweb/assets/sigmaaldrich/product/documents/105/563/pr05485w-ug-ms.pdf