illustra plasmidPrep Mini Spin Kit

Product booklet

cytiva.com

29275352 AI

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1 Introduction

Product codes

28904269 (50 purifications)

28904270 (250 purifications)

About

For the rapid extraction and purification of plasmid DNA from small scale cultures of *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. No claim or representation is intended for its use to identify any specific organism or for clinical use (diagnostic, prognostic, therapeutic, or blood banking).

It is the responsibility of the user to verify the use of the illustra™ plasmidPrep Mini Spin Kit for a specific application range, as the performance characteristics of this product have not been verified for any specific organism.

Safety

For use and handling of the products in a safe way, refer to the Safety Data Sheets.

Note: *This protocol requires the use of ethanol.*

Storage

All kit components should be stored at room temperature (20–25°C).

Expiry

For expiry date please refer to outer packaging label.

2 Components

Kit contents

Identification	Pack Size	10 purifications	50 purifications	250 purifications
	Product code	Sample Pack	28-9042-69	28-9042-70
Red	Lysis buffer type 7	3 mL	15 mL	60 mL
White	Lysis buffer type 8	3 mL	15 mL	60 mL
Blue	Lysis buffer type 9	10 mL	50 mL	220 mL
Yellow	Wash buffer type 1	1 mL Add 4 mL ethanol before use	7 mL Add 28 mL ethanol before use	26 mL Add 104 mL ethanol before use
Gray	Elution buffer type 4	2 mL	10 mL	35 mL
	illustra plasmid mini columns	10	50	250
	Collection tubes	10	50	250

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 other illustra kits e.g. the Lysis buffers supplied in the illustra plasmidPrep Mini Spin Kit are not the same as the Lysis buffers supplied in the illustra plasmidPrep Midi Flow 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.

Note: *Lysis buffer type 7 contains RNase A. The Elution buffer type 4 consists of 10mM Tris-HCI (pH 8.0).*

Materials and equipment to be supplied by user

Disposables:

DNase-free 1.5 mL microcentrifuge tubes (snap cap). One per purification.

Chemicals:

Absolute ethanol

Equipment needed

Microcentrifuge that accommodates 1.5 mL microcentrifuge tubes

Vortex mixer (optional)

3 Description

Background

The illustra plasmidPrep Mini Spin Kit is designed for the rapid extraction and purification of plasmid DNA from 1.5 mL and 3 mL cultures of *Escherichia coli (E. coli).* The procedure can be completed in approximately 9 minutes (2 × cultures) to yield plasmid DNA with a purity and quality compatible with many common molecular biology techniques, including cloning, restriction enzyme digestion, PCR amplification and DNA sequencing (see figures in *Typical output, on page 10*).

The plasmid DNA yield from a 1.5 mL culture of a freshly grown *E. coli* strain containing a high copy number plasmid (> 300 copies/cell) and grown to A_{600} approximately 2.5 is typically 6 to 9 µg (A_{260}/A_{280} > 1.8).

Users purifying low copy number plasmids (10–20 copies per cell) or large molecular weight plasmids (> 35 kbp) should follow the protocol in *Protocol for 1.5 mL and 3 mL culture volumes, on page 14* with a 3 mL culture of *E. coli*. After the harvesting of Bacterial Culture step, all further steps are identical to that for harvesting bacteria from 1.5 mL *E. coli* culture i.e., no extra buffer volumes are required.

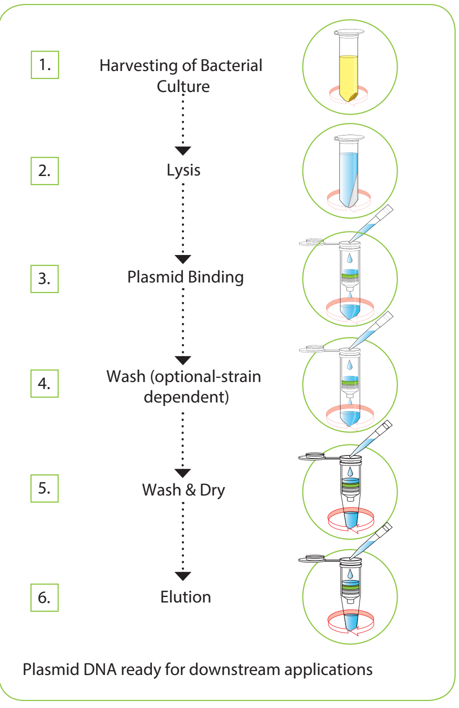
The illustra plasmidPrep Mini Spin Kit utilizes a simple plasmid DNA purification protocol, employing a modified alkaline cell lysis procedure (1–3) and a novel silica-based membrane. No organic solvents are used; instead, chaotropic salts are included to denature protein components and promote the

selective binding of plasmid DNA to the novel silica membrane (4, 5). Denatured contaminants are easily removed by subsequent washing. The purified plasmid DNA is eluted in a low ionic strength buffer, at a plasmid concentration suitable for most molecular biological applications.

The basic principle

Illustration

Use of the illustra plasmidPrep Mini Spin Kit involves the following steps:



Step procedure

Step	Comments	Component
Harvesting of Bacterial Culture	Bacteria are harvested by centrifugation and the spent medium removed.	Bacteria
Lysis	Bacterial cells are re-suspended in an isotonic solution containing RNase A.	Lysis buffer type 7
	Cells are lysed by alkali treatment; genomic DNA and proteins are denatured.	Lysis buffer type 8
	The pH of the lysate is neutralized with an acetate buffered solution, containing a chaotropic salt.	Lysis buffer type 9
	Lysate is centrifuged to pellet cellular debris, including genomic DNA, proteins and lipids.	
Plasmid Binding	The cleared cellular lysate is applied to the illustra plasmid mini column. Presence of chaotrope promotes binding of plasmid DNA binds to the membrane.	illustra plasmid mini column & Collection tube
Wash (optional strain dependent)	An optional wash step removes residual nuclease activity & carbohydrates. Recommended for EndA+ strains.	Lysis buffer type 9
Wash & Dry	A combined washing & drying step, with an ethanolic buffer, removes residual salts and other contaminants.	Wash buffer type 1
Elution	Plasmid DNA is eluted from the illustra plasmid mini column in a low ionic strength buffer.	Elution buffer type 4

Product specifications

Sample type:	1.5 mL processed bacterial culture	3.0 mL processed bacterial culture
Typical A_{600}	2.5–3.0	2.5–3.0
Time/prep[1]	Approximately 9 minutes	Approximately 9 minutes
Yield[2]	6–9 µg	9–15 µg
purity - A_{260}/A_{280}	> 1.8	> 1.8
purity - A_{260}/A_{230}	> 1.7	> 1.7

1 Actual time/prep will vary slightly depending on the user's experience with the protocol.

2 Actual yield will vary depending upon the bacterial strain used, growth conditions and the plasmid type isolated. For example, the values quoted above refer to the isolation of a 6.3 kbp highcopy number plasmid (300–500 copies/cell) extracted from *E. coli*. strain TOP10 grown overnight in Lysogeny Broth (LB) medium. LB is a nutritionally complex medium, primarily used for the growth of bacteria. (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).

Typical output

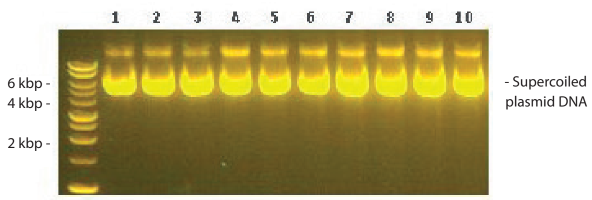


Fig 1. Undigested plasmid DNA

pCORON1002-EGFP-C1; 400 ng samples were run on a 1% (w/v) agarose gel.

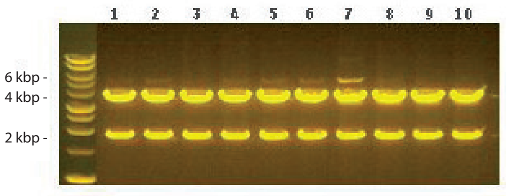


Fig 2. Digested plasmid DNA

pCORON1002-EGFP-C1; 400 ng samples were digested with 1 unit of the salt sensitive restriction enzyme HindIII, at 37°C for 1 hour.

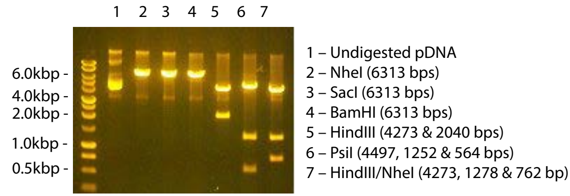


Fig 3. Multiple restriction digests

Sample 1 from above; 400 ng aliquots were digested with 5 units of the restriction endonucleases indicated, at 37°C for 1 hour.

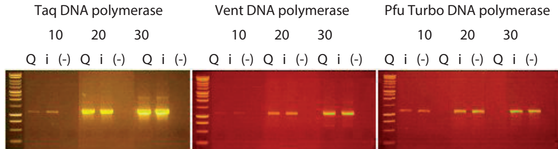
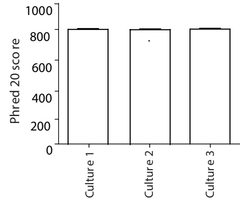


Fig 4. End-point PCR using several thermal stable DNA polymerases

Lanes Q & I represent amplification products derived from plasmid (pCORON1002-EGFP-C1) DNA samples extracted using a kit from either an alternative supplier or the illustra plasmidPrep Mini Spin Kit respectively. (-) represents no template control reactions. The numbers 10, 20 & 30 indicate the number of thermo-cycles performed. Aliquots (5 μL) of each reaction were loaded on a 1% (w/v) agarose gel.



DNA sequencing	Phred 20 scores
Culture	**illustra**
1	806 ± 15
2	818 ± 10
3	819 ± 12

Fig 5. Phred 20 scores as an indication of plasmid DNA quality.

From 3 separate cultures of *E. coli*, 6 plasmid DNA (pCORON1002- EGFP-C1) extractions were performed from 1.5 ml culture volumes. All plasmids were subjected to DNA sequencing and the Phred 20 score determined for each reaction.

4 Protocols

Factors that may affect plasmid yield and purity are outlined in *Factors affecting plasmid DNA yield and purity, on page 22*.

Note: *Buffers and mini columns ARE NOT transferable between Cytiva kits, e.g., the composition of the Wash buffer in the plasmidPrep Mini Spin Kit is not the same as the Wash buffer in the plasmidPrep Midi Flow Kit and the illustra plasmid mini columns supplied in the plasmidPrep Mini Spin Kit are not the same as the columns provided in the blood genomicPrep Mini Spin Kit.*

Preparation of working solutions

See section *Materials and equipment to be supplied by user, on page 5* and *Equipment needed, on page 5* for Materials & Equipment to be supplied by user.

Lysis buffer type 8

Ensure no precipitate is visible in the bottle containing Lysis buffer type 8.

Step	Action
1	If necessary, warm the solution in a 37°C water bath for 5 minutes. **Note:** *Lysis buffer type 8 should be stored at room temperature (20–25°C).*

Wash buffer type 1

Prior to first use, add absolute ethanol to the bottle containing Wash buffer type 1.

Note: *Once ethanol has been added, store buffer upright and airtight.*

Step	Action
1	Add 28 mL of absolute ethanol to Wash buffer type 1 in kit 28904269 (50 purifications) or add 104 mL to Wash buffer type 1 in kit 28904270 (250 purifications).
2	Mix by inversion.
3	Indicate on the label that this step has been completed.
4	For 10 purifications sample pack size, please add 4 mL absolute ethanol to Wash buffer type 1 prior to use.

Protocol for 1.5 mL and 3 mL culture volumes

Harvesting of Bacterial Culture

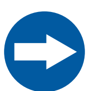
Step	Action
1	Transfer 1.5 mL from a fresh overnight culture to a microcentrifuge tube. To pellet bacteria, centrifuge at full speed (16 000 × g) in a microcentrifuge for 30 seconds. Discard supernatant and re-centrifuge. Remove any residual supernatant using a pipette.

Step	Action
2	If processing 3 mL culture volumes, repeat step 1. Pelleted DNA can be stored at -20°C if necessary. ***Note:*** *If purifying a high molecular weight or low copy number plasmid, process 3 mL culture volume.*
3	Proceed with the next part of the protocol.

Lysis

Step	Action
1	Add 175 µL Lysis buffer type 7 to the bacterial pellet and thoroughly re-suspend the pellet. ***Note:*** *Cell re-suspension can be achieved by either vortexing, pipetting up and down or by scraping the base of the microcentrifuge tube across the surface of an empty pipette tip box. Incomplete cell re-suspension will result in reduced plasmid DNA recovery.*
2	Add 175 µL Lysis buffer type 8 and mix immediately by gentle inversion (approximately 5 times) until solution becomes clear and viscous. ***Note:*** *Vigorous mixing will shear genomic DNA resulting in contamination of the final purified sample. Do not vortex. Do not allow the lysis reaction to exceed 5 minutes. Lysis buffer type 8 contains NaOH which will denature the plasmid DNA on prolonged incubation.*

Step	Action
3	Add 350 µL Lysis buffer type 9 and mix immediately by gentle inversion until the precipitate is evenly dispersed. ***Note:*** *The total column loading volume has been reduced to 700 µL compared to that described in the previous protocol. This change has been fully validated and has no impact on kit performance.* ***Note:*** *Cellular debris, genomic DNA and KDS appear as a white flocculent precipitate. Continue gentle inversion until the precipitate is evenly dispersed (approximately 10 times). The precipitate must be effectively dispersed to ensure consistent purity and yield of the isolated plasmid DNA.*



NOTICE

Do not shake or mix vigorously since genomic DNA will be sheared and co-purify with the plasmid DNA; mix by gentle inversion.

Step	Action
4	Centrifuge at full speed (approximately 16000 × g) for 4 minutes. ***Note:*** *For applications that require less stringent purity (e.g. DNA sequencing) a 3 minute flocculent spin can be performed. For applications that are more salt sensitive, a > 5 minute flocculent spin may be required.*
5	During centrifugation, for each purification that is to be performed, place one illustra plasmid mini column in one Collection tube. ***Note:*** *If required the snap-on lid can be removed without affecting the performance of the illustra plasmid mini column.*
6	Proceed with the next part of the protocol.

Plasmid Binding

Step	Action
1	Carefully transfer the cleared supernatant to the mini column (approximately 700 µL). Close the lid of the column gently.

Step	Action
2	Centrifuge at full speed (approximately 16000 × g) for 30 seconds. Discard the flowthrough by emptying the Collection tube. ***Note:*** *When purifying plasmid DNA from 3 mL culture volumes, the user may notice some residual lysate on the column after this centrifugation step. This can be ignored; proceed with the Wash & Dry step below. On washing this residual lysate will generally pass through the column with no effect on the yield or purity of the isolated plasmid DNA.* *If significant volumes of residual lysate do remain, this probably indicates that excessive flocculent material is being transferred onto the illustra plasmid mini column, blocking the silica membrane. Increase the time of the flocculent spin above to 6–10 minutes, providing that the culture* A_{600} *was approximately 2.5.* ***Note:*** *It is recommended that a pipette is used but decanting directly can be performed as an alternative. It is important to avoid transferring any cellular debris to the column as this will affect the purity of the isolated plasmid DNA.*
3	Proceed with the next part of the protocol.

Wash (optional-strain dependent)

Step	Action
1	Wash the column with 400 µL Lysis buffer type 9 and centrifuge at full speed (approximately 16000 × g) for 30 seconds. Discard the flowthrough. ***Note:*** *This step is necessary to remove potential nuclease and carbohydrate contamination when isolating DNA from E. coli strains containing the wild type EndA+ gene (e.g. HB101 or JM101) only*
2	Proceed with the next part of the protocol.

Wash & Dry

Step	Action
1	Add 400 µL Wash buffer type 1 to the column and centrifuge at full speed (approximately 16000 × g) for 1 minute. Carefully discard flowthrough and the Collection tube. ***Note:*** *After centrifugation, if any of the Wash buffer type 1 comes into contact with the bottom of the column, discard the flow through and re-centrifuge for 30 seconds. The presence of contaminating ethanol in the eluted plasmid DNA may affect the downstream applications and therefore care must be taken to ensure its complete removal.*
2	Proceed with the next part of the protocol.

Elution

Step	Action
1	Transfer the illustra plasmid mini column into a fresh microcentrifuge tube and add 100 µL Elution buffer type 4 directly onto the center of the column. ***Note:*** *If a more concentrated sample is required, add single 50 µL volume of Elution buffer type 4, incubate for 1 minute and centrifuge to elute.*
2	Incubate the column for 30 seconds at room temperature.
3	Microcentrifuge at full speed (approximately 16000 × g) for 30 seconds to recover the plasmid DNA as flowthrough in the microcentrifuge tube. ***Note:*** *If a higher yield is required, follow the elution protocol described above, but elute with two successive 50 µL elution volumes.*

If a more concentrated sample is required, do the following:

Step	Action
4	Add single 50 µL volume of Elution buffer type 4, incubate for 1 minute and centrifuge to elute.
5	Store the 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:

$RPM = 1000 \times \sqrt{(RCF/1.12r)}$

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

RPM = revolutions per min.

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.

Isolation of low copy number and large molecular weight plasmids

The procedure described in *Protocol for 1.5 mL and 3 mL culture volumes, on page 14* for the isolation of plasmid DNA from a 3 mL *E. coli* culture is that recommended for the isolation of either low copy number or large molecular weight plasmids. The 3 mL protocol has been successfully applied to the isolation of both a low copy number (10–20 copies/cell, 11 kb) and a large molecular weight plasmid (10–20 copies/cell, 35 kb). In both instances the purified plasmid DNA samples generated were of sufficient yield, purity, and quality to be compatible with most molecular biology techniques, including restriction enzyme digestion, PCR amplification and DNA sequencing. Using only 1.5 mL culture volumes may result in a prohibitively low yield of plasmid DNA.

Factors affecting plasmid DNA yield and purity

Cell density -The yield and purity of plasmid DNA isolated with the illustra plasmidPrep Mini Spin Kit can be affected by a number of external factors e.g. culture cell density, the type of plasmid (high or low copy number), the size of the insert and the host strain used.

Cell density is the most important factor and cultures grown to an extremely high density ($A_{600} > 5$) can overload the column system and result in the poor recovery of plasmid DNA in terms of yield and purity. If high cell densities are obtained, it is suggested that the user processes smaller culture volumes to ensure no deleterious effect on plasmid recovery.

The A_{600} of an overnight culture of the *E. coli* strain TOP10 (transformed with a high copy number plasmid > 300 copies/cell) and grown in LB is approximately 2.5. From a 1.5 mL volume of LB, the illustra plasmidPrep Mini Spin Kit will routinely isolate 6 to 9 µg of high quality plasmid DNA ($A_{260}/A_{280} > 1.8$).

Growth conditions -Specific factors which affect culture growth, and ultimately the density of the culture, are listed below.

Inoculation - for 1–3 mL cultures, use a fresh single *E. coli* colony from an agar plate containing the appropriate antibiotics to inoculate growth medium.

Culture medium - When incubated for an equivalent period of time, cultures grown in enriched media (e.g. 2 × YT and Terrific Broth) tend to give cell densities that are significantly higher than those achieved with LB medium. 2 × YT broth (16 g tryptone, 10 gm yeast extract, 5 gm sodium chloride per liter medium) and Terrific Broth (TB; 12 gm tryptone, 24 gm yeast extract, 4 mL glycerol, 2.31 gm KH2PO4, 12.54 gm K2HPO4) should therefore be used with caution (see Cell density notes above).

Aeration - Cultures should be well-aerated during growth. When growing cultures in a 30 mL universal container, no more than 3 mL of media should be used. Aeration will be poor if cultures are grown in 1.5 or 2.0 mL microcentrifuge tubes. Poor aeration will lead to poor culture growth, and subsequently to low yields of plasmid DNA.

Plasmid copy number - For a given length of incubation and a given medium, low copy number plasmids will give lower yields than high copy number plasmids.

Size of insert - In general, the larger the size of the insert, the lower the cell density, and lower the yield of plasmid DNA from a given culture medium. Insert size in the plasmid used to generate typical data in *Typical output, on page 10* is 783 bp.

Host strain - Strains which grow poorly or contain large amounts of nucleases or carbohydrates should be avoided. HB101 and its derivatives express endonuclease A (*EndA+*), which if not inactivated, can digest plasmid DNA. These strains may also release carbohydrates that can inhibit restriction digests (7). Note the *E. coli* strains DH5α and TOP10 facilitate the extraction of good quality plasmid DNA and are recommended for use with the illustra plasmidPrep Mini Spin Kit.

Length of incubation –For cultures grown in an enriched medium (e.g. 2xYT or TB), the length of the incubation time should not exceed 12 hours. Cultures in LB medium should be grown for at least 9 hours to obtain sufficient cell mass for processing. Cultures (in any medium) should not be grown for more than 16 hours, due to increased rates of cell death, which will affect the yield and quality of extracted plasmid DNA.

Troubleshooting guide

This guide may be helpful in the first instance, however if problems persist or for further information please contact Cytiva technical services. Visit *cytiva.com* for contact information.

Alternatively log on to *cytiva.com/illustra*.

Table 1. Problem: plasmid DNA yield is low

Possible cause	Suggestions
The bacterial culture was not fresh.	A culture should be processed in a timely manner after it has reached the required cell density. Alternatively, bacterial pellets can be stored at -20°C, prior to plasmid DNA extraction with no significant effect on purity or quality.
The total A_{600} units of the volume of culture processed was too high.	Measure the A600 of the culture before processing. If the culture density A600> 5, reduce the volume of culture processed.
The cell pellet was not completely resuspended in Lysis buffer type 7.	Cell re-suspension can be achieved by either vortexing, pipetting up/down or alternatively by scraping the base of the microcentrifuge tube across the surface of an empty pipette tip box (6).
Following the addition of Lysis buffer type 9, the sample was not adequately mixed.	After Lysis buffer type 9 is added, mix by gently inverting the tube until a flocculent precipitate appears. Continue inverting until the precipitate is evenly dispersed (10–20 inversions). The cell lysate must be broken up effectively to ensure consistent yields.
The Wash buffer type 1 was not completely removed.	After the Wash & Dry centrifugation step, if any of the ethanolic Wash Buffer comes into contact with the bottom of the column discard the flowthrough and re-centrifuge for 30 seconds. The presence of residual ethanol may affect downstream applications and must be carefully removed.

Table 2. Problem: Plasmid DNA is contaminated with genomic DNA

Possible cause	Suggestions
The sample was mixed too vigorously after adding Lysis buffers type 8 and/or 9.	Mix gently by inverting the sample 10–15 times after adding either of the solutions. Vigorous mixing may cause shearing of genomic DNA thereby facilitating its co-purification with plasmid DNA.

Table 3. Problem: Agarose gel electrophoresis shows a band migrating faster than supercoiled plasmid DNA. The fast band does not cut with restriction enzymes.

Possible cause.	Suggestions
Plasmid DNA was irreversibly denatured by Lysis buffer type 8.	The band migrating slightly faster on the agarose gel is denatured plasmid DNA. It is generated when plasmid DNA is exposed to Lysis buffer type 8 for an excessive amount of time. Plasmid DNA should be exposed to Lysis buffer type 8 for no more than a few minutes prior to the addition of the neutralizing Lysis buffer type 9. Do not allow the cell lysis reaction to proceed for > 5 minutes.

Table 4. Problem: plasmid DNA does not cut to completion or is degraded on incubation at 37ºC.

Possible cause	Suggestions
Plasmid DNA irreversibly denatured by Lysis buffer type 8 and therefore will not cut.	See *Table 3, on page 26*
Host strain possesses carbohydrates (that may interfere with restriction enzyme digestion) or residual nucleases (that are carried over into the final sample and degrade the isolated plasmid DNA).	Perform the optional Lysis buffer type 9 wash as described in the protocol. This step is necessary to remove any possible nuclease and carbohydrate contamination. If the total A600 unit of the culture used was excessively high, incubate Lysis buffer type 9 on the column for 2–3 minutes to ensure complete nuclease inactivation. Alternatively, use an EndA negative strain such as TOP10.
Wash buffer type 1 was not completely removed and therefore interfered with the restriction digest.	Discard the column flowthrough by emptying the Collection tube as described in the procedure. If necessary, re-place the column into the Collection tube and re-spin briefly (30 seconds) to remove any residual Wash buffer type 1. Note - if any of the ethanolic Wash buffer comes into contact with the bottom of the column, discard the flow-through and re-centrifuge.

6 Related products

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

If you need further information, Cytiva technical services are happy to assist. Visit *cytiva.com* for contact information.

Application	Product	Product code	Pack sizes
Purification of transfection quality plasmid DNA from *E. coli*	illustra plasmidPrep Midi Flow Kit	28904267	25 purifications
Purification of genomic DNA from small volumes of whole blood and blood cell fractions	illustra blood genomicPrep Mini Spin Kit	28904264	50 purifications
Purification of genomic DNA from animal tissues and cultured mammalian cells	illustra tissue & cells genomicPrep Mini Spin Kit	28904275	50 purifications
Purification of genomic DNA from various bacterial strains	illustra bacteria genomicPrep Mini Spin Kit	28904258	50 purifications
Small scale RNA isolation. High quality RNA from diverse sample types	illustra RNAspin Mini Kit	25050070	20 preparations
Preparation of circular DNA templates	illustra TempliPhi™ 100 Amplification kit	25640010	100 reactions
Kits containing ready-to-use mix for PCR amplification	illustra PuReTaq Ready-To-Go™ PCR Beads	27955701	96 reactions
	FideliTaq™ PCR Master Mix Plus	E71183	125 units
DNA purification from PCR and enzymes	illustra GFX™ PCR DNA & Gel Band purification kit	27960201	100 columns

Application	Product	Product code	Pack sizes
	illustra GFX 96 PCR Purification kit	25690202	10 × 96 well plates
DNA Ligation	DNA ligation System	RPN1507	50 reactions
	Ligate-IT Rapid Ligation kit	US78400	25 reactions
	Ready-To-Go T4 DNA ligase	27036101	50 reactions
Blunt-Ended PCR Cloning	Blunt-ended PCR Cloning Kit	RPN5110	40 reactions
Non-radioactive nucleic acid labeling & detection	Gene Images random-prime DNA Labeling kit	RPN3520	30 reactions
	Gene Images 3′-Oligolabeling kit	RPN5770	Labels 1000 pmol
Non-radioactive nucleic acid labeling & detection	ECL Direct Nucleic acid labeling & Detection System	RPN3000	Labels 5 µg
	Gene Images ECF Detection kit	RPN3580	2500 cm2 membrane
	Gene Images CDP-Star™ Detection kit	RPN3550	2500 cm2 membrane
Large scale purification of plasmid DNA by gel filtration	Sephacryl™ S-1000SF	17047601	750 mL
Purification of oligonucleotides following synthesis	illustra NAP™-5 Columns	17085301	20 purifications

Application	Product	Product code	Pack sizes
Dye terminator removal from automated sequencing reactions	illustra AutoSeq G-50	27534001	50 columns

7 References

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8 Quick Reference Protocol Card

Cue card

Quick Reference Protocol Card

illustra™ plasmidPrep Mini Spin Kit

28904269 (50 purifications)
28904270 (250 purifications)

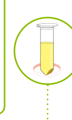
Protocol for 1.5 & 3 ml culture volumes

• Check appropriate volume of ethanol added to Wash buffer type 1

:Add :Spin :Incubate

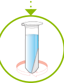
1. Harvesting of bacterial culture

- 1.5 ml bacterial culture
- 30 seconds 16 000 × g
- Pour off and discard supernatant
- Repeat for 3 ml culture volume
- 30 seconds 16 000 × g (for all culture volumes)
- Remove residual supernatant



2. Lysis

- 175 µl Lysis buffer type 7; re-suspend pellet
- 175 µl Lysis buffer type 8; gently invert
- 350 µl Lysis buffer type 9; gently invert
- 4 minutes 16 000 × g



3. Plasmid binding

- Transfer supernatant to plasmid mini column inside Collection tube
- 30 seconds 16 000 × g
- Discard flowthrough

4. Wash (optional-stain dependent)

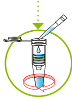
- 400 µl Lysis buffer type 9
- 30 seconds 16 000 × g
- Discard flowthrough

5. Wash & Dry

- 400 µl Wash buffer type 1
- 1 minute 16 000 × g
- Discard flow-through and Collection tube

6. Elution

- Transfer plasmid mini column to a new DNase-free microcentrifuge tube
- 100 µl Elution buffer type 4
- 30 seconds at room temperature
- 30 seconds 16 000 × g
- Retain eluant
- Store purified plasmid DNA at -20°C



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