DNA Precipitation

Remember that DNA is water-soluble, so any alcohol will work to push it out of solution. Before you select a method, consider what salts are in solution with your DNA, and what the aim of your precipitation is. Do you want to preferentially precipitate long fragments? Or are you concerned about yield and want to squeeze every last drop from your sample?

Ethanol vs. Isopropanol

Simply put, isopropanol is a more forceful precipitation agent for DNA, while EtOH is more gentle. As such, the key differences are the volume required, the effect of temperature, and the time it will take to precipitate and to dry. Both precipitations will only work if there is a salt in solution to stabilize the DNA backbone and allow DNA strands to condense together.

Ethanol	**Isopropanol**
2.5x sample volume required Good when you have space	1x sample volume required Good when tube space is limiting
Slower precipitations overall – 5 minutes absolute minimum, I like to wait at least 10	Faster precipitations – I've done 1 minute gently inverting before centrifugation and retrieved quite a lot of DNA
Salt less soluble overall	Salt more soluble overall
Will push out all fragments, even small ones	HMW DNA will come out preferentially with room temperature precipitations of Iso
More volatile, dries more quickly Pellet usually dry in 5-10 minutes	Less volatile, dries more slowly I have let larger pellets sit overnight
Gentler, best for PCR product, DNA samples of mixed quality, and general use	Harsher, best for HMW at RT, or very small fragments with cold and time

Salt Choices

Many times your protocols will not allow you to choose which salt is in solution with your DNA (or tell you at all, cough Qiagen). However, knowing the effects of the different salts can be useful:

- **Sodium chloride** is less soluble in water in solutions of both ethanol and isopropanol, so you're going to have some coprecipitation with your DNA no matter what. Consider a second 70% ethanol rinse if you are concerned about salt carryover. 0.2M is typical. Especially good for samples with SDS because NaCl will keep SDS soluble, even in 70% ethanol, so you'll pull out residual SDS from samples. If this is a concern a second 70% wash never hurts.
- **Lithium chloride** sounds great because it stays soluble in ethanol, but the carryover of chloride isn't ideal if you're going straight into any kind of PCR or RT reactions as it inhibits polymerases a bit. 0.8M is typical for RNA.
- **Sodium acetate** is great because it doesn't inhibit polymerases as much, but it's a bit more expensive and rarely used these days. 0.3M is typical, and for RNA make sure the pH is 5.2.

- **Ammonium acetate** is great if there is concern of residue mucus/slime/carbohydrates. 2M will help push out even DNTPs, but ammonium can interfere with some enzymes. I prefer to add ammonium acetate at low levels to my 70% ethanol directly to help with slime.

Time and Temperature

This is the annoying line where I say "by feel". This is nuanced and you can try 5 different ways before finding what works. I have left samples in the -20C freezer overnight, and I have also inverted a tube 3 times at room temperature and then spun immediately. Both produced the DNA I was looking for.

A note on tube orientation (yes, apparently this matters): I was always told to keep the tube on its side to allow more overall surface area for DNA to fall out on without trapping anything underneath the pellet. I also was told to keep the tube upright to ensure that the pellet forms at the bottom of the tube rather than streaking up the back. If I'm going overnight, I usually lay it down and stand it up as it come back to 0C. Compromise.

Longer precipitations are going to be better when you have either very long or very short DNA. Long precipitations at room temperature are necessary for HMW fragments (anything over 5000bp, I would wait at minimum 10 minutes). Similarly, long and very cold precipitations will aid in recovery of smaller fragments of DNA.

Know that any precipitation should be gently brought up in temperature ON ICE to 0C before spinning or your DNA will not pellet cleanly. If you have a refrigerated centrifuge (preferred) you can bring it to 4C.

For most samples, 20 minutes at -20C is a nice balance- it gets cold enough to get most of the DNA out but isn't ridiculously low. Make sure that you allow the sample to warm back up on ice for 5 minutes, and spin for at least 10 minutes. For all other samples, see next page.

	RT and Short	RT and Long	Cold and Short	Cold and Long
I want:	Large amounts of DNA, and there is plenty in the sample	Large amounts of HMW DNA, preferentially relative to short, but I don't have infinite DNA in sample	Decent amounts of DNA- maximize yield per unit time.	ALL the DNA in the sample (scales with cold)
What temp:	Benchtop rack	Benchtop rack	On ice OR in freezer at 0C, -20C, or -70C	In freezer at 0C, -20C, or -70C
Time:	5-10 minutes	30 min – 2 hours	5-20 minutes	Up to overnight/24 hours.
I'll get:	Will precipitate most of the medium-sized (>500bp) or higher DNA, if DNA concentration is high. You WILL lose some DNA.	Won't precipitate short pieces as much as long pieces.	Most of the DNA in my sample.	ALL OF IT.
Tweaks	Get a bit more by adding *nol at -20C or -80C.	Get a bit more by adding *nol at -20C or -80C.	Add cold *nol at -20C or -80C.	Add cold *nol and throw the tube in the freezer. Make sure you label the tube emphatically.
Tips	Save your first precipitation of *nol, place in new tube, and throw it in a freezer overnight to recover anything missed the first time. Useful to have more sample in the freezer just in case.	See ←	Gently invert tube a few times to increase yield.	The colder and longer, the more DNA will precipitate…ish. Past overnight usually doesn't increase yield. Gently invert tube a few times in the first few hours to increase yield.
Notes			Alcohol now viscous: bring up to 0C on ice before spinning	Alcohol now viscous: bring up to 0C on ice before spinning
Pelleting	5 minutes spin has worked	5 minute spin has worked	Spin 10 minutes MINIMUM.	Spin 10 minutes MININIMUM. Recommend 4C for 20 minutes (up to 30 minutes).