DNA-encoded library selection protocol - Alix Chan of the David Liu lab 2016-11-11

Reagents:

- His Dynabeads (or equivalent for your tag of interest)
- Yeast total RNA (10 mg/mL I have a lot of this)
- BSA (ultrapure, 50 mg/mL)
- 5M NaCl
- 1M Tris pH 8.
- 1M sodium phosphate pH 8
- Molecular biology grade (DNAse, RNAse, protease free) water.
- 4M imidazole pH 8 (or other concentrated eluent solution - glutathione, SDS, etc)

Buffers, made fresh from 5x stocks of PBST and TBST

PBST: 50 mM sodium phosphate pH 8.0, 300 mM NaCl, 0.01% Tween-20 (± 5 mM DTT) (make 1 mL/sample/selection)
TBST: 50 mM Tris-HCl pH 8, 150 mM NaCl, 0.05% Tween-20 (± 5 mM DTT) (1.1 mL/sample/selection)
Blocking: TBST with 0.1mg/mL BSA, 0.6 mg/mL yeast RNA 15 min @ 4°C. (150 µL/sample/selection)
Library: 1 pmol/50 µL, or 20 nM, in blocking buffer. (50 µL/sample/selection)
Elution: PBST with 300 mM imidazole (50 µL/sample/selection)

Selection:

1. Wash 25 µL resin (volume for His dynabeads) with 2x 300µL PBST.
2. Dilute 5-25 µg protein to 300 µL in PBST. Incubate with resin 30 min @ 4°C. (Cold room rocker, tilted just short of vertical so the tubes are almost horizontal, set @ ~20%. Some may clump, just resuspend manually).
3. Save the protein FT that didn't bind, freeze on -80. Wash with 2x 200 µL TBST.
4. Blocking: 100 µL TBST with 0.1mg/mL BSA, 0.6 mg/mL yeast tRNA. 15 min @ 4°C.
5. Incubate 1-2 pmol library in 50 µL TBST w/ BSA/tRNA, 60 min @ 4°C. Collect FT.
6. Wash 3x 200 µL TBST. Switch tubes each for each wash (resuspend, take up suspension to new tube, place on magnetic rack and remove supernatant). This prevents contamination from library in/outside the tube.
7. Elute with 50 µL PBST with 300 mM imidazole (5 min)

notes:

- store FTs/beads (samples with high library concentration) SEPARATE from the eluates. Freeze on dry ice.
- once protein is loaded onto beads, keep on ice as much as possible (until elution).