

Tips for cloning siRNA templates into pSilencer™

Important parameters for successful ligation

- Oligonucleotides should be $\geq 70\%$ full-length, and accurately quantitated: this makes it possible to anneal equimolar quantities of sense and antisense hairpin siRNA oligonucleotides to make full-length, complementary, hairpin siRNA-encoding inserts for ligation.
- Always include a minus-insert ligation control: colonies that grow after transformation with the minus-insert ligation represent “background”. The number of background colonies compared to the number of colonies from the plus-insert ligation indicates whether the ligation worked well or not. Successful plus-insert ligations will generate at least three times as many colonies as the minus-insert ligations.

Tips for transformation of *E. coli*

- Always include a non-transformed competent cell control: this negative control is a culture of your competent cells plated at the same dilutions as your transformed cells. No colonies should be seen on this plate, indicating that the ampicillin or carbenicillin in the culture medium is effective at inhibiting the growth of *E. coli* that do not contain the pSilencer plasmid.
- Use an appropriate amount of ligation product for the transformation. This amount will vary depending on how the competent cells were prepared and on the transformation method.
- Be sure that the bacterial culture medium has cooled adequately before adding 100–200 $\mu\text{g}/\text{ml}$ ampicillin or carbenicillin. Store antibiotic-containing plates at 4°C, and use them before the antibiotic loses its potency.
- Dilute the transformed cells in LB (the appropriate dilution will depend on the transformation method), and plate 2–3 dilutions. Incubate the transformants overnight, and evaluate the results promptly (or store the plates at 4°C until they are evaluated).

Evaluating *E. coli* transformants: Examine each plate

- The non-transformed control culture should yield no colonies (indicating that the ampicillin or carbenicillin in the culture medium is effective at inhibiting the growth of *E. coli* that do not contain the pSilencer plasmid)
- Identify the dilution of plus- and minus-insert ligation transformations that yields a reasonable number of well-spaced colonies. The minus-insert ligation will probably result in some ampicillin resistant colonies, but the plus-insert ligation should yield 2–10 fold more colonies than the minus-insert ligation. (Remember to take the dilution into account when calculating the percent background.)

