

Making Seed Stock

1. Identify a suitable condition from which to prepare a seed stock – this should be a drop that contains crystals and not too much precipitate. This is the seed source drop. If both subwells in a multi-subwell plate have crystals, you can use both subwells as your seed source. If you have only a small number of crystals in any one drop, but have a number of crystal-containing drops all under similar conditions, then these can be combined.
2. Make up 1ml of the reservoir solution associated with the seed source drop ('reservoir'). If you are combining different drops, then make up a reservoir solution that is at least as concentrated as the most concentrated seed source drop reservoir.
3. Put a seed-bead (1/8" Teflon bead) in a 1.5ml Eppendorf tube. Label the seed-bead Eppendorf tube (well, date, ect), and add 50 μ l of the reservoir solution to it.



Eppendorf and Seed-bead

4. Cut the tape from the seed source drop – add 1 μ l of the reservoir solution to the droplet, and then poke a crystal with one of the Hampton tools. The crystal should crush when you poke it. If it doesn't, then the crystals are too old to make good seeds, and you should select another candidate for your seed source. If the crystals start dissolving when you add the 1 μ l of reservoir, you will need to make up a new seed stock reservoir with more precipitant in it, and try again.
 5. Using a 2 μ l pipette, move the seed source droplet, and all the crystals, to the 50 μ l of reservoir solution in the seed bead Eppendorf tube.
 6. Close the lid of the tube, and vortex at full speed for a minute.
 7. Clean the Hampton tool (you can reuse it!)
 8. Add another 200 μ l of the reservoir solution to the vortexed Eppendorf, and vortex briefly (2 seconds) to mix. This is your seed stock.
 9. Using the Mosquito, set up a plate with a seeding protocol. The standard protocol will have 100nl protein, 100nl reservoir, and 40nl of your seed stock. The seed stock may have to be further diluted, or the drop ratio changed to get good crystals from the seed stock.
- Reference: J.R. Luft and G.T. DeTitta. (1999). *A Method to Produce Microseed Stock for Use in the Crystallization of Biological Macromolecules*, Acta Cryst., **D55**, 988-993