Transformation of Electrocompetent DH10B or XL-1 Blue Bacterial Cells

by TCH 8/13/01, Updated by JK Park 5/12/04

I. For Ligation Products

1) Precipitate the ligation products with ethanol followed by washing with 70% ethanol twice.
2) Let the pellet air-dry (or remove the residual liquid by briefly spinning the tube), and dissolve the pellet in **45ul** ddH_2O.
3) Thaw an aliquot of **5ul** of electrocompetent DH10B bacterial cells on ice (**Note: We are using ElectroMAX DH10B from BRL/Invitrogen; and 5ul-aliquots are kept at -80^{0}C**).
4) Meanwhile, clean 1.0mm cuvettes by power-rinsing with ddH_2O, then 70% ethanol and 100% ethanol. After air-drying, the cuvettes should be chilled on ice.
5) To a new 1.7ml microfuge tube, add **15ul** of precipitated ligation products, along with **5ul** of electrocompetent DH10B bacterial cells. Keep on ice.
6) Transfer the **20ul** DNA/bacteria mix to a pre-chilled 1.0mm cuvette. Bring down the mix to the bottom by gently tapping cuvette. Keep on ice.
7) Prepare **500ul LB** (**no antibiotics!**) in 1.5 ml microfuge tubes.
9) Perform electroporation at **1.8KV**, followed by adding 500ul LB to the cuvette.
10) Mix bacterial cells well by gently pipetting up and down, and **immediately plate** 100ul to 200ul onto 1 to 2 LB plates (containing appropriate antibiotics). [**Optional/preferred**: immediately plate 200ul onto one LB plate, and incubate the remaining cell mix (in 1.7ml microfuge tubes) at 37^{0}C water bath for 20-30min, followed by plating the mix (approx. 100-200ul) onto another LB plate].
11) Incubate at 37^{0}C incubator overnight (usually 12-15hrs).

II. For Plasmid DNA

1) You will need approx. **1.0 to 2.0ul** of miniprep plasmid DNA or **1.0-50.0ng** of purified plasmid DNA to transform DH10B or XL-1 Blue cells. Prepare the DNA in a total of **15ul** with ddH_2O.
2) You may use **5.0ul** of electrocompetent DH10B bacterial cells (or homemade electrocompetent XL-1 Blue cells) per transformation.
3) Follow **Steps 3** through **9** as described in **Section I**.
4) The transformation mix can be plated (usually using **5 to 30ul** of unincubated mix on 1-2 plates in order to get well-isolated colonies) or used to inoculate 2.0 to 10.0ml LB medium (containing appropriate antibiotics).

NOTE:

1) In order to avoid sparking/short-circuit during electroporation, it is important to precipitate the ligation products and thoroughly washing the pellet. It is also important to completely cold down the cuvettes. If it does spark, the transformation is no good, but one can add 20ul ddH_2O, re-zap it, and hope for the best!

2) Direct plating after zapping is a good way to keep background low. However, in some cases whether the ligation reaction is more difficult, a short incubation (20-30min) at 37^0C may be desirable if only a few colonies are recovered from overnight incubation.