illustra plasmidPrep Midi Flow kit

Product booklet

Table of Contents

1 Introduction

Product codes

28904267 (25 purifications)

28904268 (100 purifications)

About

For extraction and purification of transfection quality plasmid DNA from E. coli

Important

Read these instructions carefully before using the products.

Intended use

The products are intended for research use only, and shall not be used in any clinical or *in vitro* procedures for diagnostic purposes.

It is the responsibility of the user to verify the use of the illustra™ plasmidPrep Midi Flow kit for a specific application as the performance characteristics of this kit have not been verified for any specific organism.

Safety

All chemicals should be considered as potentially hazardous. For use and handling of the products in a safe way, refer to the Safety Data Sheets.

CAUTION

Lysis buffer type 11 contains Ribonuclease; sensitizer.

Lysis buffer type 12 contains Sodium Hydroxide; irritant.

Lysis buffer type 13 contains Acetic Acid; irritant.

Wear gloves and safety glasses.

Storage

This kit is shipped at ambient temperature and can be stored at room temperature (20°C to 25°C). Ensure temperature of Lysis buffers type 11 & 13 is reduced to 4°C prior to use. For convenience, Lysis buffers type 11 & 13 can be stored at 4°C.

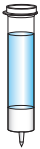
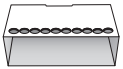
Expiry

For expiry date please refer to outer packaging label.

2 Components

Kit contents

Identification	Pack Size	5 purifications	25 purifications	100 purifications
	Product code	Sample pack	28904267	28904268
Red	Lysis buffer type 11	36 mL	180 mL	2 × 360 mL
White	Lysis buffer type 12	36 mL	180 mL	2 × 360 mL

Blue	Lysis buffer type 13	36 mL	180 mL	2 × 360 mL
Yellow	Wash buffer type 2	120 mL	2 × 300 mL	5 × 480 mL
Gray	Elution buffer type 3	24 mL	120mL	4480 mL
Green	TE buffer type 1	6 mL	30mL	120 mL
	illustra Fast-Flow plasmid 250 columns [1]	5	25	2 × 50
	Column stand [1]	Not included [1]	1	1
	Waste reservoir [1]	Not included [1]	1	1
	Adapters [2]	6	12	12

[1] The cardboard package supplied with 25 and 100 pack sizes can be converted into a column stand to aid purification. The plastic column tray can be used as a Waste reservoir.

[2] The Adapters assist the use of racks and centrifuge tubes with the illustra Fast-Flow plasmid 250 columns during the Purification step, allowing elution off the Fast-Flow column directly into a high speed centrifuge tube.

Refer to the Certificate of Analysis for a complete list of kit components.

Cytiva supplies a wide range of buffer types across the illustra nucleic acid purification and amplification range. The composition of each buffer has been optimized for each application and may vary between kits. Care must be taken to only use the buffers supplied in the particular kit you are using and not use the buffers supplied in otherillustra kits e.g. the Lysis buffers supplied in the illustra plasmidPrep Midi Flow kit are not the same as the Lysis buffers supplied in the illustra plasmidPrep Mini Spin Kit.

In order to avoid confusion and the accidental switching of buffers between kits, a numbering system has been adopted that relates to the entire range of buffers available in the illustra purification range. For example there are currently 14 Lysis buffers in the illustra range, 6 Wash buffers and 8 Elution buffers, denoted by Lysis buffer type 1–14, Wash buffer type 1–6 and Elution buffer type 1–8, respectively. Please ensure you use the correct type of Lysis, Wash and Elution buffer for your purification.

Materials and reagents to be supplied by user

Disposables:

1.5 mL DNase-free microcentrifuge tubes (1 per purification)

Ice

Chemicals:

70% Ethanol Isopropyl Alcohol (Propan-2-ol)

Sterile media and antibiotics for culture of bacteria (see *Essential preliminaries, on page 18* & *Factors affecting plasmid yield, quality & purity, on page 46* for further details)

Equipment to be supplied by user

Standard microbiological equipment and consumables, including

culture plates, loops, flasks and 37°C shaking incubator.

Tube cutter (preferably 4 mm), guillotine or scissors.

LabMate PD-10 Buffer Reservoirs can be used for easier handling and loading of volumes greater than 12 mL onto the illustra Fast-Flow plasmid 250 column.

Centrifuge(s) and tubes:

Please ensure you have a suitable centrifuge, rotor and tube types for the centrifugation steps detailed in *Table 1, on page 8*. Tubes must be able to withstand the spin speeds indicated. In addition, for the centrifugation within the Concentration and De-salination steps, the centrifuge tubes must be able to withstand alcohol. All centrifugation steps are performed at 4°C.

The different tube types are represented pictorially in the protocols as follows:

Polypropylene or polycarbonate (use for Lysis & Clarification steps)

Polypropylene or polyallomer (use for Purification, Concentration and De-salination steps)

Table 1. Centrifugation details

Plasmid copy number	Details	Bacterial Culture Preparation step	Lysis & Clarification steps	Purification step (very low copy number only)	Concentration step	De-salination step
High (low)	Sample volume (ml)	50 (150)	18		8	5
	Spin speed (× g)	5 000	20 000		15 000	15 000
	Length of spin (minutes)	15	30		30	10
	Tube composition		polypropylene or polycarbonate		polypropylene or polyallomer	polypropylene or polyallomer
Very low	Sample volume (ml)	500	45	90	8	5

Plasmid copy number	Details	Bacterial Culture Preparation step	Lysis & Clarification steps	Purification step (very low copy number only)	Concentration step	De-salination step
	Spin speed (× g)	5 000	20 000	20 000	15 000	15 000
	Length of spin (minutes)	15	2 x 20	30	30	10
	Tube composition		polypropylene or polycarbonate	polypropylene or polyallomer	polypropylene or polyallomer	polypropylene or polyallomer

3 Description

Background

The illustra plasmidPrep Midi Flow kit is designed for the extraction and purification of transfection quality plasmid DNA from Escherichia coli (E. coli). The protocol is based on a simple process, at the core of which are the 4 steps of Lysis, Clarification, Purification and De-salination. A modified alkaline Lysis procedure is followed by clarification through centrifugation, to remove precipitated genomic DNA, Potassium Dodecyl Sulphate (KDS) and other contaminants. Anion exchange Purification is facilitated by plasmid binding in high-salt conditions to the illustra Fast-Flow plasmid 250 columns. Impurities such as RNA, cell wall debris and protein are removed through successive high-salt washes. Purified plasmid is eluted from the column at a higher salt concentration. The plasmid is then concentrated and de-salinated using Isopropanol precipitation and a 70% Ethanol wash.

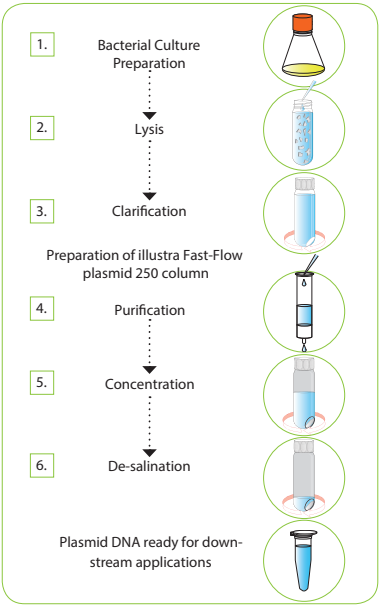
illustra Fast-Flow plasmid 250 columns are provided pre-equilibrated and require only gravity to run, reducing hands on and process time. The resin bed of the illustra Fast-Flow plasmid 250 columns is protected with a high density polyethylene Vyonfilter frit that prevents the Fast-Flow plasmid 250 media from running dry during the Purification step. The column medium has been developed to provide excellent flow characteristics, exceptional chemical and pH stability, and high selectivity and capacity for plasmid DNA.

The large effective surface area and high level of derivitization of the medium facilitate strong anionic-exchange. Since plasmid DNA binds at a high salt concentration, binding of potential impurities is minimized. Pure plasmid can be eluted in buffers containing higher salt concentrations.

The basic principle

Illustration

Use of the illustra plasmidPrep Midi Flow kit involves the following steps:



Step procedure

Step	Comments	Component
1. Bacterial Culture Preparation	*E. coli* is grown to late logarithmic phase in appropriate medium and harvested by centrifugation.	**Bacteria**
2. Lysis	Bacterial pellet is re-suspended in buffer containing RNase I. Cells are lysed in alkaline conditions with Sodium Dodoecyl Sulphate (SDS)-detergent present.	**Lysis buffer type 1 1**
	Genomic DNA and proteins are denatured; RNA is hydrolyzed.	**Lysis buffer type 1 2**
3. Clarification	High speed centrifugation causes genomic DNA, protein and KDS precipitate to form a pellet. Plasmid DNA remains in solution.	
Preparation of illustra Fast-Flow plasmid 250 column		**illustra Fast-Flow plasmid 250 column**
4. Purification	Clarified lysate is loaded onto illustra Fast-Flow plasmid 250 column. Plasmid binds to the anion exchange matrix. Wash steps remove contaminants and pure plasmid is eluted in a highsalt buffer.	**Wash buffer type 2** **Elution buffer type 3**
5. Concentration	Plasmid is concentrated by Isopropanol precipitation and recovered by high speed centrifugation.	
6. De-salination	Salt and Isopropanol are removed with a 70% Ethanol wash. Plasmid DNA is recovered by high speed centrifugation. The pellet is air dried and re-suspended in a low ionic strength buffer.	**TE buffer type 1**

Product specifications

Sample type:	*E. coli* cultures containing high, low or very low-copy number plasmids and cosmid vectors up to 50 kb.
Lysis principle	Modified alkaline lysis procedure
Purification principle	Anion-exchange chromatography
Purification format	Single-use, gravity flow illustra Fast-Flow plasmid 250 columns
Effective capacity	≥ 250 µg plasmid DNA
Sample size	From 25 mL up to 150 mL culture medium, dependent upon growth conditions, strain of E. coli and size and copy number of vector (see *Essential preliminaries, on page 18* & *Factors affecting plasmid yield, quality & purity, on page 46* for details)
Typical yield	100 to ≥ 250 µg high-copy number plasmid 20 to 250 µg low and very low-copy number plasmid
Purity A260/A280 A260/A230 % supercoiled DNA	1.8–2.0 2.0–2.5 > 70% (strain dependent)
Major subsequent applications	Transfection of most cell lines, automated sequencing, PCR and mutagenesis, enzyme modifications and cloning.

If large numbers of samples are to be processed from small volumes of bacterial cultures (1–3 mL), transfection quality DNA is not required and a yield of 6–9 µg plasmid DNA per purification is sufficient, consider use of the illustra plasmidPrep Mini Spin Kit.

Typical output

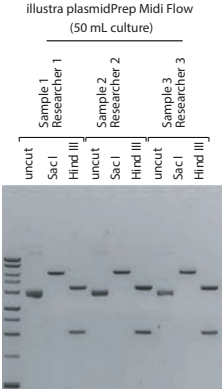


Fig 1. Purification of a 6.3 kb high copy number plasmid from E. coli TOP10 using the illustra plasmidPrep Midi Flow kit

The agarose gel shows that uncut samples consist predominantly of supercoiled monomeric plasmid DNA (pCORON1002-EGFP-C1). No endogenous nuclease activity was evident. Complete restriction enzyme digestion was effected with SacI and HindIII. 3 separate 50 mL culture volumes were processed by 3 separate researchers.

Table 2. Typical yield & purity values

Plasmid details	Culture Volume (mL)	Yield (ug)	A_{260}/A_{280}	A_{260}/A_{230}
High copy number, 6.3 kbp	50	289	1.90	2.34
Low copy number, 36 kbp	150	191	1.93	2.29

Table 3. Typical quality values

Plasmid details	Culture Volume (mL)	Endotoxin (EU/mg)	Supercoiled monomer (%)	Transfection (% COS7 cells)	Sequencing (Phred 20 read length)
High copy number, 6.3 kbp	50	0.05	91.5	72	818
Low copy number, 36 kbp	150	0.13	ND	ND	ND

High copy number and low copy number plasmids were prepared using the illustra plasmidPrep Midi Flow Kit, as indicated. ND = not determined.

4 Protocols

Note: *It is advisable to familiarize yourself with* *Materials and reagents to be supplied by user, on page 6*, *Equipment to be supplied by user, on page 7* *and* *Chapter 4 Protocols, on page 16* *before beginning the purification, ensuring all material, reagents, consumables and equipment are to hand.*

NOTICE

Ensure appropriate tubes are available for high speed centrifugation steps.

NOTICE

Buffers are NOT transferable between Cytiva illustra kits e.g. the composition of the Wash buffer in the plasmidPrep Midi Flow Kit is not the same as the Wash buffer in the plasmidPrep Mini Spin Kit. Please note type number for differentiation.

Note: *Two protocols are provided; one for the purification of up to 250 µg of high and low copy number plasmid and one for purification of very low-copy number plasmids (< 10 copies per cell). Please ensure you follow the correct protocol (see Table 4, on page 18).*

Preparation of working solutions

For Materials & Equipment to be supplied by user see *Materials and reagents to be supplied by user, on page 6* & *Equipment to be supplied by user, on page 7* .

Lysis buffers type 11 & 13

Ensure temperature of Lysis buffer type 11 and Lysis buffer type 13 is reduced to 4°C before use.

Lysis buffer type 12

Ensure no precipitate is visible in the bottle containing Lysis buffer type 12. If necessary, warm the buffer in a 37°C water bath for 5 minutes. Lysis buffer type 12 should be stored at room temperature (20°C to 25°C).

Essential preliminaries

For a more in depth analysis of the factors affecting plasmid yield, purity and quality, please see *Factors affecting plasmid yield, quality & purity, on page 46*

Culture volumes

Note: *Culture volumes of E. coli required differ depending on the plasmid copy number (see the table below). The volumes advised enable optimal performance of the Fast-Flow plasmid 250 columns. To obtain good aeration, use a sterile flask with a volume of at least 4 times the volume of the culture.*

Table 4. Relationship between plasmid copy number, culture volume, expected yield and protocol

Plasmid copy number	Maximum culture volume (mL)	Expected yield (µg)	Protocol
High (> 50)	25–50	100–250	*Protocol for purification of high-copy number and low-copy number plasmids, on page 20*
Low (10–50)	150	20–250	*Protocol for purification of high-copy number and low-copy number plasmids, on page 20*

Plasmid copy number	Maximum culture volume (mL)	Expected yield (µg)	Protocol
Very low (<10)	500	20–200	*Protocol for purification of very low-copy number plasmids, on page 31*

*If cell density exceeds 1×10^{10} cells/mL harvest only 25 mL of culture.

E. coli strain

The protocols provided have been optimized using a range of strains of E. coli. Certain strains of E. coli produce excessive carbohydrate, especially when combined with the larger culture volumes required for very low copy number plasmid purification. When purifying very low copy number plasmids, the protocol in section *Protocol for purification of very low-copy number plasmids, on page 31* should be followed, where the volume of Lysis buffers type 11, 12, & 13 used is increased to 10 mL during the Lysis step. In addition 3 washes with 10 mL Wash buffer type 2 is performed during the Purification step. Additional buffers can be made according to the recipes provided in *Buffer composition, on page 55*.

Protocol for purification of high-copy number and low-copy number plasmids

Bacterial Culture Preparation

Step	Action
1	Pick a single colony from a freshly streaked selective plate and inoculate a starter culture of 2–5 mL Lysogeny Broth (LB) medium containing the appropriate selective antibiotic. (LB can also be known as Luria broth or Luria Bertani broth). Adjust salt levels as appropriate for bacterial strain, culture conditions and salt sensitivity of antibiotic used. Incubate for approximately 8 hours, 37°C, 225 rpm. ***Note:*** *To obtain good aeration, use a sterile tube or flask with a volume of at least 4 times the volume of the culture. Dilute 1:1000 into 50–150 mL selective LB 12–18 hours 37°C 225 rpm*

Step	Action
2	Dilute the starter culture 1 in 1000 into the appropriate volume of selective LB medium (see *Table 5, on page 21*). Incubate at 37°C 12–18 hours, 225 rpm.

Table 5. Typical growth criteria with DH5α and TOP10 cells

Plasmid copy number	Maximum culture volume (mL)	Inoculum volume (µL)	Expected final cell density	Typical final A_{600}
High (> 50)	50[1]	50	$3–9 \times 10^{9}$ [1]	1.3–2.6
Low (10–50)	150	150	$3–9 \times 10^{9}$	1.3–2.6

[1] If cell density exceeds 1×10^{10} cells/mL harvest only 25 mL of culture.

Step	Action
3	To harvest cells from the medium, centrifuge for 15 minutes at 5 000 × g and 4°C. Pour off spent culture medium and use a pipet to remove any excess fluid and leave the cell pellet as dry as possible. ***Tip:*** *At this point the protocol can be stopped and continued later.*

NOTICE

The cell pellet may be stored at -20°C. Defrost thoroughly at room temperature for 5–15 minutes prior to use. Do not thaw for greater than 15 minutes, as endogenous enzyme activitiy can affect plasmid yield and purity.

Lysis

Step	Action
1	Cell resuspension-Add 6 mL Lysis buffer type 11 and thoroughly re-suspend the pellet by pipetting up and down.
2	Transfer to high speed centrifuge tube

NOTICE
Ensure tubes selected are made of polycarbonate or polypropylene, suitable for centrifugation at 20000 × g and large enough to allow complete mixing of Lysis buffers types 11–13 added in the following steps.

3	Cell lysis-Add 6 mL Lysis buffer type 12. Mix thoroughly by inverting 6 times with an incisive action. Do not vortex, as this will result in shearing of genomic DNA.
4	Incubate at room temperature (RT) for 5 minutes. The lysate will appear viscous.

NOTICE
Do not incubate for longer than 5 minutes as this may denature supercoiled plasmid DNA.

Step	Action
5	Neutralization-Add 6 mL pre-chilled Lysis buffer type 13 and immediately mix thoroughly by inverting with an incisive action 6 times. ***Note:*** *A white flocculent will form and viscosity should be reduced. If the mixture still appears viscous, more mixing is required. Do not vortex, as this will result in shearing of genomic DNA.*
6	Incubate on ice for 15 minutes.

Clarification

Step	Action
1	Spin the sample for 30 minutes at 20000 × g and 4°C.

NOTICE
Use polypropylene or polycarbonate tubes.

Preparation of Fast-Flow plasmid 250 column

Step	Action
1	Remove the top cap from the Fast-Flow plasmid 250 column. Cut off the recessed part of the bottom tip using a guillotine, 4 mm tube-cutters or scissors. Allow the excess storage liquid to flow through the column. Support the column over a suitable waste receptacle, such as the Waste reservoir provided, to catch buffer flowthrough.

Purification

Step	Action
1	Column loading-Immediately following centrifugation, carefully pipet the clarified lysate supernatant into a new polycarbonate or polypropylene tube. ***Note:*** *After centrifugation the supernatant should be clear. If supernatant is not clear, transfer to fresh tube and re-centrifuge. Immediate transfer of the clarified lysate with a pipet to a fresh tube before loading onto column is recommended to avoid disturbing the pellet. The small flecks of flocculent that remain on the surface of the clarified lysate should stick to the pipet.* ***Tip:*** *At this point you can take a 50 µL aliquot of the clarified lysate supernatant for sample analysis, see* *Purification analysis, on page 45*

Step	Action
2	Apply 10 mL of clarified lysate directly to the Fast-Flow plasmid 250 column. Apply remaining clarified lysate to column. Allow lysate to enter the resin completely by gravity flow. Purification analysis: Retain 100 µl of flowthrough. ***Tip:*** *At this point you can take a 100 µL aliquot of the flowtrough for sample analysis, see* *Purification analysis, on page 45*
3	Wash:Apply 10 mL Wash buffer type 2 to column. Allow buffer to completely enter the resin by gravity flow. ***Tip:*** *At this point you can take a 200 µL aliquot of the flowtrough for sample analysis, see* *Purification analysis, on page 45*
4	Repeat wash with additional 10 mL Wash buffer type 2 ***Note:*** *This second wash step is essential for high purity.* ***Tip:*** *At this point you can take a 200 µL aliquot of the flowtrough for sample analysis, see* *Purification analysis, on page 45*

Step	Action
5	Elute-Place a suitable polyallomer or polypropylene centrifuge tube under the column. Use the Adapters to ensure flowthrough enters tube. Apply 4 mL Elution buffer type 3 to the column and collect the flowthrough.

NOTICE

Use of polycarbonate centrifuge tubes is not recommended as they are not resistent to the alcohol used in subsequent steps. Use polypropylene or polyallomer tubes.

Tip:

At this point you can take a 40 µL aliquot of the flowtrough for sample analysis, see *Purification analysis, on page 45*

Tip:

At this point the protocol can be stopped and continued later.

Eluate may be stored at 4°C overnight or at -20°C for longer periods.

Concentration

Step	Action
1	Add 4 mL room temperature Isopropanol. Mix by inversion.

NOTICE
Ensure Isopropanol is at room temperature to minimize salt precipitation.

2	Mark the outside of the tube to aid pellet location and immediately spin for 30 minutes at 15000 × g and 4°C. The tube should be placed in the rotor such that the mark is located where the pellet is expected to form.

NOTICE
Ensure centrifugation is performed at 4°C to prevent sample overheating.

3	Carefully and immediately pour off supernatant or aspirate with a pipet. The pellet will have a glassy appearance but can be located by means of the prior tube mark. Take care not to disturb the plasmid pellet when removing supernatant.

De-salination

Step	Action
1	Wash the pellet with 5 mL room temperature 70% (v/v) Ethanol.
2	Spin for 10 minutes at 15 000 × g and 4°C.
3	Carefully pour off the supernatant or aspirate with a pipet.

NOTICE

The pellet may be distributed over the wall of the tube, and so wash the walls and pellet with 70% Ethanol prior to centrifugation. Take care not to disturb the plasmid pellet when removing supernatant.

Step	Action
4	Air dry the pellet for 5–10 minutes. Dissolve plasmid DNA in up to 1 mL TE buffer type 1. Transfer the sample to a 1.5 mL DNasefree microcentrifuge tube (user supplied).

NOTICE

Over drying the pellet will make the plasmid difficult to dissolve. Rinse the walls of the tube to ensure recovery of all plasmid DNA. Excessive pipetting may shear plasmid DNA. If pellet was over dried, heat the TE buffer to 60°C prior to addition, or gently roller mix buffer on the pellet overnight.

5	Determine plasmid yield, purity, endotoxin levels and quality as necessary (see *Plasmid yield, purity, endotoxin level & quality determination, on page 42* for details).
6	Store purified plasmid DNA at -20°C.

Protocol for purification of very low-copy number plasmids

Bacterial Culture Preparation

Step	Action
1	Pick a single colony from a freshly streaked selective plate and inoculate a starter culture of 2–5 mL LB medium containing the appropriate selective antibiotic. Incubate for approximately 8 hours, 37°C, 225 rpm. ***Note:*** *To obtain good aeration, use a sterile tube or flask with a volume of at least 4 times the volume of the culture. Dilute 1:1000 into 500 ml selective LB 12–18 hours 37°C 225 rpm*
2	Dilute the starter culture 1 in 1000 into selective LB medium – inoculate 500 mL of LB medium with 500 µL of starter culture. Incubate at 37°C 12–18 hours, 225 rpm. ***Note:*** *Final cell density should be 3–9 × 10^9/mL, with typical A_{600} values of 1.3–2.6 for standard bacterial strains such as TOP10 and DH5α*

Step	Action
3	To harvest cells from the medium, spin for 15 minutes at 5000 × g and 4°C. Pour off spent culture medium and use a pipet to remove any excess fluid and leave the cell pellet as dry as possible. ***Tip:*** *At this point the protocol can be stopped and continued later.* ***Note:*** *The cell pellet may be stored at -20°C. Defrost thoroughly at room temperature for 5–15 minutes prior to use. Do not thaw for greater than 15 minutes, as endogenous enzyme activitiy can affect plasmid yield and purity.*

Lysis

Step	Action
1	Cell resuspension-Add 15 mL Lysis buffer type 11 and thoroughly re-suspend the pellet by pipetting up and down.

Step	Action
2	Transfer to high speed centrifuge tubes.

NOTICE

Ensure tubes selected are made of polycarbonate or polypropylene, are suitable for centrifugation at 20000 × g and are large enough to allow complete mixing of Lysis buffers types 11–13 added in the following steps.

3	Cell lysis-Add 15 mL Lysis buffer type 12. Mix thoroughly by inverting 6 times with an incisive action.
4	Incubate at room temperature (RT) for 5 minutes. The lysate will appear viscous.

NOTICE

Do not incubate for longer than 5 minutes as this may denature supercoiled plasmid DNA. Do not vortex, as this will result in shearing of genomic DNA.

Step	Action
5	Neutralization-Add 15 mL pre-chiled Lysis buffer type 13 and immediately mix thoroughly by inverting with an incisive action 6 times.
6	Incubate on ice for 15 minutes. ***Note:*** *A white flocculent will form and viscosity should be reduced. If the mixture still appears viscous, more mixing is required. Do not vortex, as this will result in shearing of genomic DNA.*

Clarification

Step	Action
1	Spin the sample for 20 minutes at 20000 × g and 4°C.
2	Pour supernatant into a fresh polycarbonate or polypropylene high speed centrifuge tube.
3	Re-centrifuge for 20 minutes at 20000 × g and 4°C. After the second centrifugation, the supernatant should be clear.

Step	Action
4	Immediately pour or pipet supernatant into a fresh polycarbonate or polypropylene high speed centrifuge tube. ***Note:*** *Immediate transfer of the clarified lysate with a pipet to a fresh tube before loading onto column is recommended to avoid disturbing the pellet. The small flecks of flocculent that remain on the surface of the clarified lysate should stick to a pipet.*

Preparation of Fast-Flow plasmid 250 column

Step	Action
1	Remove the top cap from the Fast-Flow plasmid 250 column. Cut off the recessed part of the bottom tip using a guillotine, 4 mm tube-cutters or scissors. Allow the excess storage liquid to flow through the column. Support the column over a suitable waste receptacle, such as the Waste reservoir provided, to catch buffer flowthrough.

Purification

Step	Action
1	Add 45 mL Isopropanol to the clarified lysate and mix by inversion.
2	Centrifuge for 30 minutes at 20000 × g and 4°C.

Step	Action
3	Carefully remove supernatant and dissolve pellet in 4 mL TE buffer type 1 by pipetting up and down .

NOTICE
Take care not to disturb the pellet when removing the supernatant.

4	Add 6 mL Wash buffer type 2, mix by inversion. ***Tip:*** *At this point you can take a 50 µL aliquot of the clarified lysate for sample analysis, see* *Purification analysis, on page 45*
5	Apply sample directly to Fast-Flow plasmid 250 column and allow to enter media completely by gravity flow. ***Tip:*** *At this point you can take a 100 µL aliquot of the flowthrough for sample analysis, see* *Purification analysis, on page 45*
6	Wash:Apply 10 mL Wash buffer type 2 to column. Allow buffer to completely enter the resin by gravity flow. ***Tip:*** *At this point you can take a 200 µL aliquot of the flowthrough for sample analysis, see* *Purification analysis, on page 45*

Step	Action
7	Repeat wash twice with additional 20 mL Wash buffer type 2 ***Note:*** *These additional washes are essential when purifying very low copy number plasmids from large culture volumes or using bacterial strains that produce large amounts of carbohydrate. A total of 3 × 10 mL washes is required.* ***Tip:*** *At this point you can take a 200 µL aliquot of the flowthrough for sample analysis, see* *Purification analysis, on page 45*

Step	Action
8	Elute-Place a suitable polyallomer or polypropylene centrifuge tube under the column. Use the Adapters to ensure flowthrough enters tube. Apply 4 mL Elution buffer type 3 to the column and collect the eluate.

NOTICE

Use of polycarbonate centrifuge tubes is not recommended as they are not resistent to the alcohol used in subsequent steps.

Tip:

At this point you can take a 40 µL aliquot of the flowthrough for sample analysis, see *Purification analysis, on page 45*

Tip:

At this point the protocol can be stopped and continued later.

Note:

Eluate may be stored at 4°C overnight or at -20°C for longer periods.

Concentration

Step	Action
1	Add 4 mL room temperature Isopropanol. Mix by inversion.
2	Immediately spin for 30 minutes at 15000 × g and 4°C. Carefully and immediately pour off supernatant or aspirate with a pipet.

NOTICE

Ensure Isopropanol is at room temperature to minimize salt precipitation.

Ensure centrifugation is performed at 4°C to prevent sample overheating.

The pellet will have a glassy appearance and may be difficult to locate. Mark the outside of the tube before centrifugation to aid pellet location.

Take care not to disturb the plasmid pellet when removing supernatant.

De-salination

Step	Action
1	Wash the pellet with 5 mL room temperature 70 % (v/v) Ethanol.
2	Spin for 10 minutes at 15000 × g and 4°C.
3	Carefully pour off the supernatant or aspirate with a pipet.

NOTICE

The pellet may be distributed over the wall of the tube, and so wash the walls and pellet with 70% Ethanol prior to centrifugation. Take care not to disturb the plasmid pellet when removing supernatant.

Step	Action
4	Air dry the pellet for 5–10 minutes. Dissolve plasmid DNA in up to 1 mL TE buffer type 1 ***Note:*** *Over drying the pellet will make the plasmid difficult to dissolve.* *Rinse the walls of the tube to ensure recovery of all plasmid DNA.* *Excessive pipetting may shear plasmid DNA.* *If pellet was over dried, heat the TE buffer to 60°C prior to addition, or gently roller mix buffer on the pellet overnight.*
5	Determine plasmid yield, purity, endotoxin levels and quality as necessary (see *Plasmid yield, purity, endotoxin level & quality determination, on page 42* for details).
6	Store purified plasmid DNA at -20°C.

5 Appendices

RPM calculation from RCF

The appropriate centrifugation speed for a specific rotor can be calculated from the following formula:

RPM = 1000 × √(RCF/1.12r)

Where RCF = relative centrifugal force; r = radius in mm measured from the center of the spindle to the bottom of the rotor bucket; and

RPM = revolutions per minute.

For example, if an RCF of 735 × g is required using a rotor with a radius of 73 mm, the corresponding RPM would be 3 000.

Plasmid yield, purity, endotoxin level & quality determination

Yield

Purified plasmid concentration should be determined by UV spectrophotometry (A_{260}), and through comparison with a known standard by agarose gel electrophoresis and subsequent densitometric analysis. Typical yield values are given in *Table 6, on page 42* below.

Table 6. Expected yield for different plasmid types

Plasmid copy number	Maximum culture volume (mL)	Expected yield (µg)
High (> 50)	50	100–250
Low (10–50)	150	20–250
Very low (< 10)	500	20–200

Note: *An absorbance reading of 1 at 260 nm corresponds to approximately 50 ng/µL. The reliable UV spectrophotometric range should be determined for each spectrophotometer. Generally, for spectrophotometers with a 1 cm path length, A_{260} readings that lie between between 0.1 and 1.0 can be trusted and therefore appropriate dilutions (5 to 50 ng/mL) should be analyzed. For NanoDrop™ spectrophotometers, absorbance readings between 1 and 10 are reliable.*

Purity

If available, the UV spectrophotometric ratios A_{260}/A_{280} and A_{260}/A_{230} provide a limited indication of purity as measures of protein, RNA and salt contamination. Expected A_{260}/A_{280} range is 1.8–2.0, and A_{260}/A_{230} is 2.0–2.5. If there is significant contamination with protein, the A_{260}/A_{280} will be less, and accurate quantitation of plasmid DNA will not be possible by spectroscopy. Significant absorption at 230 nm can indicate salt contamination.

Endotoxin levels

Endotoxin levels can be determined by a number of methods such as the QCL-100 kit from Lonza Corp. Ltd.

Plasmid quality

Agarose gel electrophoresis and subsequent densitometric analysis can be used to determine the percentage of supercoiled DNA relative to genomic DNA and denatured supercoiled and nicked (linear and open circular) plasmid.

The majority of plasmid should be supercoiled when using the illustra plasmidPrep Midi Flow kit. If your purified sample appears not to be predominantly supercoiled, please consult *Factors affecting plasmid yield, quality & purity, on page 46* & *Troubleshooting guide, on page 49*. See *Table 7, on page 44* for guidelines of how to interpret agarose gel and spectroscopy results.

Table 7. Interpretation of agarose gel and spectroscopy results

Unexpected presence of	Agarose gel result	Spectroscopy shows
Protein	-	A_{260}/A_{280} ratio < 1.8
Salt	Plasmid fails to digest with restriction enzyme	Significant absorption at A_{230}
RNA	Small molecular weight contamination.	Raised A_{260}/A_{280} ratio
Genomic DNA	High molecular weight contamination, which upon restriction enzyme digestion produces a smear.	Unexpectedly high A_{260}
Denatured supercoiled plasmid	Band that runs faster than supercoiled plasmid and will not itself digest.	-
Nicked linear or open circular plasmid	Band that runs slower than supercoiled plasmid.	-
Oligomeric and multimeric plasmid [1]	Band that runs slower than supercoiled plasmid, which upon restriction enzyme digestion produces a linear monomeric form.	-
Deletion mutants	Smaller faster migrating species, which upon restriction enzyme digestion show an unexpectedly small size.	-

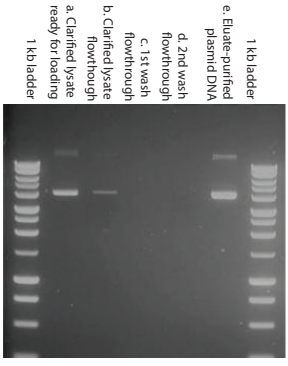
[1] Multimeric forms do not affect the quality of plasmid DNA and should not be mistaken for genomic DNA.

Purification analysis

To the samples taken during the Purification step add an equal volume of Isopropanol. Mix briefly and centrifuge at 16 000 × g in a benchtop centrifuge for 10 minutes. Decant or aspirate the supernatant, taking care not to disturb the pellet. Add 1 mL 70% Ethanol and centrifuge again for 5 minutes at 16 000 × g. Decant or aspirate the supernatant. Air dry the pellet for 3–5 minutes at room temperature, and re-suspend in 20 µL of TE buffer type 1.

Run 2–5 µL of each sample on a 1% agarose gel. If possible, assess UV absorbance data with a NanoDrop spectrophotometer. A typical gel image is shown below.

Fig 2. Typical gel image obtained using samples obtained for Purification analysis.



A 6.3 Kbp high-copy number plasmid was purified from E. coli TOP10 using the illustra plasmidPrep Midi Flow kit.

Samples are from protocol *Purification, on page 25*, at the stages indicated at the top of the gel. RNA may be visible at stages b., c. & d. as a faint low molecular weight band.

Factors affecting plasmid yield, quality & purity

Certain steps in the isolation and purification of plasmid DNA using the illustra plasmidPrep Midi Flow kit have been optimized. They are essential for the production of good quality, highly pure plasmid DNA. These steps may be influenced by a number of factors that are detailed below:

Host strains

Many bacterial strains have been developed for the propagation of plasmids and can be used in conjunction with the illustra plasmidPrep Midi Flow kit. Bacterial strains from the DH series, C600, XL1-blue TOP series, and JM series generally provide good yield and purity of plasmid. However, the following points should be considered:

Oligomeric forms

The replication machinery in certain strains can lead to the production of oligomeric and multimeric forms of plasmid. Oligomeric forms are visible on agarose gels, but are not thought to affect performance in downstream applications.

Endogenous nuclease activity

Certain strains, including early JM and HB series, can have high levels of endogenous nuclease activity, resulting in nicked or re-arranged plasmid.

Carbohydrate levels

Some strains, including TG1 and the JM series, when cultured in rich media can produce high levels of carbohydrate. It is advisable to use simple media such as LB, reduce culture volumes and harvest during late-logarithmic or early stationary phase. Follow protocol in *Protocol for purification of high-copy number and low-copy number plasmids, on page 20* when purifying high or low copy number plasmids.

When purifying very low copy number plasmids, excessive carbohydrates may be produced as a consequence of the larger culture volumes required. Follow protocol in *Protocol for purification of very low-copy number plasmids, on page 31* noting the increase in the volume of Lysis buffers type 11, 12 & 13 to 10 mL for the Lysis step and 3 × 10 mL washes during the Purification step (for buffer recipes see *Buffer composition, on page 55* sections 6.6).

If problems persist, it is advisable to switch bacterial strains.

Inoculation techniques

Always prepare plasmid from a culture that has been cultured from a single colony grown on a fresh selective agar plate. Avoid inoculating straight from liquid cultures, frozen stocks or old plates as this can lead to plasmid re-arrangement, contamination, mutation or total loss of the plasmid.

Always use a starter culture to inoculate the larger cultures as detailed in sections *Protocol for purification of high-copy number and low-copy number plasmids, on page 20* & *Protocol for purification of very low-copy number plasmids, on page 31*. This adapts the bacteria for rapid liquid culture, and ensures inoculation with a mid-logarithmic growth phase culture.

Culture medium, volume and growth

The illustra plasmidPrep Midi Flow kit has been optimized to isolate 100–250 µg plasmid DNA from cultures containing 3–9 × 10^9 cells/ mL. Increasing the cell density or culture volume will not necessarily increase yield or purity and may have a detrimental effect on plasmid quality. The use of too many cells can result in an inefficient Lysis step, leading to reduced yields, ineffective RNA digestion and genomic DNA contamination. Either dilute cultures with cell densities exceeding 9 × 10^9 cells/mL or use lower culture volumes.

For best results, use a basic culture medium, such as LB broth. The use of rich media such as Teriffic Broth (TB) and 2 × YT broth (16 g Tryptone, 10 g Yeast Extract, 5 g Sodium Chloride per liter medium) is not recommended. Cultures should be grown to late logarithmic or early stationary phase, which for most cultures of *E. coli* is 16–20 hours incubation at 37°C, given adequate aeration. Prolonged culture times may result in bacteria entering decline phase, resulting in cell rupture during incubation, and reduced plasmid yield and purity.

Plasmid characteristics

Copy number

Plasmid copy number depends upon a number of factors, including plasmid size, characteristics of the insert and level of expression, the origin of replication and the host strain. Protocols are provided for working with high, low and very low copy number plasmids; please ensure you follow the correct protocol.

Chloramphenicol (170 µg/mL) may be added to mid-logarithmic phase cultures containing low copy number relaxed plasmids to inhibit protein synthesis and replication of the host genome. The culture is then incubated for a further 4–8 hours during which plasmid replication proceeds unhindered, resulting in an accumulation of plasmid and an increase in copy number. When purifying relaxed plasmids from cultures that have been amplified using chloramphenical, follow the protocol for high copy number plasmids.

Size

Most large plasmids are low or very low copy number due to the physiological stress that replicating them places upon the cell. Larger plasmids are prone to re-arrangements and mutations, and therefore extra care should be taken to ensure cultures are fresh and harvested in early stationary phase. Larger plasmids are also more susceptible to shear forces, and so vigorous mixing and repeat pipetting should be avoided throughout the protocol (and when using the plasmid in downstream applications).

Troubleshooting guide

This guide may be helpful in the first instance, however if problems persist or for further information please contact Cytiva technical services. Contact details are on the back page. Alternatively log onto *cytiva.com/illustra*

Table 8. Problem: Low yield of plasmid

Possible cause	Suggestions
Bacterial growth conditions inappropriate	• To obtain good aeration, use a sterile flask with a volume of at least 4 times the volume of the culture. • Vigorous shaking of the culture is required (225 rpm). • Check media contains appropriate antibiotic selection
Cell density too high, resulting in poor cell lysis, or column overloading	• Harvest culture at late-logarithmic or early stationary phase. Expected A_{600} is 1.3–2.6 with 3–9 × 109 cells/mL. • Once culture reaches required density, process cells rapidly. Alternatively, store cell pellet at -20°C.
Inefficient lysis with Lysis buffer type 11	• Ensure cells thoroughly re-suspended in Lysis buffer type 11. The presence of a brown pellet at the end of the Clarification step may indicate inefficient lysis. Expected pellet color after Clarification is predominantly white.
Inadequate mixing of sample with Lysis buffer type 13	Once Lysis buffer type 13 has been added to the sample, mix by inversion until a flocculent precipitate appears. Continue inverting until the precipitate is evenly dispersed, the brown color disappears and the solution is less viscous (approximately 6 inversions).
Clarified lysate, Wash buffer type 2 or Elution buffer type 3 not allowed to draiN from illustra Fast-Flow plasmid 250 column completely	• Ensure column is allowed to drain completely before proceeding to the next step.

Possible cause	Suggestions
Pellet loss during Concentration or De-salination steps	• After the Concentration step, the pellet may be difficult to see. Mark the outside of the high speed centrifuge tube before centrifugation to allow the pellet to be located. Re-orientate the tube in the centrifuge using this mark for each subsequent spin to avoid disturbing the pellet. • Ensure that the supernatant is removed immediately. When decanting supernatant, orient the tube such that the pellet is on the upper-side and slowly decant the supernatant into a sterile container so that any lost pellet can be recovered. Alternatively, use a sterile pipet.
DNA pellet incompletely re-suspended in TE buffer type 1 after De-salination step	• Wash walls of the centrifuge tube with TE buffer type 1 to ensure maximum yield
Pellet over dried and failed to dissolve in TE buffer type 1	• If pellet was over dried, heat the TE buffer type 1 to 60°C prior to addition, or gently roller mix buffer on the pellet overnight using a horizontal roller mixer.

Table 9. Problem: Genomic DNA contamination (see ***Table 7, on page 44***)

Possible cause	Suggestions
Cell density too high, resulting in poor cell lysis, or column overloading	• Check bacterial growth conditions and cell density are correct (For high and low copy number plasmids see *Table 5, on page 21*. For very low copy number plasmids final cell density should be 3–9 × 10^9 cells/mL).
Too vigorous mixing of sample after addition of Lysis buffer type 12 and/or 13	• Mix by inversion only. Do not vortex.
Perceived contamination is multimeric supercoiled plasmid	• Genomic DNA contamination will appear as large molecular weight contamination on agarose gels that digests to form a smear. A_{260} yield may also be unexpectedly high.

Table 10. Problem: RNA contamination (see ***Table 7, on page 44***)

Possible cause	Suggestions
Cell density too high, resulting in poor cell lysis, or column overloading	• Check bacterial growth conditions and cell density are correct (For high and low copy number plasmids see *Table 4, on page 18*. For very low copy number plasmids final cell density should be 3–9 × 10^9 cells/mL).
Lysis buffer type 11 not pre-chilled before use	• For convenience, store Lysis buffer type 11 at 4°C. • If RNA is evident as small molecular weight contamination on an agarose gel and a raised A_{260}/A_{280} ratio, the plasmid may be treated with fresh RNase I or Lysis buffer type 11, loaded onto an illustra Fast-Flow plasmid 250 column and purified following the protocol from the Purification step f. Wash - Apply 10 mL Wash buffer type 2 onwards.

Table 11. Problem: Supercoiled plasmid is denatured (see ***Table 7, on page 44***)

Possible cause	Suggestions
Incubation of sample in Lysis buffer type 12 was longer than 5 minutes	• Supercoiled plasmid DNA has been irreversibly damaged. It is normal to see levels of up to 5% of plasmid exhibiting this damage. Do not incubate sample in Lysis buffer type 12 for longer than 5 minutes.

Table 12. Problem: Nicked, linear or open circular plasmid in sample (see ***Table 7, on page 44***)

Possible cause	Suggestions
Use of endonuclease containing bacterial strain	• See *Factors affecting plasmid yield, quality & purity, on page 46* for selection of host strain.
Nuclease contamination of buffers	• Wear gloves and use sterile plastic wear for all steps. Make fresh buffers according to *Buffer composition, on page 55*.
Use of a different buffer for TE buffer type 1	• Use the buffer provided in the kit.
Vigorous mixing, vortexing or excessive pipetting	• Follow advice for mixing given at each step.

Table 13. Problem: Multimeric plasmid present in sample (see ***Table 7, on page 44***)

Possible cause	Suggestions
Selected bacterial strain	• See section *Factors affecting plasmid yield, quality & purity, on page 46* for selection of host strain.

Table 14. Problem: Deletion mutant plasmid present in sample (see ***Table 7, on page 44***)

Possible cause	Suggestions
Bacterial culture allowed to grow for too long. Agar plate not fresh.	Check bacterial growth conditions and cell density are correct (For high and low copy number plasmids see *Table 5, on page 21*. For very low copy number plasmids final cell density should be 3–9 × 10^9 cells/mL).
Plasmid insert inherently unstable or toxic	Prepare plasmid from 4 or more different colonies.
Plasmid rearrangement (especially in larger constructs)	Prepare plasmid from 4 or more different colonies.

Table 15. Problem: Salt contamination (see ***Table 7, on page 44***)

Possible cause	Suggestions
Isopropanol not at room temperature for Concentration step	• Ensure Isopropanol at room temperature before addition to sample.
Sides of tube not washed with 70% Ethanol for	• Wash sides of tube carefully.
De-salination step	• To remove salt, re-precipitate DNA-add 1/10th volume 3 M Sodium Acetate pH 5.4 and 1.1 volumes of Isopropanol. Pellet by centrifugation and wash twice with 70% Ethanol.

Table 16. Problem: Protein contamination (see *Table 7, on page 44*)

Possible cause	Suggestions
Flocculent present in sample after Clarification step. May result in blocked or slow flowing illustra Fast-Flow plasmid 250 column	• Transfer the supernatant to a fresh tube prior to column loading to avoid transfer of flocculent to column.

Table 17. Problem: Endotoxin contamination (as measured with Cambrex- 1000 kit)

Possible cause	Suggestions
illustra Fast-Flow plasmid 250 column overloaded	• Cell density greater than 3–9 × 10^9 cells/mL can overload the columns and result in endotoxin contamination.
User-introduced endotoxin contamination	• Use sterile, clean tubes and plasticware throughout.

Buffer composition

Use nuclease free water and sterile plasticware for buffer preparation. Filter sterilize before use.

Identification	Buffer	Composition
Red	Resuspension Buffer 1	100 mM Tris/HCl pH 7.5 10 mM EDTA 0.4 mg/mL RNase I
White	Lysis buffer type 12	200 mM NaOH 1% SDS
Blue	Lysis buffer type 13	3 M KOAC pH 5.5
Yellow	Wash buffer type 2	50 mM Tris/HCl pH 8 1 mM EDTA 650 mM NaCl
Gray	Elution buffer type 3	50 mM Tris/HCl pH 8 1 mM EDTA 1.2 M NaCl
Green	TE buffer type 1	10 mM Tris/HCl pH 8 1 mM EDTA

6 Related products

A full range of Molecular Biology reagents can be found in the Cytiva catalog or on the web site *cytiva.com/illustra*

If you need further information, Cytiva technical services are happy to assist.

cytiva.com/contact

Application	Product	Product code	Pack sizes
Convenient buffer addition to Fast-Flow 250 plasmid column	LabMate PD10 Buffer Reservoir	18321603	10
Preparation of additional buffers	Water, nucleasefree	US70783	500 mL
Rapid extraction and purification of plasmid DNA from small scale cultures of *E.coli*	illustra plasmidPrep Midi Flow kit	28904269	50 purifications
Purification of DNA from PCR and enzymes; *multiple samples*	illustra GFX™ PCR DNA and Gel Band Purification Kit	28903470 28903471	100 purifications 250 purifications
DNA ligation	DNA ligation system	RPN1507	50 reactions
Blunt-Ended PCR Cloning	Blunt-ended PCR Cloning Kit	RPN5110	40 reactions
Sequencing reaction kit optimized for MegaBACE DNA analysis system	DYEnamic ET terminator cycle sequencing kit	US781050	100 templates
Purification of oligonucleotides and 10–50 bp DNA fragments	illustra MicroSpin™ G-25 Columns (100-150 mL sample volume)	27532501	50 purifications
Purification of oligonucleotides following synthesis	illustra NAP™-5 Columns	17085301	50 purifications
Preparation of PCR products for automated sequencing	ExoSAP-IT™	US78200	100 reactions

Application	Product	Product code	Pack sizes
Dye terminator removal from automated sequencing reactions	illustra AutoSeq G-50 Dye Termintator Removal Kit	27534001	50 purifications

7 References

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3. Sambrook, J., Fritsch, E.F. & Maniatis, T., *Molecular cloning: A laboratory Manual*, Cold Spring Harbor laboratory, 2nd eds., (1989)
4. Vogelstein, B. & Gillespie, D., *Proc. Natl. Acad. Sci*. USA 76, 615 (1979).
5. Marko, M.A., Chipperfield, R. & Birnboim, H.C., *Anal. Biochem*. 121, 382 (1982).
6. Ausubel,F.M. et al., eds., Current Protocols in Molecular Biology 1, 1.68 (1991).
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8 Quick reference protocol

Cue card

Quick Reference Protocol Card

28904267 (25 purifications)
28904268 (100 purifications)

illustra™ plasmidPrep Midi Flow Kit

Protocol for purification of high copy number and low copy number plasmid DNA

- Ensure Lysis buffer type 12 does not contain precipitate-warm to dissolve.
- Pre-chill Lysis buffer type 13 to 4°C. • Ensure suitable high speed centrifuge tubes are available.

:Add :Spin :Incubate

1. Bacterial culture preparation

- Inoculate 5 mL starter culture
- 6–8 hours 37°C, 225 rpm
- Inoculate 50 mL (high copy number plasmids) or 150 mL (low copy number plasmids) culture
- 12–18 hours 37°C, 225 rpm
- 15 minutes 5 000 × g 4°C; discard supernatant

2. Lysis

- 6 mL Lysis buffer type 11; re-suspend pellet thoroughly
- Transfer sample to high speed polycarbonate or polypropylene centrifuge tube
- 6 mL Lysis buffer type 12; invert to mix
- 5 minutes room temperature
- 6 mL Lysis buffer type 13; invert to mix
- Incubate 15 minutes on ice

3. Clarification

- 30 minutes 20 000 × g 4°C; retain supernatant

Preparation of illustra Fast-Flow plasmid 250 Column

- Remove top cap and bottom tip
- Allow storage buffer to elute completely

4. Purification

- Load clarified lysate onto column; empty column by gravity flow
- 10 mL Wash buffer type 2; empty column by gravity flow
- Repeat for a total of 2 washes
- Place suitable polyallomer or polypropylene centrifuge tube under column
- 4 mL Elution buffer type 3
- Collect eluate by gravity flow

5. Concentration

- 4 mL Isopropanol to eluate; mix by inversion
- 30 minutes 15 000 × g 4°C; carefully remove supernatant

6. De-salination

- Wash plasmid pellet 5 mL 70% Ethanol
- 10 minutes 15 000 × g 4°C; carefully remove supernatant
- Air dry pellet 5–10 minutes
- 1 mL TE buffer type 1 to dissolve plasmid DNA

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29275351 AG V:6 02/2021