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# Standard Operating Procedure

Concerning: Making Seeds

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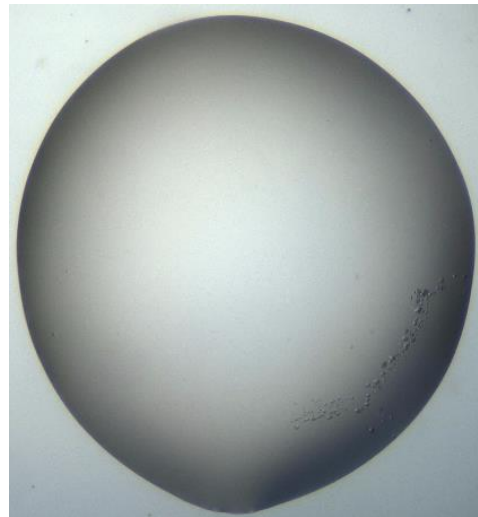
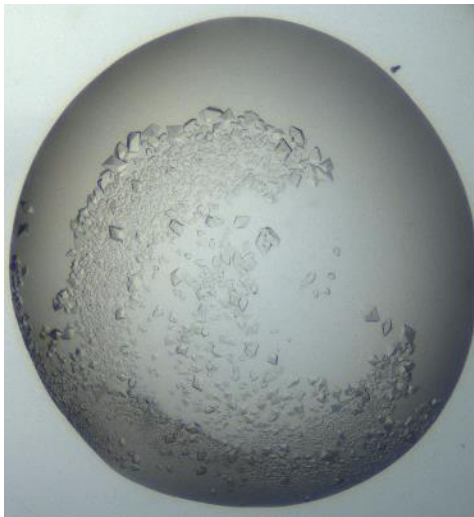
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## Seeding in C3 (Making seeds for robotic seeding)

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1. Identify a suitable condition from which to prepare seeds – this should be a drop that contains crystals and not too much precipitate. If both subwells in a multi-subwell plate have crystals, use 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 combine the droplets to make the seeds. You need to have a reasonable amount of crystal mass from which to make seeds (below, left) – a single flyspeck-sized crystal in a low volume sitting drop is not enough (below, right).



If you only have drops with homeopathic quantities of crystals try setting up more droplets under exactly the same conditions, and streak seeding from the small crystals that you have, until you produce enough crystals for a larger preparation of seeds.

2. Make up 1 mL 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. Choose what type of seed beads to use. Traditionally, a 1/8" Teflon bead was used. More recently, there has been a trend to using a few smaller beads – these can be either Teflon, ceramic, glass or stainless steel. At C3, we recommend using a few small steel shot pellets. Place one larger or a few (10?) smaller beads into a 1.5ml Eppendorf tube (Images of different types of bead from Hampton Research). In C3 we use (washed) 0.5 mm stainless steel shot, which is primarily used as an abrasive during blasting. Low protein binding glass beads used for disrupting yeast cells work well too.



4. Label the seed-bead Eppendorf tube (barcode, well, date), and add 50  $\mu\text{L}$  of the reservoir solution to it.
5. Cut the tape from the seed source drop – add 1  $\mu\text{L}$  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  $\mu\text{L}$  of reservoir, you will need to make up a new seed stock reservoir with more precipitant in it, and try again.
6. Using a 2  $\mu\text{L}$  pipette, move the seed source droplet, and all the crystals, to the 50  $\mu\text{L}$  of reservoir solution in the seed bead Eppendorf tube.
7. Close the lid of the tube, and vortex at full speed for 15 seconds.
8. Clean the Hampton tool (rinse it with water, then wipe it with ethanol), and replace it in the toolbox.
9. Add another 200  $\mu\text{L}$  of the reservoir solution to the vortexed Eppendorf, and vortex briefly (2 seconds) to mix. This is your seed stock.
10. If you only have one well with crystals, you can use the reservoir (50  $\mu\text{L}$ ) only, and skip the dilution step.

11. Using the Mosquito, set up a plate with a seeding protocol. The standard protocol will have 150nl protein, 100nl reservoir, and 50nl 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.

Notes:

The 1/8 inch seed-beads can be obtained from B J Bearings (13 Endeavour St. Warragul, VIC 3820, telephone 03 5623 5444) – 1/8" Delrin Gr1 Balls – product code KU.3.175DELRIN – about \$20 for 100).

We have used 400 µm low binding Silica beads from OPS diagnostics (Product BLBG 400-200-06) successfully as well.

Currently we are using 0.5 mm stainless steel shot from W.Granowski Pty Ltd, but this is generally only available in 25 kg quantities.