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Purifying DNA from Agarose with Homemade Glass Milk

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Protocol status: Working

I used this protocol for 20 years and it worked almost every time. I made 2 batches of glass milk in that time. It's cheap and easy.

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Abstract

You don't need an expensive kit to purify DNA. I used to make my own (very very cheap) glass milk from this protocol. It's hard to find online these days so I'm adding it here and will share. You can read more about this on an Addgene blog <https://blog.addgene.org/dna-purification-without-a-kit>

Guidelines

This method is a cheap, effective, easy way to purify DNA from bands in an Agarose gel. The DNA will be clean and can be used in almost any procedure (all that I've tried). Note that it is rumored to only work with TAE gels, but this is not correct. It works fine with TBE with very little loss. The agarose melting step takes a bit longer and might take a bit more volume but it works just fine.

Materials

MATERIALS

☒ Sodium iodide **P212121**

☒ Sodium sulfate **P212121**

☒ #1 Whatman paper **Coorstek Catalog #1001-150**

☒ Nitric acid

☒ NaCl **Merck MilliporeSigma (Sigma-Aldrich) Catalog #53014**

☒ Tris **Bio Basic Inc. Catalog #TB0196.SIZE.2.5Kg**

☒ 100% EtOH **Gold Shield Distributors Catalog #DSP-CA-151**

☒ EDTA **Fisher Scientific Catalog #16 004Y**

☒ Silica 325 Mesh

Silica 325 mesh is a flint glass powder available from ceramic shops



Safety warnings

- ⚠ Bringing Nitric Acid to an almost boil can be dangerous. Use a hood, safety equipment and don't ask a lab newbie to do this step.



1 **Preparation of Glass Powder (Steps 1-4)**

Resuspend 400 g of glass powder in 800 ml double distilled (dd) H₂O in a 2 liter flask with a stir bar
Stir for 60 minutes

2 **Collect the "fines"**

Remove and keep the supernatant by gentle pouring, leaving all settled glass. This contains fine particles which is what you want to collect. Pellet in centrifuge 10 minutes 6000 RPM

3 **Almost boil glass fines in acid to clean**

Resuspend pellet in 250 ml ddH₂O. Add Nitric Acid to 50%
Here's the tricky part. Bring close to a boil in a fume hood (! must do in fume hood!)
Allow to cool.

4 **Pellet and resuspend to generate stock aliquots for storage**

Pellet glass as before, wash 6 times with ddH₂O. Check pH and ensure it is neutral (same as water)
Resuspend with equal volume ddH₂O to the pellet volume and aliquote in 50 µl aliquots for storage in freezer so it stays suspended. It will last forever and might provide a lifetime supply.

5 **Prepare NaI Binding Solution**

- 90.8 g NaI
- 1.5 g Na₂SO₄
- in 100 ml ddH₂O.

Let dissolve but might have a few particles.

- Filter through Whatman No. 1 paper (recommend into a dark bottle)
- Put dialysis bag containing 0.5g Na₂SO₄ in bottle to keep the solution saturated.
- Wrap bottle in foil and store at 4C.
- Periodically check for discoloration. If the solution turns brown it's time for new solution. (I've never tested how it works brown, it might)

6 **Prepare Glass Milk Wash solution from stocks**

- 100 mM NaCl
- 1 mM EDTA
- 50% EtOH
- 10 mM Tris pH 7.5

Protocols recommend storage at -20C but I stored at 4C with no apparent ill effects.

7 **DNA Purification (Steps 7 - 12)**

DNA binds to glass at high salt and low temperature, elutes at low salt and higher temperature.

- Get to know the weight of your gel slices by weighing a few. 0.1, 0.5 and 1 gram for example. Once you know, you'll be able to know on sight without weighing.
- Add 2 ml Nal solution per gram of gel. This does not need to be exact.

8 **Dissolve gel**

Incubate at 37 C, mixing occasionally until agarose is totally dissolved. Might take longer for TBE gels. Can also do this in warmer bath to 50 C to speed this up.

9 **Bind to Glass**

Add 1 microliter of glass slurry per microgram of DNA. For smaller amounts, I used about 1 ul regardless, sometimes keeping a more diluted slurry for smaller amounts and so as not to waste the reagent.

Incubate DNA/glass on ice 5 mins, mixing occasionally

10 **Wash Glass with DNA bound**

- Spin suspension 5 seconds in centrifuge, remove and discard supernatant by taking off with a micro pipet.
- Wash pellet by resuspending in 250 ul Nal solution (or 10X volume of glass if larger)
- Spin, resuspend to wash pellet 2-3 times with EtOH Glass Milk Wash solution (~ same volume). Taking off supernatant with a micro pipet gently.

11 **Dry Pellet and Elute DNA**

Dry pellet well, removing all residual liquid (air dry for a short time and/or carefully use Kimwipe)

Resuspend pellet in ddH₂O or TE (> 10 microliter) and elute DNA at 37 C for >5-10 mins.

12 **Remove and keep eluted DNA in supernatant**

Spin 1 min in microfuge and remove eluted DNA in **supernatant**.
DNA is ready for ligation, restriction, radiolabelling etc.

13

If you want to purify DNA that is already in solution (not in a gel) you can add 3 volumes of Nal solution, immediately
add glass and put on ice. Follow step 10-12 to wash and elute.