SOP for Crystallization Trials

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Materials/Reagents/Equipment	**Vendor**
Disposables	
Linbro Plates	Hampton HR3-112 $319/100 plates
22mm square cover slips	
96 well Corning plate	Hampton HR3-115
Reagents	Hampton Research 1-800-452-3899
Index	HR2-130 $126
Crystal Screen HT	HR2-134 $126
Salt HT	HR2-136 $126
Wizard I&II	EBS-BWZ $ 275
Equipments	
Microcentrifuge	
Dynamic Light Scattering Machine	Protein Solutions
Pipetman P2, P1000	Rainin

Procedure

1. Do DLS on sample (look at SOP for DLS) and find a condition in which the protein is monodisperse.

2. Protein solution can be in 20-50 mM Hepes, pH 7.5, 1 mM EDTA, 0.1 M NaCl with any additives necessary to make it monodisperse. The buffer, pH and salt concentration can be adjusted based on what the protein is stable in. A good place to start in terms of concentration is: dilute protein 1:100, measure OD280 so that you obtain a reading of 0.1 (~10mg/ml). Of course, if the protein has no tyrosines/tryptophans, this will not work.

3. Determine correct protein concentration to use by using Hampton Research Crystal Screen I, drop 6. Using a Linbro plate, place 0.5 ml of solution 6 in a well. Grease top of well. On coverslip, place 0.7 µl of protein + 0.7 µl of reservoir. Flip coverslip over well, wait 30 min at room temp. If drop shows high precipitate, dilute protein 1:1 in buffer and set up again. If still too concentrated, dilute again. Continue to do so until you determine what a good concentration is that gives slight granular precipitation. If protein has to be diluted to 3 mg/ml, then use the PEG Light Screen (Hampton Research).

4. Set up Screens.

5. The initial Screening is done by using the robots Hydra Plus One or Phoenix. Eight 96 well Corning plates are set up using 0.2 µl of protein per well. The Screens used are Index, Screen I&II, Salt (Hampton Research) and Wizard (Emerald Biosciences) . Four plates are kept at room temperature and four at 4 ^{o}C

6. If protein contains more than 0.3 M NaCl, NaCl has to be added to the reservoir at a concentration of 0.3 M. The NaCl is added to the reservoir AFTER making the drops.
Same is true if the sample is in 5% glycerol. Add 5% glycerol to the reservoir after forming the drop.

7. If crystals come up in a PEG condition, try the PEG/Ion Screen (Hampton Research) (this is an excellent screen).

8. Other Screens to try if you do not obtain crystals:
PEG pH Screen Crystal Screen II