The Liu Laboratory protocol — OCP proteolysis
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OCP proteolysis:

Clean water bath and pour 4˚C storage water that is usually saved in cold room. Refill the bottle and save in cold room for next use. Switch water bath on, with setting to 4˚C, connect water bath to reaction chamber.

1. Dilute OCP in cold digestion buffer on ice (4˚C, 10 mM HEPES, pH 7.8, 200 mM NaCl) to 1 mg/ml. (Stock solution on bench shelf).

2. Load 2 ml of diluted OCP to reaction chamber (pre-chilled to 4˚C), stir on

3. Strong light on (our projector) for 10 min, photoactivation.

4. Add Trypsin resin (500) ul to the chamber. Light switching to low, incubation for 4 hours.

It's not necessary to incubate for overnight from literature.

5. Distribute digested OCP with resin to two centrifuge tubes (on ice).

6. Spin (max speed) in cold room for 5 min.

7. Carefully transfer supernatant (digested NTD, CTD) to chilled tube and save on ice,

8. Add 0.5 ml cold digestion buffer to the pellet and mix, repeat step 6, combine supernatant to Step 7.

9. Transfer digested NTD, CTD (from Step 7 and 8) to 0.22 um filtration centrifuge tube.

10. Collect and concentrate flowthrough by using 10 kDa centricon filter.

11. SDS-PAGE checking digest result.

Further isolation of NTD and CTD

Buffer exchange NTD, CTD solution to Tris-buffer (20 mM, pH 7.5) and gently mix NTD, CTD and clarify by centrifugation at 4C. Load supernatant to Q-HP column, followed by linear gradient of 0-100 mM NaCl. Fraction collection and concentrate. SDS-PAGE checking each collection, combine NTD, CTD peaks and further polishing by using SEC column.