2018-6-25 Defined spacer acquisition (DSA) pilot experiment

1. Start liquid cultures of *E. coli* BL21(DE3) ΔCR-I, GRA (ΔCRISPR-I, gfp(rev)-array) strain harboring pCas12 from plates and grow overnight at 37°C
2. In the morning, dilute the overnight grown culture 1:30 in 3 mL (100 µl) fresh LB containing 25 μg/mL Chlo (3 µl of the 25 mg/mL stock) and 100 ng/uL aTc (3 µl from 100 mg/mL stock), and grow for an additional 2 h (we can try 2, 4, 6 h for this step to increase DSA efficiency) at 37°C.
3. Spin down cells (1 mL of culture) and resuspend and wash in 1 mL ice-cold water three times to remove residual media (centrifuge at 10,000 rpm for 1 min) at 4°C.
4. Resuspend cells in 50 μL (per 1 mL of 3 mL culture) of cold water containing fwd and rev oligo strands\* each at a concentration of 3.125 μM (we can try 3.125, 6.25, 12.5, 25 μM for this step to increase DSA efficiency), and electroporate with a Bio-rad gene pulser set to 1.8 kV, 25 μF, and 200 Ω.
5. Immediately following electroporation, resuspend cells in 3 mL fresh LB, and recover cells for overnight
6. Repeat for 3 times

**\*Defined spacer oligo**

psAA33 positive control:

psAA33(fwd): 5’AAGCCCAATTTACTACTCGTTCTGGTGTTTCTCGT

psAA33(rev): 5’ACGAGAAACACCAGAACGAGTAGTAAATTGGGCTT

T7 promoter:

T7 (fwd): 5’AAGCCCTATAGTGAGTCGTATTAATTTCGCGGGAT

T7 (rev): 5’ATCCCGCGAAATTAATACGACTCACTATAGGGCTT

1. Mix 50 μM of each of fwd and rev oligos in 1:1 ratio, heat at 95C then slowly cool down to room temp to anneal oligos and get 25 μM of defined spacer oligo solution.
   1. Note – made 50 µM stocks by resuspending the ordered oligos in (2xng x 10) µl nuclease free water, vortexing & putting on heat block & repeating til dissolved
2. 2-fold serial dilution to get 25, 12.5, 6.25, 3.125 μM concentrations
3. Store at -20°C