Principal for protein crystallization

The crystallization is a process based on slow salting out and salting in. Where any soluble protein is forced to pushed out of the solution with the help of salt present in.

Reservoir is a solution where crystals grow. It contains various organic and non-organic salts.

After coming out the solution multiple protein units come together and arranged in an organised orientation. Such multiple arrangement in same direction forms Crystals.

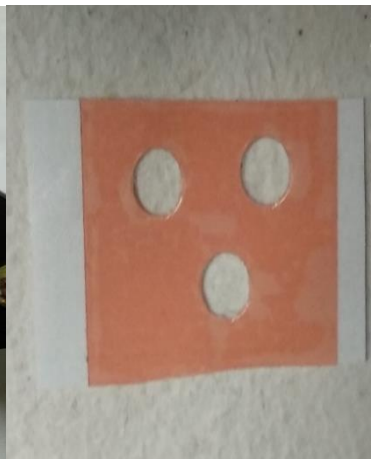
These process is done under low temperature, such that the protein units get much time to organize. Generally we use three different proportion of protein and reservoir to make crystals

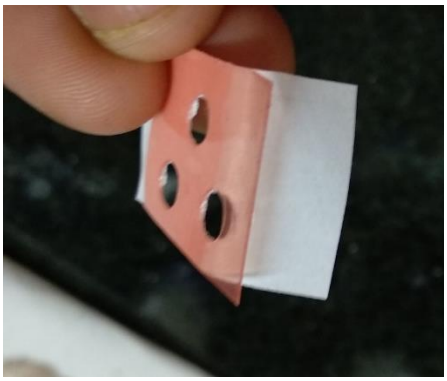
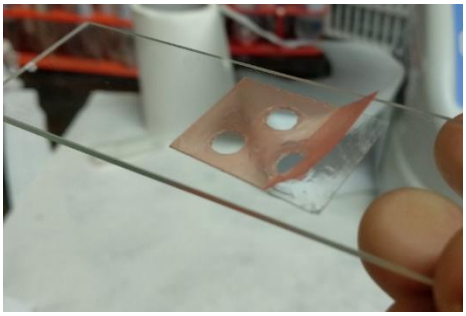
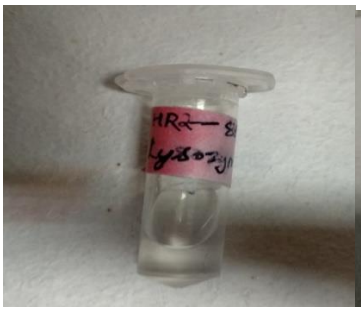
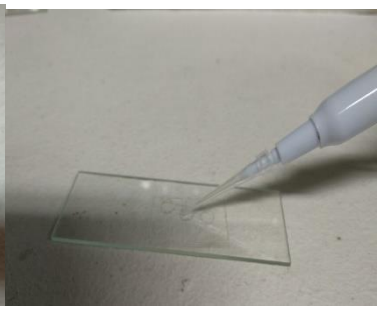
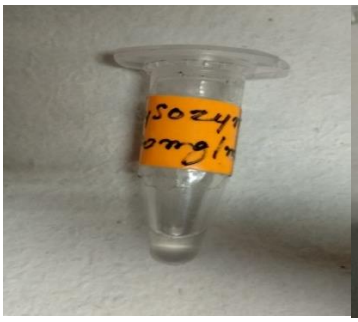
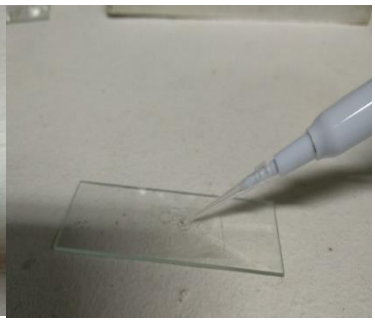
Reagents Required

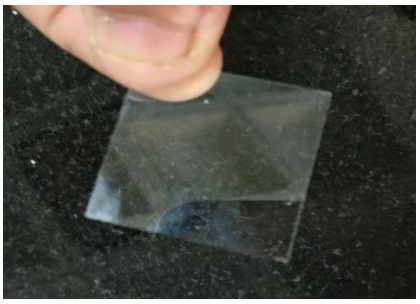
1) For dissolving lysozyme powder: 10Mm Tris-cl pH=8

2) Reservoir: PEG MME 5000 (30%), Nacl (1M), Sodium Acetate Trihydrate (0.05M)

Method:

Steps	Method	Image
1	Wipe glass slide with tissue paper.	
2	Paste Red colored transparent double sided tape on releasing paper.	
3	Make three punches in Red colored transparent double sided tape.	 

4	Cut the tape and remove the releasing paper, followed by pasting it on glass slide.	
5	Remove the red covering from the tape.	
6	Take reservoir solution and with the help of pipette, add 0.75μl, 1 μl and 0.5 μl on the slide in different punched hole created cavities.	 
7	Similarly add protein 0.75μl, 0.5μl and 1μl respectively. That is the ratio of 1:1, 1:2 and 2:1.	 

8	Immediately place a 25mm X 25mm coverslip.	
9	Place the slide for 15 minute for incubation.