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SOP-107

Plasmid DNA Ligations & Transformations

Procedures for ligation of DNA fragments and transforming *E. coli* bacteria.

Section 1

Materials and Reagents

Heating Block

Ligase Enzyme

Bacterial Plates / appropriate antibiotic

Competent Bacteria

Single colony of *E. coli* cells

LB media

50 mM $CaCl_2$ solution, ice cold

LB plates containing antibiotic

Plasmid DNA or ligation reaction

Glycerol

Beckman centrifuge

Section 2

Protocol

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Ligation Reactions

Most cloning experiments involve the insertion of a DNA fragment into a plasmid for downstream protein expression, insertion of a multiple cloning site or the addition of a property to the vector (e.g., a drug resistance marker, a promoter, a signal sequence, etc.). Ligation reactions generally precede transformation experiments.

Ligation reactions are performed under defined conditions recommended by the manufacture usually using DNA fragments generated from restriction digestion. We generally use T4 DNA Ligase from New England Biolabs (NEB).

T4 DNA Ligase Description:
Catalyzes the formation of a phosphodiester bond between juxtaposed 5' phosphate and 3' hydroxyl termini in duplex DNA or RNA. This enzyme will join blunt end and cohesive end termini as well as repair single stranded nicks in duplex DNA, RNA or DNA/RNA hybrids (1).

Source: Purified from *E. coli* C600 pcl857 pPLc28 lig8 (2).

Applications:

- Cloning of restriction fragments
- Joining linkers and adapters to blunt-ended DNA

Reaction Setup:

1. **Ligation Positive Control**

Distilled H_2O	17 ul
NEB 10X Ligation Buffer	2 ul
Vector (digested with 1 enzyme)	1 ul
T4 Ligase	1 ul
Total volume	20 ul

2. **Ligation Background Control**

Distilled H_2O	17 ul
NEB 10X Ligation Buffer	2 ul
Vector (digested with 2 enzyme)	1 ul
T4 Ligase	1 ul
Total volume	20 ul

3. Ligation Experiment

Distilled H_2O	13 ul
NEB 10X Ligation Buffer	2 ul
Vector (digested with 2 enzyme)	1 ul
Target (digested with same 2 enzyme)	3 ul
T4 Ligase	1 ul
Total volume	20 ul

Note: Can vary the ratio of target to vector fragment to enhance ligation reaction to produce desired construct.

Incubate all ligation reactions at room temperature for 10 minutes. Place on ice and prepare for transformation reactions.

Usage notes:

1. ATP is an essential cofactor for the reaction. This contrasts with *E. coli* DNA ligase which requires NAD.
2. To dilute T4 DNA Ligase that will subsequently be stored at -20°C, 50% glycerol storage buffer (Diluent Buffer A, NEB #B8001S) should be used; to dilute for immediate use, 1X T4 DNA Ligase Reaction Buffer can be used.
3. Ligation can also be performed in any of the four restriction endonuclease NEBuffers or in T4 Polynucleotide Kinase Buffer if they are supplemented with 1 mM ATP.

Application notes:
Room Temperature Ligation:
For convenience, ligations may be done at room temperature (20-25°C). For cohesive (sticky) ends, use 1 µl of T4 DNA Ligase in a 20 µl reaction for 10 minutes. For blunt ends, use 1 µl of T4 DNA Ligase in a 20 µl reaction for 2 hours or 1 µl high concentration T4 DNA Ligase for 10 minutes.

Transformation Reactions

This method is a modification of the one published in "Short Protocols in Molecular Biology," 4th edition. Plasmid DNA is precipitated unto the surface of *E. coli* bacteria after which the cells are induced to grow by heat shock.

Note: All materials that come into contact with bacteria must be sterile.
Note: Competent bacteria can be purchased and stored for use in the laboratory saving time.

The following method will produce competent *E. coli*, or cells that are ready to take up foreign DNA.

1. Inoculate a single colony of *E. coli* into 50 ml of LB media. Allow to culture to incubate at 37 ˚ C with shaking.
2. Next day inoculate a fresh 100 ml culture with 0.5 ml of overnight culture. Allow culture to incubate at 37 C with shaking until $OD_{590} = 0.375$. (You want to have log phase growing cells for transformation)
3. Pellet 30 ml of cells by centrifugation (1600 x g, 10 minutes, 4 ˚ C). Suspend each pellet in 10 ml ice cold 50 mM $CaCl_2$ solution.
4. Incubate cells on ice for 30 minutes.
5. Pellet cells by centrifugation (1100 x g, 5 minutes, 4 ˚C). Suspend each pellet in 1 ml of ice cold 50 mM $CaCl_2$ solution. These cells are now competent and ready to receive plasmid DNA. (At this time you may make the cells 15 % with glycerol and store at –80 C.)
6. Use 10 ng of plasmid or 10 ul of ligation reaction to transform 100 ul of competent cells and incubate on ice for 10 minutes.
   For transformation reactions:
   Use undigested plasmid as positive control and no plasmid for negative control.
7. Heat shock cells for 2 minutes at 42 ˚C in a water bath or heating block. Add 1 ml of LB media and incubate at 37 ˚ C for 1 hour with shaking.
8. Plate 200 ul of transformed cells on LB plates with ampicillian.
9. Next day pick colonies.

Ligation & Transformation Interpretations

Plate a positive control (whole plasmid with a known resistant marker), a negative control, just bacterial cells without a plasmid, and your experimental ligations for transformations.

The positive transformation control will tell you if your cells were competent and if they took up plasmid DNA.

The negative control will test the viability of the antibiotic in the LB plates.

The ligation positive reaction will generate colonies and tell you if your ligase is performing.

The ligation background will provide some measure of how well plasmid DNA was digested.

The number of colonies on experimental ligation plate will give some determination if reaction worked well. If ligation worked well there should be more colonies on experimental plate then background plate. In fact the ratio of experimental to background colonies generates probability of successful ligation. For example, to calculate a success rate: if there are 50 colonies on the experimental plate and 5 colonies on the background plate your background rate is 5 / 50 x 100 = 10% background or 90% insert. Essentially if you randomly pick 10 colonies from the experimental plate 9 of them will have your target DNA in the vector.

Note: You can reduce background by dephosphorylating your vector.

Section 3 References

Short Protocols in Molecular Biology. 4th Edition. Editors Frederick M. Ausubel, Roger Brent, Robert E. Kingston, David D. Moore, J.G. Seidman, John A Smith and Kevin Struhl. John Wiley & Sons, Inc. 1999

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