PROTOCOL System 2: setting up crystallization droplets (100 nL protein + 100 nL condition) from a single sample in 20 LMB plates

1. First, ensure that the system's robots are on, the nanoliter dispenser is initialized with its software open, and the liquid handler's method manager is open. Turn on the microtube-cooling carrier about 15 minutes before the main program will be started.

2. Place a test plate on the motorized SBS carrier of the plate sealer. Run the plate sealer and verify that the plate is properly sealed. Test the plate sealer three times.

3. Then, run the nanoliter dispenser to set 100-nL droplets of test solution in a test plate. Check under a microscope that the droplets are set correctly. Close the nanoliter dispenser software and remove the strip-holder block from the deck.

4. Next, insert in the custom-designed plate holder the LMB plate with the highest relative quantity of volatile reagents in its conditions. Remove the adhesive film from the plate.

5. Open the liquid handler front panel and place the unsealed plate on the deck, at the back of the first sliding carrier (Sliding carriers may be pulled out for ease of access).

6. Cover the plate with an SBS aluminum lid. Settle the lid towards the rear left corner of the carrier by applying gentle pressure to the opposite corner of the lid.

7. Unseal, load into the sliding carriers, and cover the remaining 19 plates in the same way, working from most to least volatile conditions. Once the microtube-cooling carrier is at 4 °C, as indicated by a green light, remove its cover.

8. Cut off the lid of a microtube containing (at least) 440 µL of protein sample (**Table 1**). Ensure that the sample has no foam above the meniscus, as this will interfere with the liquid detection system. Place the tube in Position 1 of the microtube-cooling carrier.

9. Place a PCR plate on the liquid handler deck in front of the plate-moving adapter carrier. Then, close the front panel.

10. After loading the deck, ensure that the nanoliter dispenser deck is clear of the strip-holder block and that the carriers at the back of the 50-µL tip stacks are clear of the aluminum SBS lids.

11. In the liquid handler 'Method Management' interface, select 'Setup plates'. Monitor the initialization of both systems and fill in the run parameters. Follow the guidelines of the Method Management interface (prompts, figures and a tip-management system help with preparation).

12. Double-check that all required components are ready, and then start the process.

13. Monitor the system as the nanoliter dispenser sets the drops in the first plate and the plate sealer subsequently seals the plate.

14. Once all 20 plates have been prepared with crystallization droplets and automatically returned to the sliding carriers, open the front panel and gently remove the plates. Check that the plates are correctly sealed before storing them for crystallization.

15. Clean the SBS lids with a 20% ethanol solution before stacking them on the left-hand side of the liquid handler for storage. Discard the PCR plate and the microtube.

16. Turn off the microtube-cooling carrier and wipe away the condensation. Leave a paper towel on top of the cooler surface to absorb further condensation. Then, replace the carrier cover and close the front panel.

Table 1. Requirements for the main program options according to user selections on the system 2. The necessary amount of protein sample, tips and microsyringes vary according to the program.

Plates		Drop size (nL)	Sample(s)		Tip requirements		Duration
Number	Type		Vol. 1 (µL)	Vol. 2 (µL)	50 µL tips	Mosquito	
10	MRC	100	240	0	80	1040	1 h 12 min
20	MRC	100	440	0	160	2080	2 h 20 min
10	MRC	100	240	240	160	2080	1 h 45 min
20	MRC	100	440	440	320	4160	3 h 05 min
10	MAXI	1000	624	0	80	560	1 h 10 min
20	MAXI	1000	1208	0	160	1120	2 h 16 min

FG Dec. 2017.