Transformation

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- **What is transformation?**

In cloning, PCR products which already were ligated into pGEM®-T Easy Vectors are cloned using *E.coli* (*JM*109) competent cell, prior to sequencing. Since transformation usually produces a mixture of relatively few transformed cells and an abundance of non-transformed cells, a method is necessary to select for the cells that have acquired the plasmid. The transformed cells with plasmid, however, need not contain the desired recombinant DNA inserted in cloning experiment. Various techniques may be further employed to screen for transformed cells that contain plasmid with an insert. Reporter genes can be used as markers, such as the lacZ gene which codes for β-galactosidase used in blue-white screening.

- **How does it work?**

Prepare two LB/ampicillin/IPTG/X-Gal plates for each ligation reaction, plus two plates for determining transformation efficiency. Equilibrate the plates to room temperature. Then, centrifuge the tubes containing the ligation reactions to collect the contents at the bottom. Add 2µl of each ligation reaction to a sterile (17 × 100mm) polypropylene tube or a 1.5ml microcentrifuge tube on ice. After that, remove tube(s) of frozen JM109 High Efficiency Competent Cells from storage and place them in an ice bath until just thawed (about 5 minutes). Mix the cells by gently flicking the tube. Avoid excessive pipetting, as the competent cells are extremely fragile. Carefully transfer 50µl of cells into each tube of prepared ligation reaction. Gently flick the tubes to mix and place them on ice for 20 minutes. Heat-shock the cells for 45–50 seconds in a water bath at exactly 42° C without shaking and immediately return the tubes to ice for 2 minutes. Later, add 950µl room-temperature SOC medium to the tubes containing cells transformed with ligation reactions and incubate for 1.5 hours at 37° C with shaking (~150rpm). Place 100µl of each transformation culture onto duplicate LB/ampicillin/IPTG/X-Gal plates. Incubate the plates overnight (16–24 hours) at 37° C. If 100µl is placed, approximately 100 colonies per plate are routinely seen using competent cells that are 1 × 108cfu/µg DNA. Use of ultra-high- efficiency competent cells may result in a higher number of background colonies. Longer incubations or storage of plates at 4° C (after 37° C overnight incubation) may be used to facilitate blue color development. White colonies generally contain inserts; however, inserts may also be present in blue colonies.

- **What is screening Transformants for Inserts (blue-white screening)?**

Successful cloning of an insert into the pGEM®-T or pGEM®-T Easy Vector interrupts the coding sequence of β-galactosidase; recombinant clones can be identified by color screening on indicator plates. However, the characteristics of the PCR products cloned into the vectors can significantly affect the ratio of blue:white colonies obtained. Usually clones containing PCR products produce white colonies, but blue colonies can result from PCR fragments that are cloned in-frame with the *lac*Z gene. Such fragments are usually a multiple of 3 base pairs long (including the 3´-A overhangs) and do not contain in-frame stop codons. There have been reports of DNA fragments up to 2kb that have been cloned in-frame and have produced blue colonies. Even if your PCR product is not a multiple of 3 bases long, the amplification process can introduce mutations (deletions or point mutations) that may result in blue colonies.

- **Why transformation is not successful?**

No colonies: A problem has occurred with the transformation reaction or the cells have lost competence. Background undigested vector and relegated non-T tailed vector should yield 10–30 blue colonies independent of the presence of insert DNA.

Low number or no white colonies containing PCR product: The PCR fragment is inserted, but it is not disrupting the *lac*Z gene. If there are a higher number of blue colonies resulting from the PCR fragment ligation than with the background control, some of these blue colonies may contain insert. Screen blue and pale blue colonies (See *Iranian Journal of Plant Physiology* 1 (4), 275 – 277).

PCR product ligation reaction produces white colonies only (no blue colonies): Ampicillin is inactive, allowing ampicillin- sensitive cells to grow. Check that ampicillin plates are made properly and used within one month. Test ampicillin activity by streaking plates, with and without ampicillin, using an ampicillin sensitive clone.

- **Contact us**

For more information or troubleshooting on your PCR purification, please do not hesitate to contact us at *ijpp@iau-saveh.ac.ir*. You can simply mention your problem by attaching your gel picture. We look forward to hearing from you soon.

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http://www.invitrogen.com/site/us/en/home/Products-and-Services/Applications/Cloning/PCR-cloning.html

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IJPP
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Books:

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A chapter in a book:

Leach, J. 1993. 'Impacts of the zebra mussel (Dreissena polymorpha) on water quality and fish spawning reefs of Western Lake Erie'. In *Zebra Mussels: biology, impacts and control*. Nalepa, T. and D. Schloesser (Eds.). Ann Arbor, MI: Lewis Publishers, pp: 381-397.

A Report:

Makarewicz, J. C., T. Lewis and **P. Bertram.** 1995. *Epilimnetic phytoplankton and zooplankton biomass and species composition in Lake Michigan 1983-1992.* U.S. EPA Great Lakes National Program, Chicago, IL. EPA 905-R-95-009.

Conference proceedings:

Stock, A. 2004. 'Signal transduction in bacteria'. Proceedings of the 2004 Markey Scholars Conference, pp: 80-89.

A thesis:

Strunk, J. L. 1991. *The extraction of mercury from sediment and the geochemical partitioning of mercury in sediments from Lake Superior.* M. Sc. thesis, Michigan State Univ., East Lansing, MI.

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