Oligo Annealing

Get 64-mer at 0.05 µM scale, desalted (no HPLC or PAGE purification necessary)

Annealing

- dissolve both oligos in 50 µl of water
- take 2µl of each oligo (forward and reverse)
- add 48µl of annealing buffer (see below)
- incubate 4 min. at 95°C
- incubate 10 min. at 70°C
- cool down oligos on your bench (can be stored at –20°C)

Phosphorylation

- take 5µl of annealed oligos
- add 12µl of water
- add 2µl of T4 ligase buffer (usually containing 1mM ATP)
- add 1µl PNK kinase
- incubate 30 min. at 37°C
- incubate 10 min. at 70°C to heat inactivate PNK

Ligation

- take 5µl of phosphorylated oligos
- add 1µl of vector (20-100ng, CIPed)
- add 11µl of water
- add 2µl of T4 ligase buffer
- add 1µl of T4 ligase (1-5U)

Transform and grow bacteria as usual
Check minipreps by digestion
Sequence since some oligos may be mutated.

Annealing buffer:

- 100mM potassium acetate
- 30mM HEPES pH7.4
- 2mM magnesium acetate