

## How to clone Targets into pCP5b for Yeast TALEN Assay:

### 1. Oligos

Order the Sense and Anti-sense oligos from IDT, usually I order 25nmole with standard desalting

For Example:

```
sense:      5' GATCtAAAAAAAAAAAAAAAAAAAACTCTCTCTCTCTCGGGGGGGGGGGGGGGGGGGGGa
DNA          tAAAAAAAAAAAAAAAAAAAACTCTCTCTCTCTCGGGGGGGGGGGGGGGGGGGGGa
DNA          aTTTTTTTTTTTTTTTTTTTTTTGAGAGAGAGAGAGAGCCCCCCCCCCCCCCCCCCCCt
Anti-sense: 5' CTAGtCCCCCCCCCCCCCCCCCCCCCGAGAGAGAGAGAGAGTTTTTTTTTTTTTTTTTTTTa
```

On sense Oligo : GATC is the BglII compatible overhang  
t is the t at the -1 position for TALEN 1  
AAAAAAAAAAAAAAAAAAAAAAAA is TALEN1  
CTCTCTCTCTCTCTC is the spacer  
GGGGGGGGGGGGGGGGGGGGG is complementary to TALEN2  
a is complementary to the t at -1 position for TALEN2

On Anti-sense Oligo : CTAG is the SpeI compatible overhang  
t is the t at the -1 position for TALEN2  
CCCCCCCCCCCCCCCCCCCC is TALEN2  
GAGAGAGAGAGAGAG is the spacer  
TTTTTTTTTTTTTTTTTTTT is complementary to TALEN1  
a is complementary to the t at -1 position for TALEN1

### 2. Vector

Digest pCP5b with BglII+SpeI+CIP  
10ug pCP5b  
30ul 10X Buffer 2 (NEB)  
3ul 100X BSA (NEB)  
10ul BglII (NEB)  
10ul SpeI (NEB)  
10ul CIP (NEB)  
? ul water (up to 300ul)

Incubate at 37°C for 1h

Expected Sizes: 10370bp and 2493bp  
Gel purify the 10kb band (use Qiagen kit)  
Quantify recovered DNA

### **3. Phosphorylation and annealing**

Suspend oligos to 100 $\mu$ M in TE

Mix:

3 $\mu$ l Sense oligo

3 $\mu$ l Anti-sense oligo

3 $\mu$ l 10X T4 DNA Ligase Buffer (NEB)

2 $\mu$ l T4 Polynucleotide Kinase (NEB)

19 $\mu$ l water

Incubate at 37°C for 30min

Add 4 $\mu$ l 0.5M NaCl

Boil for 2min

Cool slowly on bench to room temperature

Dilute 1 $\mu$ l into 499 $\mu$ l water

### **4. Ligation**

Mix:

1 $\mu$ l diluted annealed oligos

40ng pCP5b/BglII+SpeI+CIP

2 $\mu$ l 10X Ligase Buffer (NEB)

1 $\mu$ l T4 DNA Ligase (NEB)

?  $\mu$ l water (up to 20  $\mu$ l)

Incubate at Room temperature 2h

Transform 5 $\mu$ l into DH5 $\alpha$

Plate on LB+Ap

Sequence clones with P789: ACAGAAAAGCAGGCTGGGAAGCA