Optimization of crystallization conditions with additive screening

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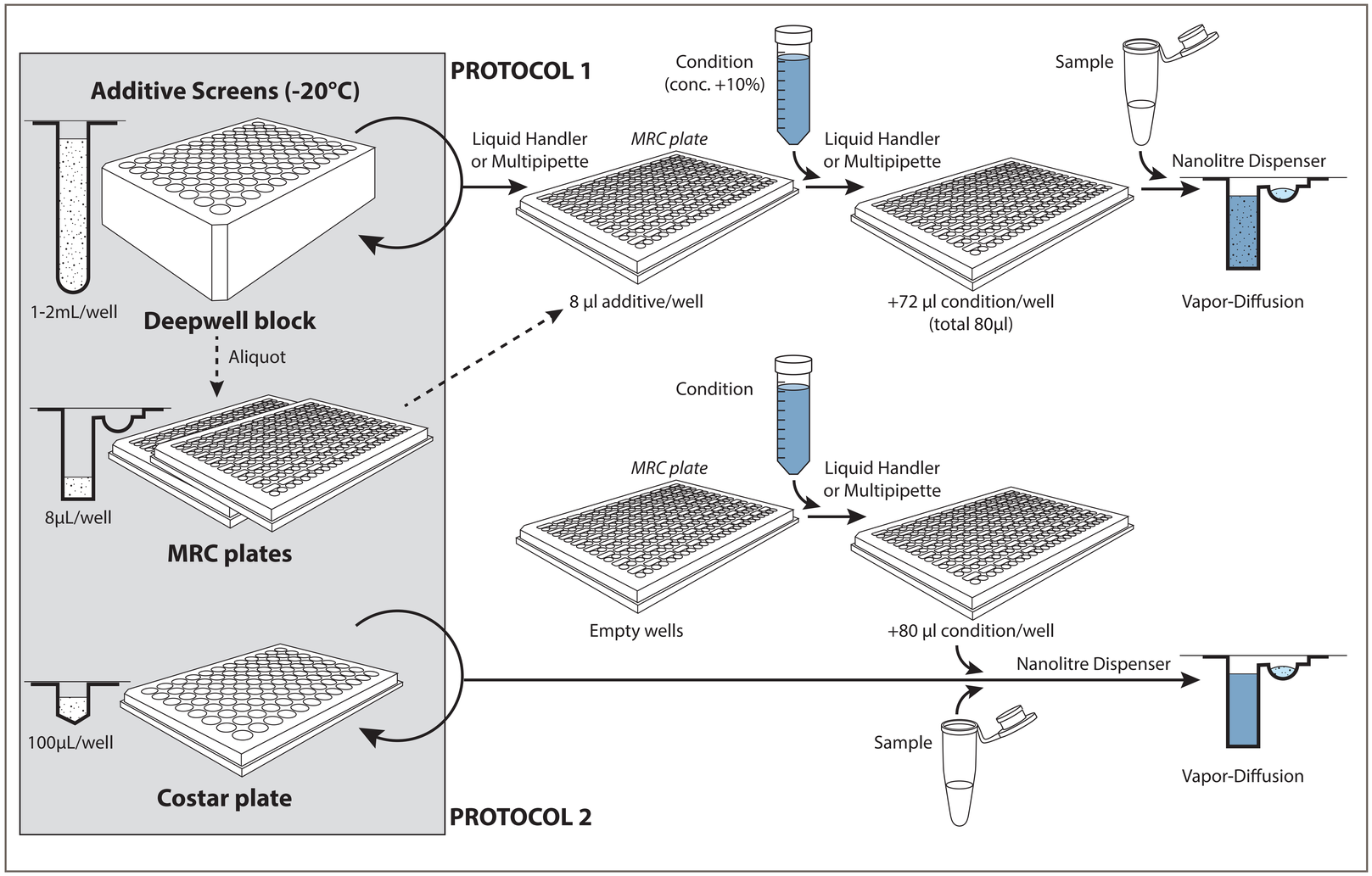
Additive screens are a popular and successful approach to the optimization of initial crystallization hits since additional reagents can be found that enhance properties of the resulting crystals.

A convenient way to perform these screens is to dispense an additive screen into the reservoirs of the crystallization plate where they are then mixed with the condition. Because of the dilution of the condition with the additive, the condition needs to be prepared at a proportionally higher concentration than initially. After this, one simply proceeds with normal set up of droplets that mix reservoir and sample in the upper-wells of the plate (*e.g.* 100 nl protein + 100 nl condition already mixed with additives).
Another way to perform additive screening conveniently is to dispense an additive screen while setting up the droplets (*e.g.* 200 nl protein + 200 nl condition + 100 nl additive). This late approach facilitates the screening at different concentrations of additives (by simply varying the volume of additive screen added) and implies a dilution of the droplets (which will also alter crystallization).
The Figure below shows the steps to perform 96-condition additive screening in MRC plates starting either with additives already in the reservoirs of an MRC plate (**'protocol 1'**) or in the wells of a cell culture plate ('Costar plate', 96 wells with V-shaped bottom, '**protocol 2**').

PROTOCOL 1. The volume of additive screen stored in MRC plate represents here 10% of the final volume in reservoirs (8 µl for a 80 µl final volume). The condition is dispensed into the reservoirs of an MRC plate with a liquid handler or manually with a multichannel pipette ('multipipette'). Later, a nanolitre dispenser is used to set up the droplets mixing sample and conditions (pre-mixed with additives) into the upper-wells.

PROTOCOL 2. Here, a nanolitre dispenser is employed to prepare droplets starting with 3 components: a Costar plate containing the additive screen (100 µl initially), the protein sample and an MRC plate pre-filled with 96 times the same crystallization condition (a liquid handler or a multipipette is used to pre-fill the plate with 80 µl, as needed here, instead of the 72 µl).



Most of the additive screens are commercially available in deep well blocks with 96 additives. The screens are normally stored at -20 °C since they are not used regularly and contain volatile and unstable compounds. The use of a frozen additive screen stored in deep well block must be planned the day before because it will take over night for all the additive solutions to thaw completely at room temperature. Also, a multitude of users might share use of the same additive screen, potentially causing problems with cross-contamination.

Finally, the height of most deep well blocks makes them entirely unsuitable for the Mosquito nanolitre dispenser. As a convenient solution to circumvent reproducibility issues and make use of our Mosquito, we purchase additive screens pre-dispensed into much smaller volumes and stored frozen in low profile plates (MRC and Costar plates).

The Visual aids department gives support to the scientists of the LMB by preparing videos , photos and figures.
The figure presented here was designed by Jo Westmoreland

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