

<b>ITB, Wageningen University</b>	EPP-SOP-WUR04
	Version 1.0

## EPP - Standard Operating Procedure

(only for selected experiments intended to transfer results from one lab to the other)

Title: Plasmid isolation from *E. coli* and *P. putida* using the Thermo Scientific GeneJET Plasmid Miniprep Kit

<u>distribution list</u>			
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# Instruction

Plasmid isolation from *E. coli* and *P. putida* using the Thermo Scientific GeneJET Plasmid Miniprep Kit

**Keywords:** Plasmid isolation – miniprep – *E. coli* – *P. putida*

## 1 Introduction / Purpose

This protocol describes how to isolate plasmids from *E. coli* and *P. putida* with the help of a kit. Both the short version and the long version of the manual are in this protocol.

## 2 Equipment and chemicals

### 2.1 Equipment

- Microcentrifuge for 1.5 ml eppendorf tubes.
- Thermo Scientific GeneJET Plasmid Miniprep Kit #K0502 or #K0503.

### 2.2 Bacterial strains

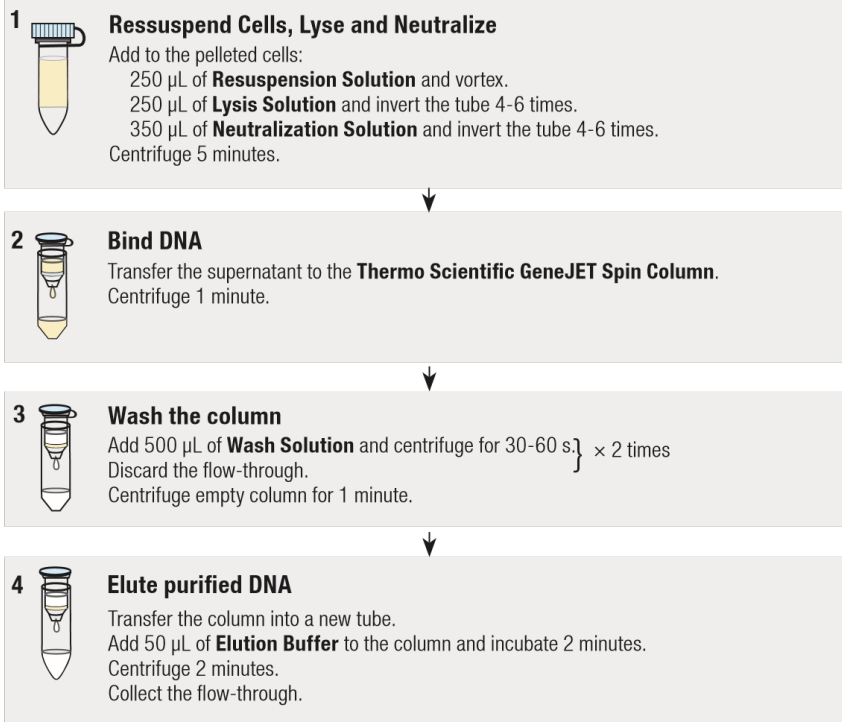
- *E. coli* or *P. putida* that you want to isolate the plasmid from.

## 3 Media and buffers

Appropriate culture medium for the bacterial strains that you want to isolate the plasmid from.

## 4 Procedures

- Inoculate 2 ml of appropriate medium with the bacterial strain that you want to isolate the plasmid from. Incubate overnight.
- Harvest the bacterial culture by centrifugation at 8000 rpm (6800 x g) in a microcentrifuge for 2 minutes at room temperature. Decant the supernatant and remove all remaining medium.
- Proceed with the steps described in the manual. The short version is on the next page, the long version starts at the page thereafter.



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Long version of the manual:



## PRODUCT INFORMATION

# Thermo Scientific GeneJET Plasmid Miniprep Kit #K0502, #K0503

[www.thermoscientific.com/onebio](http://www.thermoscientific.com/onebio)

#K\_\_\_\_ Lot \_\_\_\_\_

### CERTIFICATE OF ANALYSIS

Thermo Scientific GeneJET Plasmid Miniprep Kit has been tested in the isolation of pUC19 DNA from *E. coli* according to the PURIFICATION PROTOCOL described on p.p. 4-5. The quality of isolated DNA has been evaluated in a set of tests: spectrophotometric determination of DNA purity and concentration, agarose gel electrophoresis, digestion with Thermo Scientific FastDigest restriction endonucleases and automated sequencing.

Quality authorized by:

A handwritten signature in black ink, appearing to read "JZ", positioned above the name Jurgita Zilinskiene.

Jurgita Zilinskiene

Rev.11

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## COMPONENTS OF THE KIT

GeneJET Plasmid Miniprep Kit	#K0502 50 preps	#K0503 250 preps
Resuspension Solution	15 mL	70 mL
Lysis Solution	15 mL	70 mL
Neutralization Solution*	20 mL	100 mL
Wash Solution (concentrated)	20 mL	100 mL
RNase A (10 mg/mL)	0.15 mL	0.7 mL
Elution Buffer (10 mM Tris-HCl, pH 8.5)	4 mL	30 mL
GeneJET Spin Columns	50	250
Collection Tubes (2 mL)	50	250

\*Wear gloves when handling Neutralization Solution. Do not add bleach to sample preparation waste.

## STORAGE AND STABILITY

GeneJET™ Plasmid Miniprep Kit can be stored for up to 12 months at room temperature (15-25°C). For longer storage, it is recommended to keep the kit at 4°C.

If precipitate forms in the buffers during storage, it should be redissolved by incubating the buffers at 37°C before use.

After addition of RNase A, the Resuspension Solution can be used for 6 months when stored at 4°C.

**Note. Close the bag with GeneJET Spin Columns tightly after each use!**

## DESCRIPTION

The GeneJET Plasmid Miniprep Kit is designed for rapid and cost-effective small-scale preparation of high quality plasmid DNA from recombinant *E. coli* cultures. The kit utilizes an exclusive silica-based membrane technology in the form of a convenient spin column. Each GeneJET spin column can recover up to 20 µg of plasmid DNA. The kit can be successfully used for efficient purification of any size plasmids and cosmids. The actual plasmid yield and optimal culture volume depend on the plasmid copy number and medium used for cultivation.

## PRINCIPLE

Pelleted bacterial cells are resuspended and subjected to SDS/alkaline lysis (1) to liberate the plasmid DNA. The resulting lysate is neutralized to create appropriate conditions for binding of plasmid DNA on the silica membrane in the spin column (2). Cell debris and SDS precipitate are pelleted by centrifugation, and the supernatant containing the plasmid DNA is loaded onto the spin column membrane. The adsorbed DNA is washed to remove contaminants, and is then eluted with a small volume of the Elution Buffer (10 mM Tris-HCl, pH 8.5).

The purified plasmid DNA is ready for immediate use in all molecular biology procedures such as conventional digestion with restriction enzymes, fast digestion with FastDigest® restriction enzymes, PCR, transformation and automated sequencing.

## IMPORTANT NOTES

### •Buffer Preparation

- Add the provided **RNase A** solution to the **Resuspension Solution** and mix. After addition of RNase A, the Resuspension Solution can be used for 6 months when stored at 4°C.
- Add ethanol (96-100%) to the **Wash Solution** prior to first use:

	#K0502 50 preps	#K0503 250 preps
Wash Solution (concentrated)	20 mL	100 mL
Ethanol 96%	35 mL	170 mL
Total volume	55 mL	270 mL

- Check the Lysis Solution and the Neutralization Solution for salt precipitation before each use. Redissolve any precipitate by warming the solution at 37°C, then cool back down to 25°C before use. Do not shake the Lysis Solution too vigorously.
- Both the Lysis Solution and the Neutralization Solution contain irritants. Wear gloves when handling these solutions. See p. 8 for SAFETY INFORMATION.
- When using EndA+ strains, use Wash Solution I (#R1611) which is available to purchase separately.

### •Growth of Bacterial Cultures

- Pick a single colony from a freshly streaked selective plate to inoculate 1-5 mL of LB medium supplemented with the appropriate selection antibiotic. Incubate for 12-16 hours at 37°C while shaking at 200-250 rpm. Use a tube or flask with a volume of at least 4 times the culture volume.
- Harvest the bacterial culture by centrifugation at 8000 rpm (6800 × g) in a microcentrifuge for 2 min at room temperature. Decant the supernatant and remove all remaining medium.

Do not overload the column:

For **high-copy-number plasmids** (see Table 1), do not process more than **5 mL** of bacterial culture. If more than 5 mL of such a culture are processed, the GeneJET spin column capacity (20 µg of dsDNA) will be exceeded and no increase in plasmid yield will be obtained.

For **low-copy-number plasmids** (see Table 1), it may be necessary to process larger volumes of bacterial culture (up to **10 mL**) to recover a sufficient quantity of DNA.

**Table 1.** Copy numbers of various vectors

• High-copy 300-700 copies per cell	Low-copy 10-50 copies per cell	Very low-copy up to 5 copies per cell
pUC vectors pBluescript vectors pGEM vectors pTZ vectors pJET vectors	pBR322 and derivatives pACYC and derivatives	pSC101 derivatives and

## PURIFICATION PROTOCOLS

### •Note

- Read IMPORTANT NOTES on p.3 before starting.
- All purification steps should be carried out at **room temperature**.
- All centrifugations should be carried out in a table-top microcentrifuge at **>12000 × g** (10 000-14 000 rpm, depending on the rotor type).

Use 1-5 mL of *E. coli* culture in LB media for purification of **high-copy** plasmids.

For **low-copy** plasmids use up to 10 mL of culture.

### •Protocol A. Plasmid DNA purification using centrifuges

Step	Procedure
1	Resuspend the pelleted cells in <b>250 µL of the Resuspension Solution</b> . Transfer the cell suspension to a microcentrifuge tube. The bacteria should be resuspended completely by vortexing or pipetting up and down until no cell clumps remain. <b>Note.</b> Ensure RNase A has been added to the Resuspension Solution (as described on p.3)
2	Add <b>250 µL of the Lysis Solution</b> and mix thoroughly by inverting the tube 4-6 times until the solution becomes viscous and slightly clear. <b>Note.</b> Do not vortex to avoid shearing of chromosomal DNA. Do not incubate for more than 5 min to avoid denaturation of supercoiled plasmid DNA.
3	Add <b>350 µL of the Neutralization Solution</b> and mix immediately and thoroughly by inverting the tube 4-6 times. <b>Note.</b> It is important to mix thoroughly and gently after the addition of the Neutralization Solution to avoid localized precipitation of bacterial cell debris. The neutralized bacterial lysate should become cloudy.
4	Centrifuge for 5 min to pellet cell debris and chromosomal DNA.
5	Transfer the supernatant to the supplied GeneJET spin column by decanting or pipetting. Avoid disturbing or transferring the white precipitate. <b>Note. Close the bag with GeneJET Spin Columns tightly after each use!</b>



Step	Procedure
6	Centrifuge for 1 min. Discard the flow-through and place the column back into the same collection tube. <b>Note.</b> Do not add bleach to the flow-through, see p.8 for Safety Information.
7 for EndA+ strains only	<i>Optional:</i> use this preliminary washing step only if EndA+ strains which have high level of nuclease activity are used. Wash the GeneJET spin column by adding 500 $\mu$ L of Wash Solution I (#R1611, diluted with isopropanol) and centrifuge for 30-60 sec. Discard the flow-through. <b>Note.</b> This step is essential to remove trace nuclease activity.
8	Add <b>500 <math>\mu</math>L of the Wash Solution</b> (diluted with ethanol prior to first use as described on p.3) to the GeneJET spin column. Centrifuge for 30-60 seconds and discard the flow-through. Place the column back into the same collection tube.
9	Repeat the wash procedure (step 8) using <b>500 <math>\mu</math>L of the Wash Solution</b> .
10	Discard the flow-through and centrifuge for an additional 1 min to remove residual Wash Solution. This step is essential to avoid residual ethanol in plasmid preps.
11	Transfer the GeneJET spin column into a fresh 1.5 mL microcentrifuge tube (not included). Add <b>50 <math>\mu</math>L of the Elution Buffer</b> to the center of GeneJET spin column membrane to elute the plasmid DNA. Take care not to contact the membrane with the pipette tip. Incubate for 2 min at room temperature and centrifuge for 2 min. <b>Note.</b> An additional elution step (optional) with Elution Buffer or water will recover residual DNA from the membrane and increase the overall yield by 10-20%. For elution of plasmids or cosmids >20 kb, prewarm Elution Buffer to 70°C before applying to silica membrane.
12	Discard the column and store the purified plasmid DNA at -20°C.

•**Protocol B. Plasmid DNA purification using vacuum manifolds**

Step	Procedure
1	Perform cell lysis and lysate clearing steps according to steps 1 - 4 in Protocol A on page 4.

2	Prepare the vacuum manifold according to the supplier's instructions. Place the GeneJET spin column(s) onto the manifold.
3	Transfer the supernatant to the supplied GeneJET spin column by decanting or pipetting. Avoid disturbing or transferring the white precipitate. <b>Note. Close the bag with GeneJET Spin Columns tightly after each use!</b>
<b>Step</b>	<b>Procedure</b>
4	Apply the vacuum to draw the sample through the column. Switch off the vacuum after the entire sample has passed through the column.
5 for EndA+ strains only	<i>Optional:</i> use this preliminary washing step only if EndA+ strains which have high level of nuclease activity are used. Wash the GeneJET spin column by adding 500 $\mu$ L of Wash Solution I (#R1611, diluted with isopropanol) and apply the vacuum to draw the solution through the column. <b>Note.</b> This step is essential to remove trace nuclease activity.
6	Add <b>500 <math>\mu</math>L of the Wash Solution</b> (diluted with ethanol prior to first use as described on p.3) to the GeneJET spin column.
7	Apply the vacuum to draw the solution through the column. Switch off the vacuum after the solution has passed through the column.
8	Repeat the wash procedure (steps 6-7) using <b>500 <math>\mu</math>L of the Wash Solution</b> .
9	Transfer the column to collection tube and centrifuge for an additional 1 min to remove residual Wash Solution. This step is essential to avoid residual ethanol in plasmid preps.
10	Transfer the GeneJET spin column into a fresh 1.5 mL microcentrifuge tube (not included). Add <b>50 <math>\mu</math>L of the Elution Buffer</b> to the center of GeneJET spin column membrane to elute the plasmid DNA. Take care not to contact the membrane with the pipette tip. Incubate for 2 min at room temperature and centrifuge for 2 min. <b>Note.</b> An additional elution step (optional) with Elution Buffer or water will recover residual DNA from the membrane and increase the overall yield by 10-20%. For elution of plasmids or cosmids >20 kb, prewarm Elution Buffer to 70°C before applying to silica membrane.
11	Discard the column and store the purified plasmid DNA at -20°C.

## TROUBLESHOOTING

Problem	Possible cause and solution
<p><b>Low yield of plasmid DNA</b></p>	<p><b>Bacterial culture too old</b>            Inoculate a fresh batch of antibiotic-containing growth medium with a freshly-isolated single bacterial colony from an overnight plate. Cultivate the cells for no more than 16 h at 37°C while shaking in LB media. Reduce the cultivation time to less than 12 h when using rich media like TB.</p> <p><b>Incomplete lysis of bacterial cells</b>            It is essential that the cell pellet is completely resuspended in the Resuspension Solution prior to lysis. No cell clumps should be visible before the addition of the Lysis Solution. Check the Lysis Solution for salt precipitation before each use. Redissolve any precipitate by warming the solution to 37°C, then mix well before use. Cell cultivation in LB media is recommended. Reduce culture volume when using a rich cultivation media like TB.</p> <p><b>Inefficient elution of DNA</b>            The Elution Buffer must be dispensed to the center of the membrane for efficient elution.</p>

<p><b>Contaminated DNA preparation</b></p>	<p><b>Residual ethanol</b> Ensure that step 10 of the protocol is performed.</p> <p><b>RNA in the eluate</b> Ensure that RNase A is added to the Resuspension Solution before the first use.</p> <p><b>Genomic DNA in the eluate</b> To avoid shearing and liberation of genomic DNA, do not vortex or shake the cells during lysis and neutralization (steps 2 and 3), mix by gentle inversion of the tube. Do not lyse the cells (step 2) for more than 5 min. Do not cultivate cells longer than 16 h in LB media or 12 h in TB media.</p> <p><b>Additional band of denatured plasmid</b> Denatured plasmid DNA migrates ahead of supercoiled DNA. It is not suitable for following enzymatic manipulations such as restriction digestion. To avoid denaturation, do not lyse the cells (step 2) for more than 5 min.</p>
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## SAFETY INFORMATION



### Lysis Solution

•**Danger**

**Hazard statements:**

H314 Causes severe skin burns and eye damage.

H319 Causes serious eye irritation.

**Precautionary statements:**

P260 Do not breathe dust/fume/gas/mist/vapours/spray.

P303+P361+P353 IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing.

Rinse skin with water/shower.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P310 Immediately call a POISON CENTER or doctor/physician.

P405 Store locked up.

P501 Dispose of contents/container in accordance with local/regional/national/ international regulations.



- **Lysis Solution C Corrosive**

Hazard-determining components of labelling: sodium hydroxide **Risk phrases:**

34 Causes burns.

**Safety phrases:**

20 When using do not eat or drink.

23 Do not breathe gas/fumes/vapour/spray.

26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

36/37 Wear suitable protective clothing, gloves and eye/face protection.

45 In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible).

60 This material and its container must be disposed of as hazardous waste.



- **Neutralization Solution**

**Warning**

**Hazard statements:**

H302 Harmful if swallowed.

H315 Causes skin irritation.

H319 Causes serious eye irritation.

**Precautionary statements:**

P280 Wear protective gloves/protective clothing/eye protection/face protection.

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P321 Specific treatment (see on this label).

P362 Take off contaminated clothing and wash before reuse.

P301+P312 IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell. P501

Dispose of contents/container in accordance with local/regional/national/international regulations.



- **Neutralization Solution Xn Harmful**

Hazard-determining component of labeling: guanidinium chloride **Risk phrases:**

R22 Harmful if swallowed.

R36/38 Irritating to eyes and skin.

**•Safety phrases**

S23 Do not breathe gas/fumes/vapour/spray.

S26 In case of contact with eyes, rinse immediately with plenty of water and seek medical advice.

S36/37 Wear suitable protective clothing and gloves.

S60 This material and its container must be disposed of as hazardous waste.

## REFERENCES

1. Birnboim H.C., and Doly, J. (1979) A rapid alkaline lysis procedure for screening recombinant plasmid DNA. *Nucleic Acids Res.* 7, 1513 -1522.
2. Vogelstein, B. and Gillespie, D. (1979) Preparative and analytical purification of DNA from agarose. *Proc. Natl. Acad. Sci. USA* 76, 615-619.

### •PRODUCT USE LIMITATION

This product is developed, designed and sold exclusively for research purposes and in vitro use only. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Please refer to [www.thermoscientific.com/onebio](http://www.thermoscientific.com/onebio) for Material Safety Data Sheet of the product.

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## 5 remarks/troubleshooting

## 6 Biosafety

Safety information for hazardous chemicals is described in the Thermo Scientific manual above.

## 7 Acknowledgement



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