illustra plasmidPrep Mini Spin Kit

For the rapid extraction and purification of plasmid DNA from small scale cultures of *E. coli*.

Product booklet

Code: 28-9042-69 (50 purifications)
28-9042-70 (250 purifications)

Page finder

1. Legal

Restrictions for product use

The **illustra™ plasmidPrep Mini Spin Kit** has been designed, developed, and sold **for research purposes only.** They are suitable **for *in vitro* use only.** 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.

Contact your GE Healthcare representative for the most current information (see back cover for contact details).

http://www.gelifesciences.com

Amersham Place, Little Chalfont,
Buckinghamshire. HP7 9NA UK.

2. Handling and storage

2.1. Safety warnings and precautions

Warning: For research use only.

Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products. Suitable protective clothing such as laboratory overalls, safety glasses and gloves should be worn. Care should be taken to avoid contact with skin or eyes; if contact should occur, wash immediately with water (see Material Safety Data Sheet(s) and/or Safety Statement(s) for specific recommendations).

Warning: This protocol requires the use of ethanol.

2.2. Storage

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

2.3. Expiry

For expiry date please refer to outer packaging label.

3. Components

3.1. Kit contents

Identification	Pack Size Cat. No.	10 purifications Sample Pack	50 purifications 28-9042-69	250 purifications 28-9042-70
	Lysis buffer type 7	3 ml	15 ml	60 ml
	Lysis buffer type 8	3 ml	15 ml	60 ml
	Lysis buffer type 9	10 ml	50 ml	220 ml
	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
	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.

GE 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-HCl (pH 8.0).

3.2. Materials and equipment to be supplied by user

Disposables:
DNase-free 1.5 ml microcentrifuge tubes (snap cap). One per purification.

Chemicals:
Absolute ethanol

3.3. Equipment needed

Microcentrifuge that accommodates 1.5 ml microcentrifuge tubes
Vortex mixer (optional)

4. Description

4.1. Introduction

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 1–5).

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 section 5.3. 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.

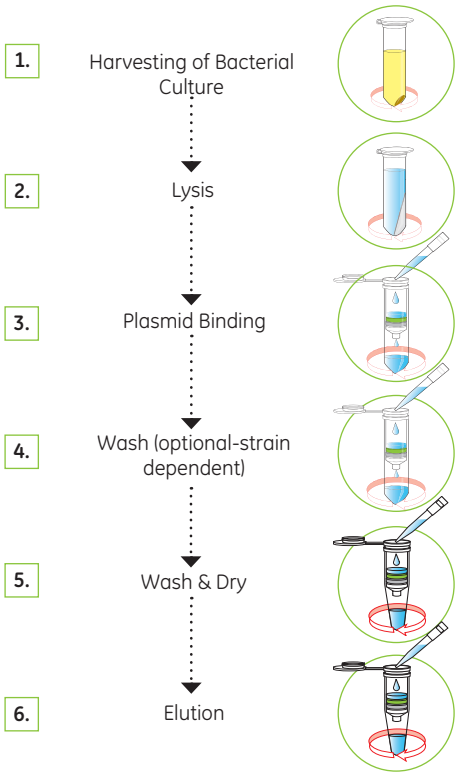
The **illustra plasmidPrep Mini Spin Kit** utilizes a simple plasmid DNA purification protocol, employing a modified alkaline cell lysis procedure (1–3) and a novel silica-based membrane.

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.

4.2. The basic principle

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



Plasmid DNA ready for downstream applications

Step	Comments	Component
1. Harvesting of Bacterial Culture	Bacteria are harvested by centrifugation and the spent medium removed.	**Bacteria** 
2. 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.	
3. 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** 
4. Wash (optional strain dependent)	An optional wash step removes residual nuclease activity & carbohydrates. Recommended for *EndA*$^{+}$ strains.	**Lysis buffer type 9**
5. Wash & Dry	A combined washing & drying step, with an ethanolic buffer, removes residual salts and other contaminants.	**Wash buffer type 1**
6. Elution	Plasmid DNA is eluted from the illustra plasmid mini column in a low ionic strength buffer.	**Elution buffer type 4**

4.3. Product specifications

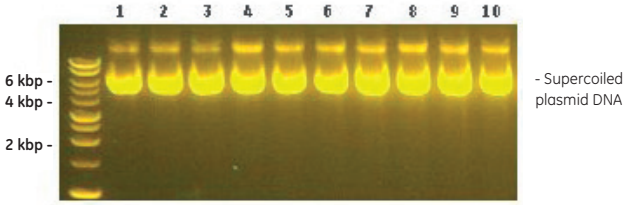
Sample type:	1.5 ml processed bacterial culture	3.0 ml processed bacterial culture
Typical A_{600}	2.5–3.0	2.5–3.0
Time/prep*	Approximately 9 minutes	Approximately 9 minutes
Yield**	6–9 µg	9–15 µg
purity - A_{260}/A_{280}	> 1.8	> 1.8
purity - A_{260}/A_{230}	> 1.7	> 1.7

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

** 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 high-copy 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).

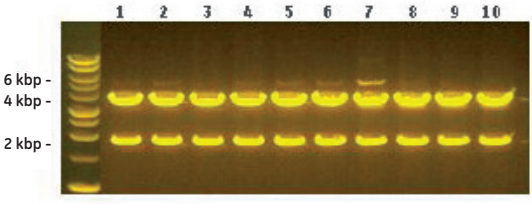
4.4. Typical output

Figure 1. Undigested plasmid DNA



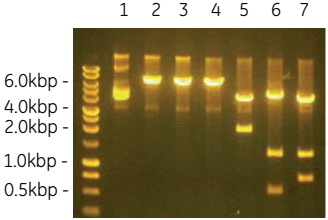
pCORON1002-EGFP-C1; 400 ng samples were run on a 1% (w/v) agarose gel.

Figure 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.

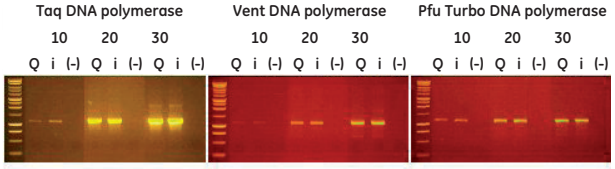
Figure 3. Multiple restriction digests



1 – Undigested pDNA
2 – NheI (6313 bps)
3 – SacI (6313 bps)
4 – BamHI (6313 bps)
5 – HindIII (4273 & 2040 bps)
6 – PsiI (4497, 1252 & 564 bps)
7 – HindIII/NheI (4273, 1278 & 762 bp)

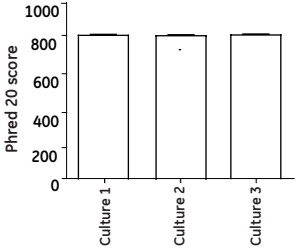
Sample 1 from above; 400 ng aliquots were digested with 5 units of the restriction endonucleases indicated, at 37°C for 1 hour.

Figure 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.

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



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

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.

5. Protocols

Factors that may affect plasmid yield and purity are outlined in Section 6.1.

Note: Buffers and mini columns ARE NOT transferable between GE 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.

Use of icons

The Key below describes the purpose of the icons used throughout the protocol booklet.

This icon is used to highlight particularly critical steps within the protocol that must be adhered to. If this advice is not followed it will have a detrimental impact on results.

This icon is used to highlight technical tips that will enhance the description of the step. These tips may indicate areas of flexibility in the protocol or give a recommendation to obtain optimum performance of the kit.

5.1. Preparation of working solutions

See section 3.2 and 3.3 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. If necessary, warm the solution in a 37°C water bath for 5 minutes. 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. Add 28 ml of absolute ethanol to Wash buffer type 1 in kit 28-9042-69 (50 purifications) or add 104 ml to Wash buffer type 1 in kit 28-9042-70 (250 purifications). Mix by inversion. Indicate on the label that this step has been completed. For 10 purifications sample pack size, please add 4 ml absolute ethanol to Wash buffer type 1 prior to use.

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

5.2. Protocol for 1.5 ml and 3 ml culture volumes

1. Harvesting of Bacterial Culture

a. 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.

30 seconds
16 000 x g

b. If processing 3 ml culture volumes, repeat step a. 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.

2. Lysis

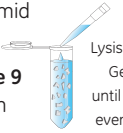
a. Cell re-suspension- Add 175 µl **Lysis buffer type 7** to the bacterial pellet and thoroughly re-suspend the pellet.

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.

b. Cell Lysis - 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.

c. Neutralisation - 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.

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

4 minutes
16 000 × g

d. Flocculent spin-Centrifuge at full speed (approximately 16 000 × 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.

e. 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.

3. Plasmid Binding

a. Column lysate loading-Carefully transfer the cleared supernatant to the mini column (approximately 700 µl). Close the lid of the column gently. Centrifuge at full speed (approximately 16 000 × g) for 30 seconds. Discard the flowthrough by emptying the Collection tube.

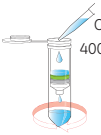
Note: When purifying plasmid DNA from 3 ml culture volumes, the user may notice some residual lysate on the column after this centrifugation step. This can be ignored; proceed with the Wash & Dry step below. On washing this residual lysate will generally pass through the column with no effect on the 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.

4. Wash (optional-strain dependent)

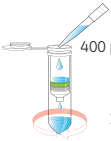
a. Wash the column with 400 µl **Lysis buffer type 9** and centrifuge at full speed (approximately 16 000 × g) for 30 seconds. Discard the flowthrough.



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.

5. Wash & Dry

a. Add 400 µl **Wash buffer type 1** to the column and centrifuge at full speed (approximately 16 000 × 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.

6. Elution

a. 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. Incubate the column for 30 seconds at room temperature. Microcentrifuge at full speed (approximately 16 000 × 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, add a single 50 µl volume of **Elution buffer type 4**, incubate for 1 minute and centrifuge to elute.

b. Store the purified plasmid DNA at -20°C.

6. Appendices

6.1. 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.

6.2 Isolation of low copy number and large molecular weight plasmids

The procedure described in section 5.2 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.

6.3. 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 section 4.4 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

6.4. Troubleshooting guide

This guide may be helpful in the first instance, however if problems persist or for further information please contact GE Healthcare technical services. Visit http://www.gelifesciences.com for contact information.
Alternatively log on to http://www.gehealthcare.com/illustra.

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 A_{600} of the culture before processing. If the culture density A_{600} > 5, reduce the volume of culture processed.
The cell pellet was not completely re-suspended 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.

Problem: plasmid DNA yield is low (continued)

Possible cause	Suggestions
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.

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.

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.

Problem: plasmid DNA does not cut to completion or is degraded on incubation at 37°C.

Possible cause	Suggestions
Plasmid DNA irreversibly denatured by Lysis buffer type 8 and therefore will not cut.	• See previous
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 A_{600} 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.

6.5. Related products available from GE Healthcare

A full range of Molecular Biology reagents can be found on the GE Healthcare web site and in the catalog: http://www.gehealthcare.com/illustra

If you need further information, GE technical services are happy to assist. Visit http://www.gelifesciences.com for contact information.

Application	Product	Product code	Pack sizes
Purification of transfection quality plasmid DNA from *E. coli*	illustra plasmidPrep Midi Flow Kit	28-9042-67	25 purifications
Purification of genomic DNA from small volumes of whole blood and blood cell fractions	illustra blood genomicPrep Mini Spin Kit	28-9042-64	50 purifications
Purification of genomic DNA from animal tissues and cultured mammalian cells	illustra tissue & cells genomicPrep Mini Spin Kit	28-9042-75	50 purifications
Purification of genomic DNA from various bacterial strains	illustra bacteria genomicPrep Mini Spin Kit	28-9042-58	50 purifications
Small scale RNA isolation. High quality RNA from diverse sample types	illustra RNAspin Mini Kit	25-0500-70	20 preparations

Application	Product	Product code	Pack sizes
Preparation of circular DNA templates	illustra TempliPhi™ 100 Amplification kit	25-6400-10	100 reactions
Kits containing ready-to-use mix for PCR amplification	illustra PuReTaq™ Ready-To-Go™ PCR Beads	27-9557-01	96 reactions
	FideliTaq™ PCR Master Mix Plus	E71183	125 units
DNA purification from PCR and enzymes	illustra GFX™ PCR DNA & Gel Band purification kit	27-9602-01	100 columns
	illustra GFX 96 PCR Purification kit	25-6902-02	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	27-0361-01	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

Application	Product	Product code	Pack sizes
Non-radioactive nucleic acid labeling & detection	ECL Direct Nucleic acid labeling & Detection System	RPN3000	Labels 5 µg
	Gene Images ECF Detection kit	RPN3580	2500 cm^2 membrane
	Gene Images CDP-Star™ Detection kit	RPN3550	2500 cm^2 membrane
Large scale purification of plasmid DNA by gel filtration	Sephacryl™ S-1000SF	17-0476-01	750 ml
Purification of oligonucleotides following synthesis	illustra NAP™-5 Columns	17-0853-01	20 purifications
Dye terminator removal from automated sequencing reactions	illustra AutoSeq G-50	27-5340-01	50 columns

7. References

1. Birnboim, H.C. & Doly, J., *Nucl. Acids Res.* 7, 1513 (1979).

2. Ish-Horowicz, D. & Burke, J.F., *Nucl. Acids Res.* 9, 2989 (1981).

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. Voo, K.S. & Jacobsen B.M., Biotechniques, 24, 240-243 (1998).

7. Ausubel, F.M. et al., eds., Current Protocols in Molecular Biology 1, 1.68 (1991).

Intentionally left blank

GE Healthcare offices:

GE Healthcare Bio-Sciences AB
Björkgatan 30, 751 84 Uppsala,
Sweden

GE Healthcare Europe GmbH
Munzinger Strasse 5, D-79111 Freiburg,
Germany

GE Healthcare Bio-Sciences Corp.
100 Results Way, Marlborough,
MA 01752
USA

GE Healthcare Dharmacon.
2650 Cresent Drive, Lafayette,
CO 80026
USA

Hyclone Laboratories, Inc,
925 W 1800 S, Logan
UT 84321
USA

GE Healthcare Japan Corporation
Sanken Bldg. 3-25-1, Hyakunincho,
Shinjuku-ku, Tokyo 169-0073,
Japan

For your local office contact information, visit
www.gelifesciences.com/contact

GE Healthcare UK Limited
Amersham Place
Little Chalfont, Buckinghamshire,
HP7 9NA, UK

http://www.gelifesciences.com

imagination at work

28-9042-71

29275352 AG 06-2017

Quick Reference Protocol Card

illustra™ plasmidPrep Mini Spin Kit

28-9042-69 (50 purifications)
28-9042-70 (250 purifications)

Protocol for 1.5 & 3 ml culture volumes

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

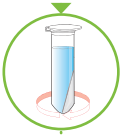
:Add :Spin :Incubate

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

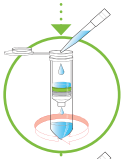


3. Plasmid binding

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

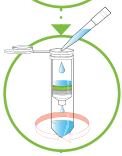
4. Wash (optional-stain dependent)

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



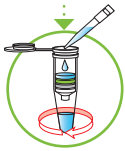
5. Wash & Dry

- 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



28-9042-71

29275352 AG 06-2017

http://www.gelifesciences.com

GE Healthcare UK Limited.
Amersham Place, Little Chalfont,
Buckinghamshire, HP7 9NA UK