How to clone in pSico and pSicoR

We order 5'phosphorylated, PAGE purified oligos designed using the program PSICOLIGOMAKER 1.5 (made by Andrea Ventura and available on the Jacks' website).
Oligos are resuspended in distilled water to a final concentration of 100μM.

Oligos annealing:

-ddH2O	23μl
-sense oligo	1μl
-antisense oligo	1μl
-2X annealing buffer	25μl

- incubate 4 minutes at 95°C
- incubate 10 minutes at 70°C
- slowly cool down the annealed oligos to 4˚C (Store at –20˚C)

2X Annealing buffer:
-200 mM potassium acetate
-60 mM HEPES-KOH pH 7.4
-4 mM Mg-acetate

Ligation:

- Dilute 1 μl of annealed oligos in 19 μl of water
- Ligate 1 μl of diluted annealed oligos to 50-100 ng of HpaI-XhoI digested pSico or pSicoR in a 10μl reaction. (Usually we do not dephosphorylate the vector, although it might help in case of partial digestion).
- Incubate at room temperature for 3 hours and transform 2μl of ligation.
- Digest minipreps with SacII-NotI (pSico) or XhoI-XbaI (pSicoR). Positive clones will release a fragment approximately 50 bp larger than the fragment released by the empty vector (710 vs 660 bp for pSico; 400 vs 350 bp for pSicoR). We use a 2% agarose gel to detect the shift. Positive clones should be sequence verified.

For **pSico** we use the following sequencing oligo (it is a reverse oligo and maps downstream to the cloning site):
5'- CAAACACAGTGCACACCACGC

For **pSicoR** we use the following sequencing oligo (it is a forward oligo and maps immediately upstream to the U6 promoter):
5'- TGCAGGGGAAAGAATAGTAGAC

GOOD LUCK WITH YOUR EXPERIMENTS.

Andrea Ventura and Alex Meissner