Reproducing HWI HTCSC crystallization screening hits

Use a Greiner 72-well microbatch plate, treated hydrophilic (Hampton Research HR3-121)
alternative (untested): Nunc Microwell 72-well minitrays

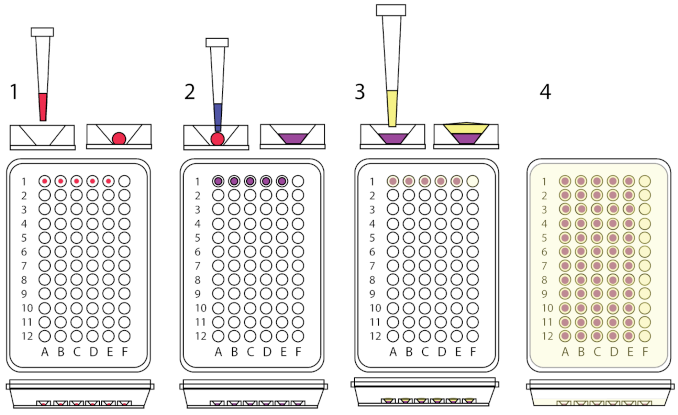
Set up 4-5 replications each of experiments (a) through (c) by following the protocol below:

(a) 1 μL protein + 2 μL cocktail
(b) 1 μL protein + 1 μL cocktail
(c) 2 μL protein + 1 μL cocktail

Step 1: Add the cocktail drop to each of the replicate experiment wells in a row of the plate. You can use a microscope to watch the drop deliveries to ease set up if desired.
Step 2: Add the protein drop (do not mix) by touching the pipette tip to the cocktail drop and dispensing the solution to each of the replicate experiments.
Step 3: Add 20 μL of Paraffin oil (PX0045-3 EMD chemicals) to each well, after each row of replicate experiments is completed.
Step 4: Once all of the drops have been set up for a given plate, cover all of the wells in the plate with an additional 5-6 milliliters of the Paraffin oil. Incubate the plate at 23°C.

Notes:

- If crystals appeared in 4-6 weeks, mineral oil may work better as the evaporation rate is lower.
- Approximately 1/4 to 1/3 of the time different results occur (hence the replications).
- Reproduction rate is >80%.



Optimization

Introduce small variations in cocktail/protein/precipitant/buffer/water ratio

- Gradient screen pH 0.2-0.5 intervals
- Precipitant concentration 2-5% increments (or 10-25 mM if a salt)

Vapor Diffusion

Hanging drop or sitting drop

- Set up several drops on each cover slip or several drops in sitting drop wells to test different protein/precipitant ratios.
- Set up reservoirs with both cocktail and 1:1 cocktail and buffer
- Replicate

Reproduction rate is lower ~30% and crystals not as high visual quality.
Vapor diffusion is better suited for an additive screen.