Plasmid isolation

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- **What is Plasmid Prep?**

Isolation of plasmid DNA from *E. coli* is a common routine in research laboratories to purify a specific sequence, since they can easily be purified away from the rest of the genome. You will perform a widely-practiced procedure that involves alkaline lysis of cells. This protocol often referred to as a plasmid "mini-prep," yields fairly clean DNA quickly and easily.

- **How does it work?**

After cloning of the desired fragment using the bacterial competent cells, the appropriate colonies will be sub-cultured in different universal bottles containing 6 ml of LB/ampicillin (100 µg/ml) at 37° C shaking water bath (~150 rpm) overnight. Then, 2 ml of the mentioned LB/ampicillin/JM109 cell cultures will be transferred to a micro-centrifuge tube and centrifuged for 2,656 x g for 3 minutes. This step will be repeated two more times in the same tube, filling the tube again with more bacterial culture. The purpose of these additional steps is to increase the starting volume of cells, so that more plasmid DNA can be isolated per prep. Afterwards, the supernatants will be discarded and the pellets dried. Two hundred µl of solution I (50 mM glucose, 25 mM Tris-HCl, pH8 and 10 mM EDTA) will be added to each pellet, mixed and vortexed vigorously to dissolve the pellets. Next, 200µl of freshly prepared solution II (1% SDS and 0.2 M NaOH) will be added and then will be left at room temperature for 4 minutes. Later, 200 µl of solution III (60 ml of 5 M potassium acetate, 11.5 ml of glacial acetic acid and 28.5 dH2O) will be added and the mixtures will be left on ice for 15 minutes. The mixtures will be centrifuged for 10 minutes at 17,382 x g and the supernatant will be transferred to new 1.5 ml micro-centrifuge tubes. After that, 5 µl of 50 mg/ml RNase A will be added and incubated in a 37° C water bath for 3 hours. After RNase A incubation, 600 µl of phenol will be added, and the mixtures will be vortexed and centrifuged at 17,382 x g for 3 minutes. The supernatants will be removed and placed in new micro-centrifuge tubes. Next, 600 µl of chloroform will be added, the mixtures will be vortexed and centrifuged again at 17,382 x g for 3 minutes. The aqueous layers will be removed and 0.1 volume of 3 M sodium acetate and 2.5 volume of absolute ethanol will be added to each tube. The mixtures then will be placed on ice for 20 minutes. Later, it will be centrifuged at 17,382 x g for 15 minutes. The supernatants will be discarded and 1 ml of 70 % ethanol will be added to each one and centrifuged for 15 minutes at 17,382 x g. The supernatant is discarded by pipetting and the pellet is vacuum dried. Lastly, 30 µl of dH2O will be added to each pellet and the samples are left overnight at 4° C.

- **What is it used for?**

The plasmid mini-prep can be used to quickly find out whether the plasmid is correct in any of several bacterial clones. The yield is a small amount of impure plasmid DNA, which is sufficient for analysis by restriction digest and for some cloning techniques.

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Sambrook, J., and Russell, D. W. (2001). Molecular Cloning: A Laboratory Manual (3rd ed.). Cold Spring Harbor Laboratory Press. New York: USA.

http://www.promega.com/resources/protocols/technical-bulletins/101/pureyield-plasmid-miniprep-system-protocol/

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Books:

Durbin, R., S. R. Eddy, A. Krogh and **G. Mitchison.** 1999. *Biological Sequence Analysis: Probabilistic Models of Proteins and Nucleic Acids*. Cambridge: University Press.

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.

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