CIS Display Selection

-The Han Lab

First, prepare the plasmid pNbLib-His-RepA-cis-ori as below:

1) PCR Nb-Lib from original NbLib-final with: #3131, #3132, #3133 and #3134; ~480bp
2) PCR RepA-cis-ori with: #3129, #3130 and #2503; ~1.3kb
3) Fuse them with: #2502 and #2503; ~1.8kb
4) Digest it with Nhe1/Xho1, and ligate it into Nhe1/Xho1 of pYG7002 to make pNbLib-His-RepA-cis-ori

Second, to generate the NbLib-Final2 for in vitro transcription and translation:
1. In vitro transcription and translation with S-30 lysate system (30min at 30C) (20 ug purified NbLib-Final2 was added to 250 ul S-30 lysate reaction for 1st round, then 5 ug library DNA in 100 ul S-30 lysate reaction)
2. His-purification
3. Dilute 10-fold with blocking buffer (2-4% Marvel, 0.1 mg/ml herring sperm DNA, 2.5 mg/ml heparin, in PBS or TBS)
4. Solid-phase selection:
a. Immobilize the targets (10 ug/ml, for AAV9, AAVrh10, 10^11 vg =~ 622 ng) on 4 ml of NUNC Star immunotubes (Fisher Scientific), coated overnight at 4C.
b. Wash twice and block for 1 h at room temperature with blocking buffer
c. Wash twice with PBS
d. Incubate with the diluted transcription/translation reactions for 1 h at room temperature
e. Wash 6-12 times with PBS/0.1% Tween-20
f. Wash 6-12 times with PBS
g. Elute DNA with 500 ul PB solution (Qiagen) and purified by using QIAquick PCR purification kit (Qiagen)
h. Recover the selected library with PCR using #2502 and #Nb-R; atcctagggccccAGACGACACCGTGACTTGCGTGCCCTG; ~430bp
i. Ligate it into RepA-cis-ori (PCR with #3129 and #2503) with Not1, PspOM1, T4 DNA ligase
j. Repeat steps 1-4 for 3-5 more rounds of selection.

(The end)


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