MULTIMERIZATION OF OLIGONUCLEOTIDE CASSETTES
Adapted from BV's Cookbook, TCH 1/27/02

1. When designing the oligos add some spacer sequences between the actual binding sites to avoid creation of artificial tandem repeats. Also design some oligos with mutations of critical nucleotides as controls.
2. A useful vector to insert your favorite binding site is pBV-Luc (created by Jian Yu and Luis da Costa), which has a very low background activity in SW480 and other CRC cells. This vector has a convenient Kpn I site for insertion of the concatemerized binding sites.
3. Oligos should be gel purified when > 30 nt. Dissolve oligos to a final concentration of 2 ug/ul.

Kinasing Reaction:
15 ul H_2O
20 ul annealed (or 10 ul separated) oligos
5 ul 10 mM ATP
5 ul 10x kinase buffer
5 ul polynucleotide kinase
50 ul, 37°C 1 h

Annealing Reaction:
40 ul sense oligo
40 ul antisense oligo
80 ul total, 95C 3 min, then ramp to 25°C over 25 min (e.g. Perkin Elmer PCR machine: program 7)
Isolate via 12% non-denaturing polyacrylamide gel, dissolve in 50 ul water.

Concatemerization:
1 ul from previous reaction
2 ul 5x ligase buffer
6 ul H_2O
1 ul ligase (NEB)
10 ul for 1 h at RT -> isolate different 1 to x-mers via 12% PA gel, dissolve in 10 ul TE

Insertion of concatamers into vector:
4 ul concatemers
1 ul vector (e.g. Kpn I digested and calf intestine alkaline phosphtaseedpGL3-OF, cipped)
10 ul Rapid Ligase-Buffer 1

2 ul   Rapid Ligase-Buffer 2
2 ul H_2O
1 ul   rapid Ligase (Boehringer Mannheim)
20 ul, 1 h RT (also: control ligation without concatemers), PC8, EtOH-precipitate and transform into DH10B.

Sequence clones with potential wild type and mutant binding sites (e.g., 7 of each).