

### Setting up a classical hanging drop vapour diffusion crystal growth experiment

- 1 Grease the rims of the wells of a 24 well vapour diffusion plate (XRL) – (To avoid evaporation of reagents and to improve reproducibility set up 1 well at a time and close each reagent tube after use.) -
- 2 Pipette 0.5 ml of reagent 1 from the screen into well A1.
- 3 Using a 22 mm round coverslip place a 1 $\mu$ l drop of sample into the centre of the coverslip.
- 4 Add a volume, equal to the sample size, of screen reagent 1 from well A1 to your sample.
- 5 Simply allow the two drops to diffuse together; do not mix.
- 6 Invert the coverslip quickly over well A1 to minimize evaporation and make sure the coverslip is sealed on the well edge by giving it a slight twist back and forward.
- 7 Continue with the appropriate reagents in remaining wells. (Reagent 2 to A2 etc.)
- 8 Note which reagent you used in which well.
- 9 Repeat this procedure using the remaining reagents in the wells.

### Incubation for crystal growth trials

Place the trays in a constant temperature room or cabinet. Many proteins vary in solubility as a function of temperature. Crystallizations have been reported from 0° - 60°C., but are usually carried out at 4°C or room temperature. For initial screening choose whichever will give you the most constant conditions.

### Systematic screens

Whilst a complete screening kit can be set up at the same time protein sample may well be in short supply. An advantage of systematic screens is that there are different ways to use such kits, depending on the amount of protein (and time) available. With a limited amount of protein set up six drops (1-2  $\mu$ l of protein solution, plus 1-2  $\mu$ l of the least concentrated salt condition). Wait for a couple of days, then transfer the coverslip onto the reservoir of next concentration. In this way, both protein and crystallising agent concentrate. This method is especially useful when working with low protein concentration (< 5 mg/ml). This can be done with the six salts, but also by testing just 2 conditions per pH, e. g. sodium chloride and ammonium sulphate at pH 4.5, ammonium sulphate and sodium acetate at pH 9.0, MPD and a PEG at pH = pl.

If precipitation occurs already when setting up the drop, add reservoir solution to the drop until it becomes clear.