Isopropanol DNA Precipitation - Best for DNA Concentration

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[1]Realizing Increased Photosynthetic Efficiency (RIPE)

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Protocol to precipitate extracted DNA from an aqueous solution to increase concentration or resolubilize in a different storage buffer.

*Use isopropanol DNA precipitation if your DNA is suspended in a very large volume, if your DNA concentration is low, or you are trying to concentrate large molecular weight DNA fragments and remove smaller fragments.

*Use ethanol DNA precipitation if you are trying to remove salt contamination or precipitate small DNA fragments.

Additional Resources:

- New England Biolabs, "DNA Precipitation: Ethanol vs. Isopropanol". June 23, 2015
- Green, Michael R. and Joseph Sambrook, "Precipitation of DNA with Isopropanol". doi:10.1101/pdb.prot093385Cold Spring Harb Protoc2017.
- Qiagen, "How can I precipitate genomic DNA using isopropanol?".

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DNA precipitation performed at room temperature minimizes co-precipitation of salt that interferes with downstream applications.

Reagents:

- Isopropanol, room temperature
- 70% Ethanol,

*Measure 70 ml of ethanol and 30 ml of distilled water separately and then mix.Do not measure 70 ml of ethanol and then bring to volume.

NOTE: 70% ethanol is hygroscopic. When opened the ethanol will both evaporate and absorb water over time. Re-use eventually will be at a lower concentration. There is also miscibility of ethanol and water. For example, measuring out 70 mL of ethanol and topping off to 100 mL with water will generate ~65% ethanol. Measuring 70 mL ethanol and 30 mL water separately, then combining them will generate ~95 mL of 70% ethanol.

- Sodium acetate (3 M, pH 5.2): 0.246g/mL (0.738g/3mL dH2O)
- DNA resuspension solution.

*Molecular Grade Water: **Safest option for downstream applications.**

*TE buffer (pH 8.0), 100 mL: **Allows easier resuspension of DNA pellet. Inhibits DNases. Good for long term storage but may interfere with enzymes in downstream applications.**

A	B	C
Reagent	**Volume**	**Final concentration**
1M Tris-Cl (pH 8.0)	1 mL	10 mM
0.5M EDTA (pH 8.0)	0.02 mL (20 ul)	0.1 mM
Distilled H2O	99 mL	

1)Mix all components. Autoclave to sterilize.

A) 1 M Tris-Cl (pH 8.0), 10 mL

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A	B	C
Reagent	**Volume**	**Final concentration**
Tris base	1.21 g	10 M
Distilled H2O	10 mL	

i) Weigh out 1.21 g Tris and add to a 15 mL centrifuge tube.

ii) Measure out 8 mL of distilled water and add to the tube.

iii) Stir to mix the solution.

iv) Add a pH meter into the solution to observe the pH.

v) Slowly add concentrated hydrochloric acid (HCl) solution using a Pasteur pipette to reduce the pH to 8.0, or another desired pH. Be careful not to add too much at a time, since the pH will change rapidly.

vi) Once the desired pH has been reached, top up the solution to 10 mL using distilled water.

B) 0.5M EDTA pH 8.0, 5 mL

A	B	C
Reagent	**Volume**	**Final concentration**
EDTA disodium salt, dihydrate	0.93 g	0.5 M
Distilled H2O	5 mL	

i) Weigh out 0.93 g EDTA disodium salt, dihydrate and add to a centrifuge tube.

ii) Measure out 3.5 mL distilled water and add to the tube.

iii) Shake to mix. The EDTA salt will not go into solution until the pH reaches 8.0.

iv) Add a pH meter into the solution to observe the pH.

v) To dissolve the salt, add sodium hydroxide (NaOH) pellets to the solution. Add a few pellets at a time and wait until the pellets have fully dissolved before adding more. It may take around 0.1 g of NaOH pellets before the pH is at 8.0.

vi) Once fully dissolved (this will take some time so be patient), top up the solution to 1 mL using distilled water, if necessary.

Materials:

- Analytical balance
- pH meter
- Graduated cylinder
- Pipette, single channel, 1-100 ul
- Pipette tips, 1-100 ul
- 1.5 mL microcentrifuge tube
- Microcentrifuge
- Paper towel or kim wipe

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1 Transfer desired amount of aqueous DNA solution to a fresh microtube. (70 ul)

2 Add 3.0 M pH 5.2 sodium acetate to the DNA solution to result in a final concentration of 0.3 M sodium acetate. (7 ul)

3 Add 0.6–0.7 volume of isopropanol at room temperature and mix well. (50 ul)

4 Point caps of tubes out to help identify pellet location below cap. Centrifuge the sample immediately at 10,000–15,000 x g for 20–30 min in a microcentrifuge tube at 4°C.

5 Identify the pelleted DNA. Carefully decant the supernatant fluid into a fresh labeled tube without losing the pellet.

If concerned about losing DNA pellet during decanting, the supernatant can be stored in a fresh tube until the recovery of the precipitated DNA is confirmed.

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6 Wash the DNA pellet by adding 500 ul or more (depending on the size of the preparation) of room-temperature 70% ethanol. Flick tube and invert several times until pellet is removed from the side of the tube and all sides can be washed.

Thorough 70% ethanol washing is critical to remove residual isopropanol which can hinder the redissolution of the DNA.

7 Centrifuge the sample at 10,000–15,000 x g for 20–30 min at 4°C.

8 Visually identify the pellet. Pour out ethanol. Tap tube gently on paper towel, being sure to keep track of the pellet.

9 

Optional: For high quality DNA applications requiring very clean DNA extractions, repeat steps 6-8. More ethanol washes will increase quality but will negatively affect recovery.

10 After last ethanol wash, use a pipette to remove any ethanol that remains after decanting.

11 Allow samples to air dry at room temperature for 10-20 min. When pellet turns from white to clear the pellet is dry. Do not over dry.

Overdrying of the pellet will make resuspension of the DNA more difficult.

12 Resuspend the DNA pellet in Tris, TE (pH 8.0), or molecular grade water. Incubate in **4 °C** overnight for more complete resuspension. For long term storage, store at **-20 °C** .

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