Transformation protocol

Reaction 1 (cloning and transforming your gene)

1. All reactions and tube must be on ice
2. Prepare the ligation reaction mixture by combining (in order) the following components:

   1.5 μl StrataClone Cloning Buffer

   0.5 μl StrataClone Vector Mix amp/kan

   (**Tube A** containing 1.5 μl Cloning and Buffer 0.5 μl Vector Mix)

   1 μl of PCR product (gene purified from gel)

3. Mix gently by repeated pipetting, and then incubate the ligation reaction at room temperature for 5 minutes. When the incubation is complete, place the reaction on ice.
4. Add 1 μl of the cloning reaction mixture to the tube of thawed competent cells. Mix gently (**do not mix by repeated pipetting**).
5. Incubate the transformation mixture on ice for 5–20 minutes.
6. Heat-shock the transformation mixture at 42°C for 45 seconds.
7. Incubate the transformation mixture on ice for 2 minutes.
8. Add 200 μl of pre-warmed LB medium to the transformation reaction mixture.
9. Allow the competent cells to recover for at least 1 hour at 37°C with agitation. (Lay the tube of cells on the shaker horizontally for better aeration.)

Reaction 2 (cloning and transforming control gene)

1. Prepare the ligation reaction mixture by combining (in order) the following components:

1.5 μl StrataClone Cloning Buffer

0.5 μl StrataClone Vector Mix amp/kan

1 μl of PCR product (gene purified from gel)

(**Tube B** containing all three components)

2. Mix gently by repeated pipetting, and then incubate the ligation reaction at room temperature for 5 minutes. When the incubation is complete, place the reaction on ice.
3. Add 1 μl of the cloning reaction mixture to the tube of thawed competent cells. Mix gently (**do not mix by repeated pipetting**).
4. Incubate the transformation mixture on ice for 5–20 minutes.
5. Heat-shock the transformation mixture at 42°C for 45 seconds.
6. Incubate the transformation mixture on ice for 2 minutes.
7. Add 200 μl of pre-warmed LB medium to the transformation reaction mixture.
8. Allow the competent cells to recover for at least 1 hour at 37°C with agitation. (Lay the tube of cells on the shaker horizontally for better aeration.)

Reaction 3 (transforming control vector)

(**Tube C** containing control puc18 vector)

1. Add 1 μl of the vector to the tube of thawed competent cells. Mix gently (**do not mix by repeated pipetting**).
2. Incubate the transformation mixture on ice for 5–20 minutes.
3. Heat-shock the transformation mixture at 42°C for 45 seconds.
4. Incubate the transformation mixture on ice for 2 minutes.
5. Add 200 μl of pre-warmed LB medium to the transformation reaction mixture.
6. Allow the competent cells to recover for at least 1 hour at 37°C with agitation. (Lay the tube of cells on the shaker horizontally for better aeration.)