SELEX protocol

Note:

1. All solutions should be vortexed before adding.
2. Gloves are always important in experiment

Binding buffer:

1. Tris pH7.5 50mM
2. KCl 5mM
3. NaCl 100mM
4. $MgCl_2$ 1mM

Binding buffer (with Tween20):

Tween 20 0.02%

Elution buffer=1XPCR buffer

Library Preparation:

1. ssDNA/PCR product are dissolved in 500ul of binding buffer.
   Note: ssDNA:10uM SELEX probe 1ul/ PCR product: depending on how dark the band is and how many tubes are being added, usually adding to the total of 5ul.
2. The library was denatured at 95℃ for 5 min, cooled immediately in ice for 15min.

SELEX

1. **Binding**: The library is transferred to the SELEX tube, the reaction is then performed on the 3D rotary mixer for 1 hr in 4℃ fridge. Centrifuged at 13,000rpm, 2mins, then collect the supernatant as flow through (FT) to a clean eppendorf.
   Note that: To avoid drawing out the gels on the buttom of the SELEX tube when collecting the supernatant, centrifuge with the unite position with the cap ear on the outer side, then carefully place the tip at the opposite side of the cap ear when drawing flow through, or the other solution in the latter steps.
2. **Washing**: Add 350ul of binding buffer into SELEX tube, then shake the SELEX tube up and down like making hand-shaken drinks for 60-80 times, this is to wash down the unbind ssDNA strains.Centrifuged at 13,000rpm, 1min then collect the supernatant as washing 1(W1) to a clean eppendorf.
3. Repeat the second step for three more times to collect washing 2(W2), washing 3(W3), and washing 4(W4).
   Note that: On the third repeat of washing, add binding buffer with tween 20 to wash down the unbind strains completely, but not the last repeat in case tween 20 effects the quality of elution.
4. **Elution**: Add 350ul of elution buffer in to SELEX tube, then shake the SELEX tube up and down like making hand-shaken drinks for 60-80 times, this is to grab the ssDNA bound on the gels down into the elution buffer. Centrifuged at 13,000

rpm, 1min, then collect the supernatant as elution1(E1) to a clean eppendorf.

5. Repeat the fourth step for four more times to collect elution2(E2), elution 3(E3), elution4(E4) and elution 5(E5).
6. DNA prescription