

## Overview of Ion Exchange Chromatography

This chapter describes our kits for midi and maxi preparation of ultrapure plasmid DNA.

The method:

- Eliminates CsCl gradient ultracentrifugation which requires days.
- Avoids use of phenol/chloroform extraction
- Uses gravity flow columns, no centrifugation required

For a general overview of these products, continue reading this article, or for detailed information of the single product turn to the page which describes the product in detail.

If you are interested in	For preparing	See page
Genopure Plasmid Midi Kit	Plasmid DNA from 5 to 30 ml bacterial culture suitable for transfection, PCR, restriction analysis/Southern blotting, sequencing, cloning	122
Genopure Plasmid Maxi Kit	Plasmid DNA from 30 to 150 ml bacterial culture for transfection, PCR, restriction analysis/Southern blotting, sequencing, cloning	129

The isolation method is based on a modified alkaline lysis protocol and can be divided into the following steps:

- Harvest and disruption of the bacterial cells
- Precipitation of the bacterial "chromosomal" DNA
- Clarification of the bacterial lysate
- Adsorption of the plasmid DNA to the column matrix
- Removal of residual impurities by wash steps
- Elution of the plasmid DNA by high salt conditions
- Concentration and salt removal by alcohol precipitation

The isolation method is optimized for cultures grown in LB media; other rich media may require increased volumes of Suspension-, Lysis- and Neutralization Buffer, and an additional wash step.

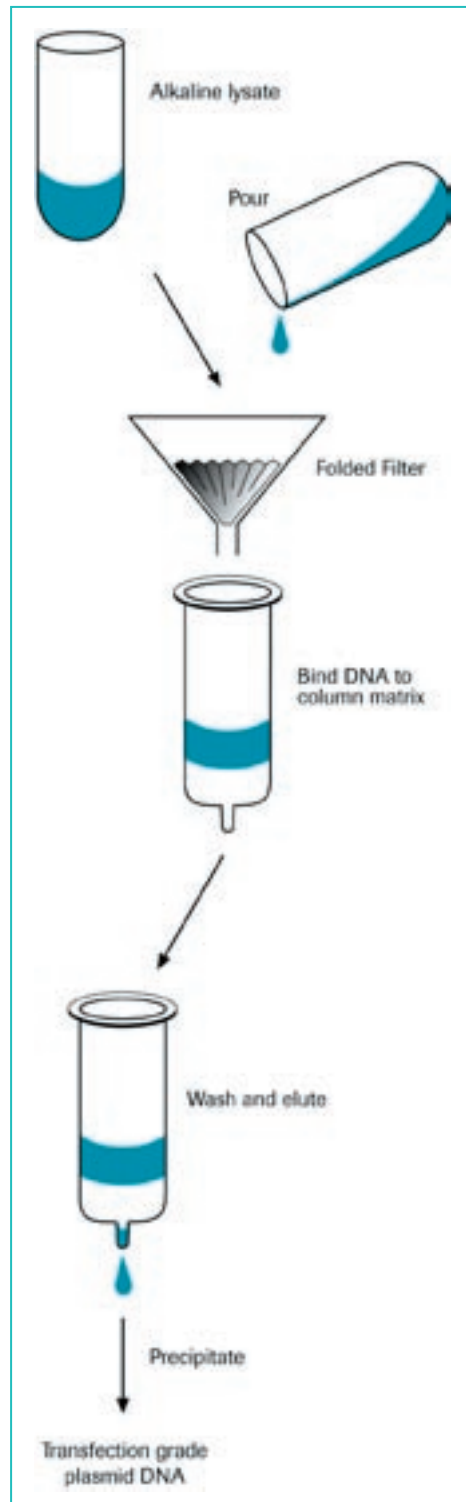
The isolation procedure is suitable for all plasmid sizes; lysates of larger constructs (up to 100 kb) should be cleared by filtration to avoid shearing.

The yield of plasmid DNA preparations is dependent on several parameters, *e.g.*, quality of the bacterial culture growth, amount of used culture suspension for the preparation, plasmid type used etc.

As a rule of thumb the typical yield of a high copy number plasmid is about 3 – 5 µg of DNA per ml of original bacterial culture (pUC, pTZ, pGEM in common host strains as XL-1 blue, HB101, JM 109).

The typical yield of low copy number plasmids is about 0.2 – 1 µg of DNA per ml of original bacterial culture. It is recommended to use the supplementary Genopure Buffer Set for low copy number plasmids in combination with the respective Genopure Plasmid Kit.

Both kits are supplied with folded filters to eliminate the time-consuming centrifugation step after the alkaline lysis. In approximately 2 min (midi) or 10 min (maxi), respectively, of unattended running cellular debris and potassium dodecylsulphate precipitates are held back by the filter thereby avoiding shearing of large DNA constructs. Besides the significant reduction of preparation time another advantage of filtration is that even small SDS precipitates which cannot be separated by conventional centrifugation are completely removed.



**Figure 35: Genopure plasmid purification procedures.**

Schematic representation of clearing the lysate by filtration with subsequent plasmid purification.

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