Mobius™ and UltraMobius™ 1000 Plasmid Kits

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About the Kits

Introductory Mobius™ 1000 Plasmid Kit	2 rxn	70854-3
Mobius 1000 Plasmid Kit	10 rxn	70853-3
	25 rxn	70853-4
Introductory UltraMobius™ 1000 Plasmid Kit	2 rxn	70907-3
UltraMobius 1000 Plasmid Kit	10 rxn	70906-3
	25 rxn	70906-4

Description

The Mobius 1000 Plasmid Kits are designed for fast, convenient, and economical isolation of up to 1 mg ultrapure plasmid DNA from 100 ml overnight bacterial culture using high-copy number plasmids (1). For low-copy number plasmids, the kit can isolate up to 200 µg DNA from 250-ml culture, and up to 1 mg from 500-ml to 1.5-liter cultures using a scalable protocol. Based on an alkaline lysis procedure, combined with streamlined anion exchange chromatography, the Mobius method produces plasmid DNA suitable for all molecular biology applications, including restriction analysis, *in vitro* transcription, sequencing, and transfection.

The Mobius resin is a patented, high-capacity Fractogel® support comprised of porous methacrylate beads of high surface area and rigidity to which anion exchange groups are grafted via flexible polyelectrolyte chains (tentacles). This feature enables the ionic groups to adopt an optimal configuration for interaction with the phosphate backbone of DNA, resulting in increased DNA binding capacity (> 1 mg plasmid/ml bed volume). In addition, the purely organic Mobius resin leads to substantially less copurification of endotoxins as compared to silica based anion exchange supports. The Mobius Kits incorporate a novel ClearSpin™ filtration device that minimizes the duration of centrifugation steps required for the clarification of lysates.

The Mobius 1000 Plasmid Kits are available in two formats. For the majority of plasmid DNA applications, such as routine transfection, cloning, sequencing, and transformation of competent *E. coli*, the standard Mobius 1000 Plasmid Kit provides DNA with sufficiently high purity and very low endotoxin levels (< 500 EU/mg). In applications where extremely low endotoxin levels are required, such as microinjection and transfection of endotoxin-sensitive cell lines, the UltraMobius 1000 Plasmid Kit is recommended. The UltraMobius 1000 Plasmid Kit incorporates an additional reagent to reduce endotoxin levels to below 20 EU/mg plasmid DNA.

Components

- Mobius™ Protocol Quick Start Guide (Reference Card)
- 2, 10 or 25 Mobius 1000 Columns
- 2, 10 or 25 ClearSpin™ Filters
- ClearSpin Adapter (2 included in 10 and 25 rxn kits, none in the Intro Kit)
- Mobius Buffer Kit

Intro. Kit	10 and 25* rxn Kits		
0.16 ml	1 ml	RNase A Solution	
16 ml	100 ml	Bacterial Resuspension Buffer	(1)
16 ml	100 ml	Bacterial Lysis Buffer	(2)
16 ml	100 ml	Mobius Neutralization Buffer	(3)
5 ml	30 ml	Detox Agent (UltraMobius only)	(4)
20 ml	125 ml	Mobius Equilibration Buffer	(A)
40 ml	250 ml	Mobius Wash Buffer	(B)
10 ml	65 ml	Mobius Elution Buffer	(C)
1 ml	12.5 ml	TE Buffer	

**Two Buffer Kits are included with the Mobius 1000 Plasmid Kit for 25 purifications.*

Storage

The unopened components of the kit are stable for more than 1 year at room temperature (21–25°C). After combining the RNase A and Bacterial Resuspension Buffer, this solution should be stored at 4°C and is stable for 6 months.

Required equipment and reagents

- Isopropanol
- Ethanol (70%) prepared with nuclease-free sterile water
- High-speed centrifuge compatible with 50-ml conical tubes
- Sterile 2-, 5-, and 10-ml pipets
- Sterile 15- and 50-ml conical centrifuge tubes
- Sterile water

Before You Begin

Plasmids and host strains

*The purification of long chain nucleic acids (> 20 kbp) by anion exchange chromatography is protected by EP 0 268 946 and corresponding patents. Purchase of the Mobius Kits does not include a license under these patents. The use of the pETcoco vectors is covered under U.S. Patent No. 5,874,259 and other patents pending. This product is sold under license from the University of Wisconsin–Madison and is limited solely for research purposes.

The Mobius™ 1000 Plasmid Kits can be used to purify a variety of plasmids* from many different *E. coli* host strains. Most commonly used plasmids fall into either high-copy number (> 200 copies per cell) or low-copy number (10–40 copies per cell) categories, depending on the plasmid replicon. The term "mid-copy" has also been applied to plasmid having between 10 and 40 copies per cell, to distinguish them from plasmids maintained at very low copy numbers (e.g., pETcoco™ vectors). Here we refer to the pETcoco plasmids as "single-copy" when maintained in the presence of 0.2% glucose, and "low-copy" when copy number is amplified to approximately 40 copies/cell in the presence of 0.01% arabinose. The copy number category determines the scale of starting culture that is recommended for the Mobius 1000 Standard Protocol; 100 ml for high-copy number plasmids and 250 ml for low-copy number plasmids. Table 1 classifies some commonly used plasmids according to copy number.

Table 1

Plasmid series	Copy Number	Protocol
pACYC, pACYCDuet™	10–12	Low-copy
pBAC™, pBACgus	> 500	High-copy
pBluescript®	> 500	High-copy
pBR322	15-20	Low-copy
pCDF, pCDFDuet™	20-40	Low-copy
pCITE®	> 500	High-copy
PCOLADuet™	~40	Low-copy
pET	~40	Low-copy
pETBlue™	> 500	High-copy
pETcoco™	~40*	Low-copy*
pETDuet™	~40	Low-copy
pGEM®	> 500	High-copy
pLysS, pLysE, pLacI	10–12	Low-copy
pRSF, pRSFDuet™	> 200	High-copy
pSCREEN™	> 500	High-copy
pSTBlue	> 500	High-copy
pT7Blue	> 500	High-copy
pTriEx™	> 500	High-copy
pUC	> 500	High-copy

*The single-copy pETcoco plasmid must be amplified to a low-copy plasmid using arabinose to prior to plasmid purification. See User Protocol TB333

Although most host strains can be used successfully with the Mobius method, the quality of the plasmid DNA can vary based on specific hosts used. The Novagen NovaBlue strain produces very high quality plasmid and has been used as the standard to qualify endotoxin levels with the Mobius Kits. Useful mutations for plasmid preparation affect the genes *endA* (eliminates endonuclease I activity, which improves the quality of plasmid DNA) and *recA* (eliminates general recombination and inhibits the formation of plasmid multimers).

Symptoms of host-related problems include impurities due to excess carbohydrates that can inhibit some enzymatic procedures, excess nicked plasmid due to high endonuclease levels, and difficulty in restriction digestion and/or transformation of restriction-plus hosts due to incompatible modification of the DNA. In these cases, it may be worthwhile to transform the plasmid into another host such as DH1 or NovaBlue. The NovaBlue strain (genotype: *endA1 hsdR17*(r_{K12}^{-} m_{K12}^{+})

supE44 thi-1 recA1 gyrA96 relA1 lac F' [*proA*$^+$*B*$^+$ *lacI*^{q}*ZΔM15* ::Tn*10*(Tet^R)]) is available from Novagen as competent cells, Singles™ Competent Cells, GigaSingles™ Competent Cells, HT96™ Competent Cells, or as a glycerol stock.

Culture conditions

Culture conditions can dramatically affect plasmid yield and quality. The Mobius™ procedure has been optimized for cultures in LB broth (see recipe below). Richer media, such as Terrific Broth, may produce more cell mass, but the yield and quality of plasmid may suffer due to greater levels of cellular and media components that can interfere with plasmid binding and separation on the anion exchange resin.

Preparation of LB broth: Per liter, combine 10 g tryptone, 10 g NaCl, and 5 g yeast extract. Add deionized water to approximately 900 ml and dissolve with stirring. Adjust pH to 7.5 with 1 N NaOH. Bring volume to 1 L with water, and autoclave. Cool to 60°C before adding appropriate antibiotic (e.g., 50 µg/ml carbenicillin).

Cultures should always be grown in the presence of appropriate antibiotic to maintain selection of the plasmid. Particular care should be taken with the ampicillin-resistance marker β-lactamase, which is present on many commonly used plasmids. During growth, cells secrete β-lactamase into the medium where it can rapidly degrade the antibiotic. Degradation of ampicillin is also enhanced during the late stages of cell growth when the pH of the culture drops. This effect can be alleviated by using carbenicillin (Cat. No. 69101-3) instead of ampicillin, as carbenicillin is less susceptible to degradation under acidic conditions.

Starter cultures for plasmid isolation are prepared by inoculating 2–5 ml LB medium containing antibiotic from freshly streaked, well-isolated colonies grown on LB agar plates containing antibiotic. After shaking at 250–300 rpm 37°C for 8 h, dilute the cells 1:200–1:500 into a culture flask containing prewarmed LB broth containing antibiotic and shake at 37°C for 12–16 h. For proper aeration, use a culture flask that has a volume at least 4-fold greater than the culture volume (e.g., a 500-ml Erlenmeyer flask for a 100-ml culture). Flasks intended for bacterial growth (e.g., Fernbach flasks) or flasks with baffles are also suitable, although not required.

As a matter of routine, glycerol stocks should be prepared from cultures grown from single colonies to an OD_{600} of approximately 0.5 to avoid the overgrowth of non-plasmid bearing cells.

Amplification of low-copy number plasmids

Although the Mobius 1000 Kits provide a reproducible method for purification of low-copy number plasmids grown in LB broth without amplification, it is possible to increase the copy number of ColE1-derived plasmids through manipulation of culture conditions, which may increase the yield of difficult plasmids. Plasmids, such as pBR322, can be induced to increase their copy number through the addition of chloramphenicol to the growth medium, which inhibits host protein synthesis while plasmid replication continues (2). Although this phenomenon is not clearly understood, historically investigators added 170 µg/ml chloramphenicol to cultures in log phase (OD_{600} = 0.4) followed by 12–16 h additional incubation prior to harvest (3). More recently, superior amplification of pBR322-type plasmids was reported using 10–20 µg/ml chloramphenicol to partially inhibit protein synthesis (4); in this case amplification appeared to depend on the *relA* gene product. Other strategies such as amino acid starvation and growth temperature have also been reported to cause plasmid amplification (5-7). Protocols for the amplification of low-copy number plasmids must be developed empirically under the desired growth conditions. For evaluation of plasmid amplification with a specific vector/host combination, the recommended conditions are growth in LB to an OD_{600} of 1–2, addition of 10–20 µg chloramphenicol/ml culture volume, and 12–16 h incubation at 37°C. Note that the NovaBlue strain carries the *relA1* mutation, so is unsuitable for amplification by this method.

Standard and Scalable Protocols

Two protocols are available for the isolation of plasmid DNA using the Mobius™ 1000 and UltraMobius™ 1000 Kits. As stated in "About the Kits," the Standard Protocol purifies greater than 1000 µg (1 mg) plasmid from 100 ml culture for high-copy number plasmids, and 200–250 µg plasmid for low-copy number plasmids. The Standard Protocol for the Mobius 1000 Plasmid Kit uses one ClearSpin™ Filter and one Mobius 1000 Column.

The Scalable Protocol is intended for use with low-copy number plasmids and can be scaled up to 1.5 L bacterial culture for increased yield. Use of the Scalable Protocol will reduce the number of plasmid isolations possible with one kit, since larger amounts of buffer components are required to prepare the clarified cell lysates. The Mobius Buffer Kit is available separately (Cat. No. 70855-3) to replenish kit components, if necessary.

Table 2

Plasmid type	Protocol	Culture volume	Expected yield
High-copy number	Standard	100 ml	> 1 mg
Low-copy number	Standard	250 ml	200–250 µg
Low-copy number	Scalable	0.5 to 1.5 liter	Up to 1 mg

Both the Standard and Scalable Protocols refer to the optional use of Detox Agent for the treatment of clarified lysates prior to chromatography. Detox Agent is supplied with the UltraMobius 1000 Plasmid Kits, and is not required for the Mobius 1000 Plasmid Kit.

Bacterial endotoxins

Endotoxins, also referred to as lipopolysaccharides (LPS), an essential component of the outer membrane of gram-negative cells such as *Escherichia coli* and *Salmonella typhimurium*, are present at about 3.5×10^6 copies/cell (8). In wild-type *E. coli*, the LPS molecule is composed of three basic components: an O polysaccharide side-chain, a Core Oligosaccharide and Lipid A. The amphipathic nature of the LPS molecule allows it to form micelles, as well as embed itself in the outer membrane, maintaining the hydrophilic, negatively charged O and Core polysaccharides on the exterior. In the commonly used *E. coli* strains, K-12 and B, the O polysaccharide is absent. B strains have an additional deletion of the Core polysaccharide. During the lysis of bacterial cells with detergent, LPS and other membrane components are solubilized. Since LPS has a natural affinity for silica surfaces, the isolation of plasmids using a silica-based solid phase can often result in the copurification of endotoxin. Other purification procedures such as CsCl banding and standard alkaline lysis can also result in the copurification of endotoxins. Endotoxin contamination in plasmid isolates can cause cytotoxicity in transfection and microinjection procedures (9).

In contrast to other commercial plasmid purification media that use a silica matrix, the Mobius 1000 Columns are packed with a highly hydrophilic ion exchange bead that allows plasmid DNA to be selectively purified without co-purification of LPS. The result is that plasmids isolated with the standard Mobius 1000 Plasmid Kits are substantially lower in LPS level than isolates from other kits as measured by the *Limulus* amoebocyte lysate assay (1).

Lowest endotoxin levels with UltraMobius 1000 Plasmid Kit

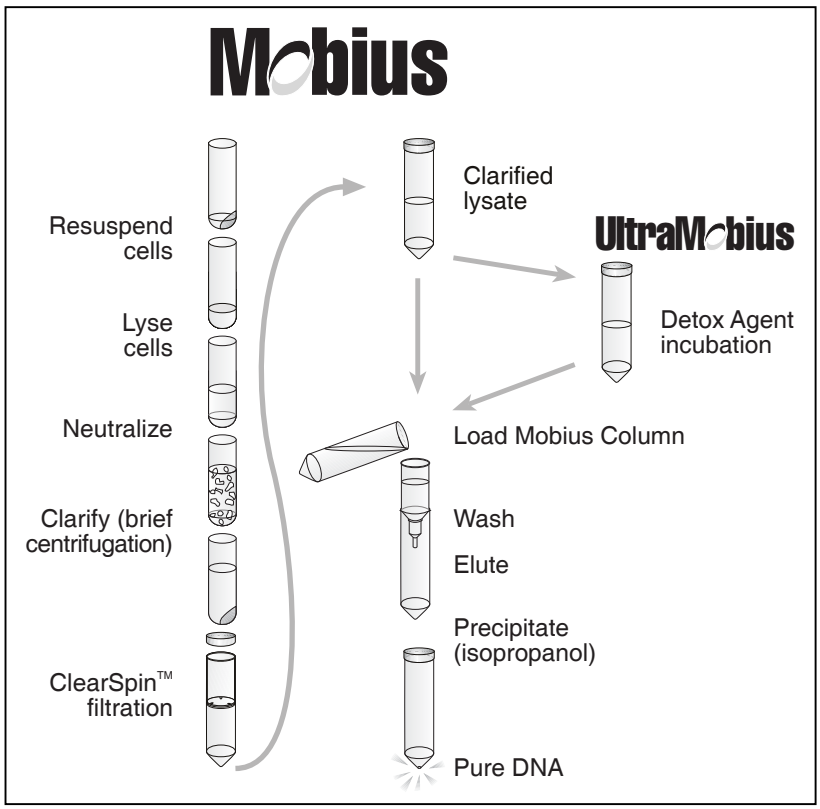
The UltraMobius 1000 Plasmid Kit combines the Mobius 1000 Columns with Detox Agent treatment, resulting in exceptionally low levels of endotoxin contamination. The UltraMobius protocol is nearly identical to the standard plasmid isolation procedure, requiring only the addition of Detox Agent to the cleared lysate and a brief (15 min) incubation on ice prior to chromatography. Use endotoxin-free water for preparing the 70% EtOH wash, and fresh sterile disposable centrifuge tubes. Endotoxin contamination can occur if glassware or solutions are sterilized in an autoclave previously used for killing bacterial cultures; therefore, it is best to avoid using autoclaved glassware and solutions.

Mobius™ 1000 Columns and ClearSpin™ Filters

The Mobius 1000 Column utilizes a proprietary high capacity anion exchange resin comprised of porous beads of high surface area and rigidity. The matrix is a modified synthetic copolymer, to which the ion exchange moieties are grafted via polyelectrolyte chains (tentacles). The flexible tentacles not only increase the resin capacity, providing extremely high plasmid DNA binding capacities, but also reduce non-specific binding and improve resolution relative to conventional chromatography resins. The resin is packed between polyethylene frits that prevent the column from running dry during chromatography. The Mobius resin is packed in a custom molded chromatography column with several convenient features. The column can be held upright in most commercially available disposable 15- or 50-ml conical centrifuge tubes (such as Falcon 352097 and 352098). During plasmid purification, fractions can be collected directly into sterile tubes without the use of a ring stand or special rack. The columns are shipped in 20% ethanol and can be stored at room temperature.

The ClearSpin Filter is a convenient filtration aid intended for the clarification of the neutralized lysates prior to chromatography. After neutralization and a 2 min centrifugation at 10,000 × g to remove the bulk of the precipitate, the ClearSpin Filter is used to remove fine particulate materials from the lysate. ClearSpin Filters have a working volume of up to 27 ml. While the ClearSpin Filters are supplied with their own collection tube, the filter dimensions are compatible with most commonly used 50-ml conical tubes. However, note that the 50-ml conical tubes from Sarstedt (Cat. No. 62.547 and Cat. No. 62.548) are not compatible with the ClearSpin Filters, due to their slightly smaller inside diameter. The filters bind in these tubes and are difficult to remove.

ClearSpin Filters can withstand a maximum centrifugal force of 5000 × g and are compatible with both fixed angle and swinging bucket rotors.



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Mobius™ 1000 and UltraMobius™ 1000 Standard Protocol

100-ml culture (high-copy number plasmid)

250-ml culture (low-copy number plasmid)

The Standard Protocol is intended for routine purification of high- and low-copy number plasmids. The yields for high-copy number plasmids typically exceed 1 mg. For low-copy number plasmids, the yields are typically around 200–250 µg. When larger amounts of low-copy number plasmids are required, the Mobius 1000 Scalable Protocol (page11) is recommended. The Scalable Protocol is designed for processing up to 1.5 L bacterial culture and typically yields up to 1 mg low-copy number plasmid. The Scalable Protocol requires the use of larger volumes of buffer than the Standard Protocol. The supplied component volumes have been optimized for the Standard Protocol. The Mobius Buffer Kit (Cat. No. 70855-3) is available separately.

Notes: ***Prior to first use of kit:***

A. *Add 1.0 ml (entire volume) RNase A solution to Bacterial Resuspension Buffer (bottle 1). Spin tube in microcentrifuge briefly to collect contents in bottom of tube and transfer entire volume to a bottle of Resuspension Buffer using a sterile pipet. After addition of RNase, mark the box on bottle as a reminder, and store the reagent at 4°C.*

Before starting a plasmid isolation:

B. *Examine Bacterial Lysis Buffer (bottle 2) for precipitation. If a precipitate is observed, warm bottle briefly at 30–40°C until precipitate dissolves.*

Note: *Lysis buffer contains Sodium Hydroxide! Wear gloves, lab coat, and protective eyewear. Clean any exposed skin thoroughly with soap and water. If exposure to the eye occurs, rinse eyes with copious amounts of water.*

C. *Chill Mobius Neutralization Buffer (bottle 3) on ice for at least 10 min prior to use.*

Culture growth

1. From a freshly streaked plate, transfer a single bacterial colony to 5-ml liquid LB culture containing the appropriate antibiotic (refer to page 5 for preparation). Incubate at 37°C for 8 h in shaking incubator at 300 rpm.
2. Transfer 0.5 ml of this culture into 100-ml (high-copy number plasmid) or 250-ml (low-copy number plasmid) sterile LB in an appropriately sized flask containing antibiotic (refer to guidelines for flask volume, page 6). Incubate at 37°C for 12–16 h in shaking incubator at 300 rpm. The OD_{600} at harvest should be approximately 3–5 when using sterile medium as a spectrophotometer blank.
3. Harvest cells by centrifugation at 5000 × g for 10 min. Carefully decant supernatant, and invert and hold tube for several seconds to drain medium. Blot mouth of inverted tube on paper towel to remove any residual medium. It is convenient to decant into bleach solution to sterilize the supernatant before disposal.

Note: *The use of 250- or 500-ml centrifuge bottles is highly recommended for cell harvesting, lysis and initial clarification. It is important to use bottles with sufficient capacity to allow adequate mixing during resuspension, lysis, and neutralization steps. The use of smaller bottles can reduce plasmid yield.*

Tip: *Cell pellets can be stored frozen at –70°C for several weeks, if desired.*

Preparation of cleared lysate

1. Chill Mobius™ Neutralization Buffer (bottle 3) on ice for 10 min.
2. Resuspend bacterial pellet in 8 ml Bacterial Resuspension Buffer (bottle 1). Swirl centrifuge bottle, pipet up and down and gently vortex until there are no visible clumps and pellet is completely resuspended. Failure to fully resuspend bacterial pellet can reduce plasmid yield.
3. Add 8 ml Bacterial Lysis Buffer (bottle 2) to the fully resuspended bacteria. Recap tubes and tip or swirl gently to mix, until lysate appears cleared and viscous. **Do not vortex!** Vortexing can cause shearing of genomic DNA, resulting in reduced plasmid yield and genomic DNA contamination. Incubate lysate at room temperature for 5 min.

Notes: *Gentle but complete mixing is critical for maximum plasmid yields. The resulting lysate should appear viscous, bubbly, and translucent, but nearly clear. "Pockets" of unlysed cells in the mixture display a stringy, opaque appearance and signify insufficient mixing. Do not exceed the recommended 5 min incubation. Excessive exposure to alkaline conditions can contribute to nicking and irreversible denaturation of plasmid DNA.*

Keep Bacterial Lysis Buffer tightly capped when not in use. Atmospheric CO_2 will decrease the effectiveness of the buffer.

4. Add 8 ml chilled Mobius Neutralization Buffer (bottle 3). Recap. Mix thoroughly by inversion to form a uniform flocculent precipitate. The mixture should become less viscous as genomic DNA, protein, detergent and cell debris are precipitated. Incubate mixture on ice for 5 min.

Note: *Properly mixed neutralized lysate should have two components: white flocculent precipitate and reduced viscosity solution. The presence of stringy, viscous debris within the neutralized lysate indicates poor mixing, which can lead to reduced plasmid yield.*

5. During incubation, remove upper and lower caps of Mobius 1000 Column. Decant excess storage buffer. Place column in 50-ml conical tube. Add 10 ml Mobius Equilibration Buffer (bottle A) into column reservoir and verify "gravity flow," which may take a few moments to begin. Allow entire 10-ml volume to flow through column. The column will not run dry, because the flow will cease when buffer meniscus reaches the top frit of column.
6. Centrifuge neutralized lysate at 10,000 × g for 2 min (room temperature or 4°C) to remove the bulk of the insoluble material. Remove cap of ClearSpin™ Filter unit (fitted in the collection tube) and decant cleared lysate supernatant into it. Replace cap and tap ClearSpin unit gently on bench top to initiate flow through the filter. If observed flow rate is low, loosen cap briefly and retighten.
7. Immediately centrifuge ClearSpin Filter in a suitable clinical or tabletop centrifuge at 2000 × g for 3 min to filter the lysate (the ClearSpin Filter should not be used without the initial centrifugation of the neutralized lysate, Step 6). If a clinical centrifuge is unavailable, adequate results can be obtained by allowing gravity transfer of the majority of the liquid phase to the lower chamber, although the plasmid yield will be slightly lower. Discard filter unit, and retain filtrate.

Note: *For agarose gel analysis (optional, refer to page 15), remove a 24-µl (for high-copy) or 60-µl (for low-copy) sample of clarified lysate. Maintain fraction on ice or store at –20°C.*

Tip: *Clarified, neutralized lysate can be frozen at –20°C or –70°C and stored for later processing, if desired. Frozen lysates must be clarified after thawing by centrifugation (10,000 × g for 10 min) prior to loading Mobius 1000 Column to remove any formed precipitate.*

8. **UltraMobius 1000 Plasmid Kit only:** Add 2.4 ml Detox Agent (bottle 4) to cleared lysate. Mix gently. Incubate on ice for 15 min.
9. **UltraMobius 1000 Plasmid Kit only**: Centrifuge Detox Agent treated lysate for 10 min at 10,000 × g.

Mobius™ 1000 Column chromatography

1. Place equilibrated Mobius 1000 Column in new 50-ml conical tube and transfer entire clarified lysate volume to column reservoir. Allow entire volume to flow through column by gravity. This may require up to 30–45 min. The column does not need to be attended during this period.
2. Transfer Mobius 1000 column to new 50-ml conical tube. Add 20 ml Mobius Wash Buffer (bottle B) to column reservoir and allow entire volume to flow through column by gravity.

For agarose gel analysis (optional, refer to page 15), remove 24 µl (for high-copy) or 60 µl (for low-copy) sample of flow through and a 20 µl (for high-copy) or 80 µl (for low-copy) sample of wash. Maintain fractions on ice, or store at –20°C.

3. Transfer loaded Mobius 1000 Column to new 15-ml conical tube and elute plasmid DNA by adding 5 ml Mobius Elution Buffer (bottle C) into reservoir. Allow entire volume to flow through column by gravity. Collect entire volume in a single fraction.

Note: Optional Stopping Point: The eluted DNA may be stored overnight at 4°C and precipitated the following day.

Note: For agarose gel analysis (optional, refer to page 15), remove 5 µl (for high-copy) or 20 µl (for low-copy) sample of the eluate.

Precipitation of plasmid DNA

1. Remove column from tube and transfer entire volume eluted sample into 30-ml polycarbonate centrifuge tube. Add 3.5 ml isopropanol to eluted sample to precipitate plasmid DNA. Mix gently and immediately centrifuge at 15,000 × g for 20 min (room temperature or 4°C).
2. Carefully aspirate and discard supernatant, avoiding contact with tube wall. It may be helpful to mark the exterior position of the tubes with a permanent marking pen to aid in locating the DNA pellet. Isopropanol-precipitated DNA pellets are often diffuse, translucent, and spread over a large portion of the exterior tube wall. Addition of Pellet Paint® or Pellet Paint NF Co-Precipitant prior to addition of isopropanol can aid in visualizing precipitated material.

Note: Pellet Paint and Pellet Paint NF Co-Precipitant have not been tested for compatibility with DNA transfection or DNA microinjection into eukaryotic cells.

3. Wash DNA pellet by adding 3 ml 70% ethanol (room temperature) and swirling tube gently.
4. Centrifuge tube at 15,000 × g for 10 min (room temperature or 4°C).
5. Decant supernatant and invert tube(s) on a clean paper towel to remove residual ethanol.
6. Air or vacuum-dry pellet until visible liquid has evaporated. Avoid excessive drying, which can make dissolving plasmid DNA more difficult. Dissolve pellet in a total volume of 0.5–1.0 ml TE Buffer or nuclease-free H_2O. To ensure complete recovery, use two or three successive volumes endotoxin-free TE or H_2O and use a pipet to break up pellet while washing tube walls. Transfer plasmid solution to a clean, labeled 1.5-ml microcentrifuge tube and store at –20°C.

Note: For ultra-low endotoxin levels, TE or water must be endotoxin-free.

Mobius™ 1000 and UltraMobius™ 1000 Scalable Protocol

0.5- to 1.5-liters culture low-copy number plasmid

The Scalable Protocol is designed for processing up to 1.5 L bacterial culture and typically yields up to 1 mg low-copy number plasmid, using one Mobius 1000 Column. The Scalable Protocol requires the use of larger buffer volumes, limiting the number of isolations possible with a 10-reaction kit to two isolations. However, the Mobius Buffer Kit (Cat. No. 70855-3) is available separately to use with the remaining Mobius 1000 Columns.

A key step differentiating the Scalable Protocol from the Standard Protocol is precipitation of plasmid DNA from the neutralized, clarified lysate using isopropanol. The pellet is then resuspended in water prior to loading on the Mobius 1000 Column, eliminating the need to run large volumes of lysate through the column. See Table 3 for appropriate buffer volumes.

Table 3 Scalable Protocol

Culture Volume	500 ml	750 ml	1000 ml	1250 ml	1500 ml
Resuspension Buffer (1)	16 ml	24 m	32 ml	40 m	48 ml
Lysis Buffer (2)	16 ml	24 m	32 ml	40 m	48 ml
Mobius Neutralization Buffer (3)	16 ml	24 ml	32 ml	40 m	48 ml
Detox Agent (4)	4.8 ml	7.2 ml	9.6 ml	12 ml	14.4 ml
Isopropanol	33.6 ml	50.4 ml	67.2 ml	84 ml	100.8 ml
Resuspend in:	5 ml	7.5 ml	10 ml	12.5 ml	15 ml
# of Isolations per Buffer Kit	6	4	3	2	2

Culture growth

1. From a freshly streaked plate, transfer a single bacterial colony to 5 ml liquid LB culture containing the appropriate antibiotic. Incubate at 37°C for 8 h in a shaking incubator at 300 rpm.
2. Dilute culture 500-fold in sterile LB in an appropriately sized flask containing antibiotic (refer to page 5 for flask volume guidelines). Incubate at 37°C for 12–16 h in shaking incubator at 300 rpm. The OD_{600} at harvest should be approximately 3–5 when using sterile medium as spectrophotometer blank.
3. Harvest cells by centrifugation at 5000 × g for 10 min. Carefully decant supernatant and hold tube, inverted, for several seconds to remove residual medium. It is convenient to decant into bleach solution to sterilize the supernatant prior to disposal.

The use of 250- or 500-ml centrifuge bottles is highly recommended for cell harvesting, lysis and initial clarification. It is important to use bottles with sufficient capacity to allow adequate mixing during resuspension, lysis and neutralization steps. The use of smaller bottles can reduce plasmid yields.

Tip: Cell pellets can be stored frozen at –70°C for several weeks, if desired.

Preparation of cleared lysate

1. Chill Mobius™ Neutralization Buffer (bottle 3) on ice for 10 min.
2. Resuspend bacterial pellet in the appropriate volume (refer to Table 3) of Bacterial Resuspension Buffer (bottle 1). Swirl centrifuge bottle, pipet up and down and gently vortex until there are no visible clumps and pellet is completely resuspended. Failure to fully resuspend bacterial pellet can reduce plasmid yield. If multiple bottles were used, pool resuspended cells into one bottle large enough to accommodate subsequent additions of Bacterial Lysis Buffer and Mobius™ Neutralization Buffer (Steps 2 and 3).
3. Add the same volume of Bacterial Lysis Buffer (bottle 2) to the fully resuspended bacteria. Recap tubes and tip or swirl gently to mix, until the lysate appears cleared and viscous. **Do not vortex!** Vortexing can cause shearing of genomic DNA, resulting in reduced plasmid yield and genomic DNA contamination. Incubate lysate at room temperature for 5 min.

Note: Gentle but complete mixing is critical for maximum plasmid yields. The resulting lysate should appear viscous, bubbly, and translucent, but nearly clear. "Pockets" of unlysed cells in the mixture display a stringy, opaque appearance and signify insufficient mixing. Do not exceed the recommended 5 min incubation. Excessive exposure to alkaline conditions can contribute to nicking and irreversible denaturation of plasmid DNA.

4. Add the same volume chilled Mobius Neutralization Buffer (bottle 3). Recap. Mix gently to form a uniform flocculent precipitate. The mixture should become less viscous as genomic DNA, protein, detergent, and cell debris are precipitated. Incubate mixture on ice for 5 min.

Note: Properly mixed, neutralized lysate should have two components: white flocculent precipitate and the reduced viscosity solution. The presence of stringy, viscous debris within the neutralized lysate indicates poor mixing, which can lead to reduced plasmid yield.

4. Centrifuge neutralized lysate at 10,000 × g for 2 min (room temperature or 4°C) to remove the bulk of insoluble material.
5. Place ClearSpin™ Filter unit into ClearSpin Adapter and place over a fresh 250-ml centrifuge bottle. Fill the ClearSpin unit with clarified lysate from Step 4. Tap the ClearSpin Filter gently to initiate flow through the filter.

Note: If the observed flow rate is low, place a clean piece of laboratory film over the top of the ClearSpin Filter and press down with the heel of your palm to initiate flow; then remove the film.

6. Refill ClearSpin Filter with clarified lysate as necessary, until entire volume has been filtered. Discard filter unit and retain filtrate.

Note: For agarose gel analysis (optional, refer to page 15), remove 60 µl sample of clarified lysate. Maintain fraction on ice, or store at –20°C

7. **UltraMobius 1000 Plasmid Kit only:** Add appropriate volume Detox Agent (bottle 4) (see Table 3) to cleared lysate. Mix gently. Incubate on ice for 15 min.
8. Add appropriate volume isopropanol (refer to Table 3) to clarified, filtered lysate. Mix gently. Immediately centrifuge at 15,000 × g for 20 min (room temperature or 4°C).
9. Carefully decant supernatant and invert tube on clean paper towel to remove residual isopropanol.
10. Resuspend pellet in appropriate volume of sterile water (refer to Table 3). Be sure to wash wall of tube, and completely dissolve pellet.

Note: Optional stopping point: The resuspended DNA may be stored at 4°C and processed the following day.

Note: For agarose gel analysis (optional, refer to page 15), remove 6 µl sample of resuspended pellet. Maintain fraction on ice, or store at –20°C.

Mobius™ 1000 Column chromatography

1. During resuspension in Step 10 above, remove upper and lower caps of a Mobius 1000 Column and place in 50-ml conical tube. Add 10 ml of Mobius Equilibration Buffer (bottle A) into the reservoir and verify that the column is flowing by gravity. The flow of buffer may take a few moments to begin. Allow the entire 10 ml volume to flow through the column. The column will not run dry, because the flow will cease when the buffer meniscus reaches the top frit of the column.
2. Place equilibrated Mobius 1000 Column in new 50-ml conical tube. Transfer entire DNA solution to column reservoir. Allow entire volume to flow through column by gravity. This may require up to
30–45 minutes. The column does not need to be attended to during this period.
3. Transfer loaded Mobius 1000 Column to new 50-ml conical tube. Cap and retain flow through fraction for agarose gel analysis. Add 20 ml Mobius Wash Buffer (bottle B) to column reservoir and allow entire volume to flow through column by gravity.
4. Transfer Mobius 1000 Column to new 15-ml conical tube and elute plasmid DNA by adding 5 ml Mobius Elution Buffer (bottle C) into reservoir and allow entire volume to flow through column by gravity. Collect entire volume in a single fraction.

Note: Optional Stopping Point: The eluted DNA may be stored at 4°C and precipitated the following day.

Note: For agarose gel analysis (optional, refer to page 15), remove 10 µl sample of eluate. Maintain fraction on ice, or store at –20°C.

Precipitation of plasmid DNA

1. Remove the column from the tube and transfer the entire volume of the eluted sample into a 30-ml polycarbonate centrifuge tube. Add 3.5 ml of isopropanol to the eluate to precipitate the plasmid DNA. Mix gently and immediately centrifuge at 15,000 × g for 20 min (room temperature or 4°C).
2. Carefully aspirate and discard the supernatant, avoiding contact with the tube wall. It may be helpful to mark the exterior position of the tubes with a lab marker to aid in locating the DNA pellet. Isopropanol-precipitated DNA pellets are often diffuse, translucent, and spread over a large portion of the exterior tube wall. Addition of Pellet Paint® or Pellet Paint NF Co-Precipitant prior to the addition of isopropanol can aid in visualizing the precipitated material.

Note: Pellet Paint and Pellet Paint NF Co-Precipitant have not been tested for compatibility with DNA transfection or DNA microinjection into eukaryotic cells.

3. Wash DNA pellet by adding 3 m 70% (v/v) ethanol and swirling the tube gently.
4. Centrifuge tube at 15,000 × g for 10 min (room temperature or 4°C).
5. Decant supernatant and invert tube on clean paper towel to remove residual ethanol.
6. Air or vacuum dry pellet until visible liquid has evaporated. Avoid excessive drying, which can make dissolving plasmid DNA more difficult. Resuspend pellet in 0.5–1.0 ml TE or DNase-free H_2O. To ensure complete recovery, use two or three successive volumes of TE or H_2O and use a pipet to break up pellet while washing tube walls. Transfer plasmid solution to a clean, labeled 1.5-ml microcentrifuge tube and store at –20°C.

Determination of Plasmid Yield and Purity

UV absorbance measurements

DNA yield can be determined spectrophotometrically by measuring the absorbance of a dilution of purified plasmid at 260 nm. The normal calculation for dsDNA is A_{260} = 1.0 = 50 µg/ml, for a 1-cm path length cuvette. Assuming a plasmid yield of 1 mg resuspended in 1 ml, the expected absorbance of an undiluted sample will be approximately A_{260} = 20. For optimal accuracy, target absorbance for UV spectrophotometer readings should be between 0.1–0.5. Therefore, sample plasmid DNA should be diluted from 1:40 to 1:200 in deionized water prior to measuring UV absorbance. The recorded A_{260} should be multiplied by the dilution and the extinction coefficient (50 µg/ml/1.0 A_{260}) to obtain the concentration of plasmid DNA in µg/ml.

Note that results of A_{260} readings are accurate only in the absence of significant contamination by RNA and other UV-absorbing materials. Critical samples should also be analyzed by agarose gel analysis to confirm that the UV absorbing material is, in fact, plasmid and that the absorbance reading is consistent with the yield observed on an agarose gel.

Additional information about purity can be obtained by reading the absorbance at 280 nm and determining the A_{260}/A_{280} ratio. Nucleic acids have an average absorbance maximum of approximately 260 nm, whereas proteins (assuming a normal distribution of aromatic residues) have an average absorbance maximum of 280 nm. Pure DNA and typical Mobius™ plasmid isolates have A_{260}/A_{280} ratio of 1.75–1.95. Preparations contaminated with protein have significantly lower ratios of 1.3–1.5, while higher ratios (greater than or equal to 2.0) may indicate the presence of significant levels of RNA.

Agarose gel analysis

Fractions from the purification of plasmids using Mobius™ Kits can be analyzed by agarose gel electrophoresis. Prior to gel analysis, samples must be precipitated with isopropanol to remove salts that interfere with electrophoresis. The recommended sample volumes in Table 4 represent approximately 0.1% (high-copy number plasmids) and 0.4 % (low-copy number plasmids) of the total volume of the fraction being analyzed. The precipitated DNA is invisible within the tube due to the small amounts of DNA present (about 1 µg) in each pellet. To avoid the loss of samples during handling, add Pellet Paint® or Pellet Paint NF Co-Precipitant to each sample prior to precipitation (see Step 3, below). Pellet Paint Co-Precipitant allows the precipitated material to be easily located on the tube wall and can be used to track the DNA pellet during washing and to confirm complete resuspension.

Preparation of Mobius purification fractions for gel analysis

1. Consult Table 4 to determine appropriate volumes. Pipet indicated volume of each of the fractions into clean microcentrifuge tubes.
2. Add water to each fraction to 100 µl total volume. Required volumes of water are indicated below.
3. (optional): If using Pellet Paint Co-Precipitant, add 2 µl to each fraction.
4. Add 100 µl isopropanol to each fraction. Mix well. Centrifuge 15 min in a microcentrifuge at maximum speed.
5. Carefully decant or aspirate supernatant. Wash pellet with 0.5 ml 70% ethanol. Spin at maximum speed for 3 min.
6. Carefully remove supernatant and dry sample pellets. Place open tubes in heat block at 50–70°C or in a centrifugal vacuum unit to accelerate drying.
7. Resuspend each pellet in 10 µl TE buffer.
8. Add 2 µl 6X DNA Gel Loading Buffer (Cat. No. 69046-3) to each tube.
9. Load 6 µl of each sample on 0.8–1% agarose gel containing ethidium bromide.

Table 4

High-copy number plasmid - Standard Protocol

Fraction	Sample volume	Additional water	Resuspend	Gel load volume
ClearSpin™ Lysate	24 µl	76 µl	10 µl	6 µl
Flow-through	24 µl	76 µl	10 µl	6 µl
Wash	20 µl	80 µl	10 µl	6 µl
Elution	5 µl	95 µl	10 µl	6 µl

Low-copy number plasmid - Standard Protocol

Fraction	Sample volume	Additional water	Resuspend	Gel load volume
ClearSpin Lysate	60 µl	40 µl	10 µl	6 µl
Flow-through	60 µl	40 µl	10 µl	6 µl
Wash	80 µl	20 µl	10 µl	6 µl
Elution	20 µl	80 µl	10 µl	6 µl

Low-copy number plasmid - Scalable Protocol

Fraction	Sample volume	Additional water	Resuspend	Gel load volume
ClearSpin Lysate	60 µl	40 µl	10 µl	6 µl
Resuspend pellet	6 µl	94 µl	10 µl	6 µl
Flow-through	10 µl	90 µl	10 µl	6 µl
Wash	40 µl	60 µl	10 µl	6 µl
Elution	10 µl	90 µl	10 µl	6 µl

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Troubleshooting Guide

Problem	Probable cause	Solution
Low plasmid yield	Low plasmid copy number	Try plasmid amplification with chloramphenicol as described on page 5.
	Insufficient cell pellet resuspension	Repeat isolation, being careful to vortex until all visible clumps are gone.
	Inadequate lysis	Be certain that Bacterial Lysis Buffer and cell suspension are fully mixed to facilitate complete lysis of bacterial cells. Ensure that the Bacterial Lysis Buffer is tightly capped when not in use.
Plasmid in wash fraction	Evaporation of Wash Buffer resulting in excessive salt concentration and plasmid elution during wash	Replace Mobius™ Wash Buffer.Close bottle lid tightly after use to avoid evaporation.
Plasmid contaminated with RNA	RNase A not added to Resuspension Buffer	Repeat procedure, add RNase A to Bacterial Resuspension Buffer prior to use. Store buffer at 4°C after use.
Plasmid contaminated with genomic DNA	Excessive mixing during lysis and neutralization steps	Repeat procedure. Be more gentle when mixing fractions during lysis and neutralization steps
Slow column flow rates	High cell mass contributing to clogging of column	Reduce cell culture volume or increase centrifugation time to 30 min at 15,000 × g to pellet fine precipitates prior to chromatography.

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