

## Travelling Salesman Protocol #1:

### Prep

Pre-thaw 30uL of beads, wash 3x with 100uL of wash buffer each to prepare.

### Anchor

Drain wash

+3 uL A-Ori

+17 uL wash buffer

Resuspend

Incubate 20min

### Wash

2x, 100uL

### Ligation 1

Drain wash

+3 uL ligase buffer

+17.5 uL water

+3 uL Amp

+4 uL Kan

+1.5 uL Chlor

+1 uL ligase

Incubate 30min

### Wash

2x 100uL

### Linker

Drain wash

+2 uL ligase buffer

+16 uL water

+1 uL linker (concentrated stock)

+1 uL ligase

Incubate 30min

### Wash

3x 100uL

### Ligation 2

Drain wash

+3 uL ligase buffer

+19.5 uL water  
+1 uL aCP  
+4 uL RFP  
+1.5 uL GFP  
+1 uL ligase  
Incubate 30min

Wash  
2x 100uL

Linker  
Drain wash  
+2 uL ligase buffer  
+16 uL water  
+1 uL linker (concentrated stock)  
+1 uL ligase  
Incubate 30min

Wash  
3x 100uL

Ligation 3  
Drain wash  
+3 uL ligase buffer  
+19.5 uL water  
+1 uL aCP  
+4 uL RFP  
+1.5 uL GFP  
+1 uL ligase  
Incubate 30min

Wash  
2x 100uL

Linker  
Drain wash  
+2 uL ligase buffer  
+16 uL water  
+1 uL linker (concentrated stock)  
+1 uL ligase  
Incubate 30min

Wash

3x 100uL

#### Ligation 4

Drain wash

+3 uL ligase buffer

+17.5 uL water

+3 uL Amp

+4 uL Kan

+1.5 uL Chlor

+1 uL ligase

Incubate 30min

#### Wash

2x 100uL

#### Tail End

Drain wash

+2 uL ligase buffer

+16 uL water

+1 uL Poly-T

+1 uL ligase

Incubate 30min

#### Wash

3x 100uL

#### Elution

Prep storage tube with 2uL of 50X TE

Drain wash

+20 uL NaOH (10mM)

Resuspend in under 50 seconds.

Transfer NaOH to storage tube, mix.

Discard beads.