

## Combigem Library Construction Protocol (Updated May 2017)

Below is a step-by-step guide for building your combinatorial gRNA library:

### 1. Generation of plasmid library in *E. coli*:

#### a. 1<sup>st</sup> round reaction:

1. Digest pAWp28 (Addgene plasmid #73850) with *BbsI* + *MfeI*. Use ~4.2kb fragment.
2. Anneal forward and reverse oligos (Forward and Reverse) to create double-stranded inserts.

Reaction to phosphorylate and anneal each pair of oligos:

1 ul Oligo F (100μ M)  
1 ul Oligo R (100μ M)  
1 ul 10X T4 Ligation Buffer (NEB)  
6.5 ul ddH<sub>2</sub>O  
0.5 ul T4 PNK (NEB)  
10 ul total

Put the phosphorylation/annealing reaction in a thermocycler using the following parameters:

37o C 30 min

95o C 5 min and then ramp down to 25oC at 5o C/min

3. Mix all annealed and phosphorylated oligos from Step 2 in 1:1 ratio.
4. Dilute annealed and phosphorylated oligos from Step 3 at a 1:200 dilution into H<sub>2</sub>O.
5. Clone using ligation, incubate at room temperature for >1 hour.

50 ng digested plasmid from Step 1  
1 ul diluted oligo duplex from Step 4  
1 ul 10X T4 Ligase Buffer (NEB)  
0.5 ul T4 Ligase (NEB)  
x ul ddH<sub>2</sub>O  
10 ul total (x number of reactions required to aim at >100-fold representation)

6. Transform the vector constructs into *E. coli* competent cells. Inoculate into LB liquid culture with 50 μg/ml of carbenicillin (Teknova) for overnight culture. Take a portion of transformed bacterial cells and spread on LBA agar plate for overnight culture.
7. Determine the number of colonies on plate to evaluate library coverage. Isolate colonies for sequencing to check the library quality.

#### b. 2<sup>nd</sup> round reaction:

1. Digest pAWp28-guide vectors (the product from (a)) with *BbsI*. Use ~4.3kb fragment.
2. Annealing of Oligo S1+ S2.
  - S1: 5'-  
GTTTGTAGAGCTAGAAATAGCAAGTTAAAATAAGGCTAGTCCGTTATCAACTTGAAAAA  
GTGGCACCGAGTCGGTGCTTTTTTGGATCCGCAACGGA-3'
  - S2: 5'-

GAATTCCGTTGCGGATCCAAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAA  
CGGACTAGCCTTATTTTAACTTGCTATTCTAGCTCTA-3'

3. Clone using ligation  
(conditions same as described in (a.)).

**c. One-wise library construction:**

- i. Digestion of barcoded gRNA library (the product from (b)) with MfeI+BglII. Take ~400bp fragment.
- ii. Digestion of pAWp12 (Addgene #72732) with EcoRI+BamHI. Take the vector backbone.
- iii. Ligation of the above two fragments.

Ligation products were transformed to *E. coli* competent cells. (Fold representation > 100).

**d. Two-wise library construction:**

- i. Digestion of barcoded gRNA library (the product from (b)) with MfeI+BglII. Take ~400bp fragment.
- ii. Digestion of one-wise library with EcoRI+BamHI. Take the vector backbone.
- iii. Ligation of the above two fragments.

Ligation products were transformed to *E. coli* competent cells. (Fold representation > 100).

**For further details, please check out our two publications:**

- Wong, S.L.A., Choi, C.G.G., Cui, C.H., Pregernig, G., Milani, P., Adam, M., Perli, S.D., Kazer, S.W., Gaillard, A., Hermann, M., Shalek, A.K., Fraenkel, E., Lu, T.K. Multiplexed barcoded CRISPR-Cas9 screening enabled by CombiGEM. PNAS 113(9), 2544-2549 (2016).
- Wong, S.L.A., Choi, C.G.G., Cheng, A.A., Purcell, O., Lu, T.K. Massively parallel high-order combinatorial genetics in human cells. Nature Biotechnol. 33(9), 952-961 (2015).