# Montage Plasmid Miniprep$_{96}$ Kit

Suitable for use with Qiagen® 9600 & 3000 Robotics Systems

## User Guide

GENOMICS

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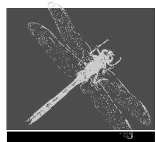


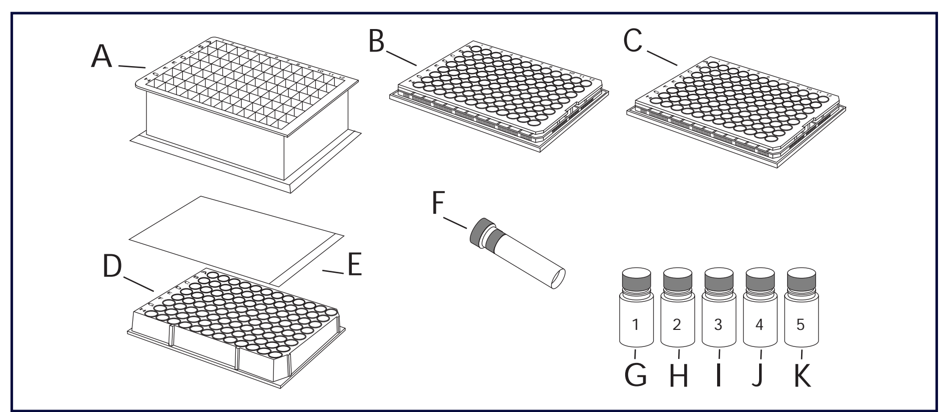
# Introduction

The Montage Plasmid Miniprep$_{96}$ Kit provides all of the reagents and materials necessary to purify plasmid DNA using a simple protocol that eliminates lengthy bind/elute methods and centrifugation. Employing unique separation technology, Millipore has developed a line of easy-to-use DNA plasmid miniprep kits that yield DNA suitable for the most sensitive downstream applications. In addition, this new technology has significantly reduced the time required for processing samples. Following bacterial lysis, three short filtration steps are all that is required to prepare 96 clean DNA samples from each plate. The plasmid DNA is retained by Millipore's proprietary size-exclusion membrane while proteins and contaminants are filtered through to waste. Adhere to the protocols provided with this kit to ensure the generation of DNA samples that can be used for applications such as cloning, DNA sequencing, transformation, and PCR.

## Automation with Montage Plasmid Miniprep$_{96}$ Kits

Millipore has enhanced the Montage Plasmid Miniprep$_{96}$ Kit's compatibility with automated systems by making it possible to collect purified samples from the top side of the plates in the kits and by eliminating lengthy centrifugation steps. This user guide provides information for automation of Montage Plasmid Miniprep$_{96}$ Kits on Qiagen® robotics systems.

# Kit Components




| Letter | Part | Function |
|---|---|---|
| A | 96-well culture block | Growth of host bacteria |
| B | Montage PLASMID$_{96}$ plate | Purification of plasmid DNA |
| C | Lysate Clearing plate | Clearing of bacterial lysate |
| D | V-bottom storage plate | Storage of plasmid DNA samples |
| E | Sealing tape | Sealing of plasmid DNA samples |

| Letter | Part | Function |
|---|---|---|
| F | RNase A | Required additive for Solution 1 |
| G | Solution 1 | Cell resuspension |
| H | Solution 2 | Cell lysis |
| I | Solution 3 | Neutralization |
| J | Solution 4 | Wash |
| K | Solution 5 | Resuspension/storage of plasmid DNA |

## Additional Equipment Required

- Pipettor
- Vacuum manifold (Millipore Cat. No. MAVM 096 OR)
- Centrifuge (for deep well culture block)
- Incubator shaker

Information about obtaining the following items is available through the Millipore web site: www.millipore.com/automation.

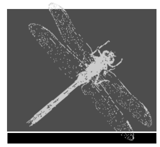
- Millipore manifold adapter and tubing assembly
- Software program for plasmid prep application

## Precautions

- Montage plates are disposable, single-use-only devices.
- This kit is for research use only. Not for use in clinical applications.
- Avoid contact with Solution 2 to prevent skin irritation.
- Montage plates consist of a polystyrene plate that is sealed to a polyethylene underdrain, forming 96 independent (individually sealed) wells. **Do not separate the underdrain from the polystyrene plate. Separation will result in plate failure and well leakage.**

## Storage Conditions

The kit reagents should be stored at 15 °C to 30 °C. However, following the addition of RNase A to Solution 1, this solution must be stored at 2 °C to 8 °C and should be used within six months (not to exceed expiration date on the kit).



# Procedures for Plasmid DNA Miniprep

The protocols described below include a modified version of a common alkaline lysis method for isolation of plasmid DNA from bacteria[1]. All of the necessary reagents are provided with this kit, including the final storage buffer (Solution 5).

## Bacterial Host Cultures

Choosing a bacterial host strain is a critical part of plasmid preparation. Endonuclease I is a 12kDa protein encoded by the *end*A gene of *E. coli.* This protein degrades double-stranded DNA and can reduce the stability of plasmid preparations if contaminating levels of this endonuclease are substantial[4]. Many *E. coli* strains carry a mutation in the *end*A gene that inactivates Endonuclease I. These strains are referred to as EndA negative ($EndA^-$) and are preferable hosts when preparing plasmid DNA. The table below lists some common host strains.

*Table 1. $EndA^-$ and $EndA^+$ strains of* E. coli .

| $EndA^-$ | $EndA^+$ |
|---|---|
| DH1 | BL21(DE3) |
| DH5a | CJ236 |
| JM109 | HB101 |
| SRB | JM101 |
| XL1-Blue | Q358 |
| XLO | TB1 |

## Culture Media and Antibiotics

Cultures grown for plasmid preparation should always be picked from a single colony taken from a plate containing a selective agent. Antibiotics should be used at every stage of growth[3], because many plasmids do not contain the par locus that ensures equal segregation of plasmids during cell division. In addition, the stability of many antibiotics decreases during culturing. Therefore, exceeding the prescribed culturing times may result in decreased DNA yields and/or reduce the quality of the purified samples. High copy number plasmids such as pUC, pBluescript, and pGEM[2] are recommended for use with this kit in order to achieve optimal plasmid yields.

The recommended medium for culturing bacteria prior to purification of plasmid DNA using the Montage Plasmid Miniprep$_{96}$ Kits is 2x Luria-Bertani (Miller) broth. This culture medium has twice the content of tryptone and yeast extract compared to normal LB (Miller) broth but has the same salt content. The formulation per liter of media is as follows:

| Component | Amount |
|---|---|
| Tryptone | 20g |
| Yeast Extract | 10g |
| NaCl | 10g |

Richer media such as Terrific Broth or Super Broth can be used for culturing to increase cell densities and plasmid yields. However, the kit performance has been optimized using the 2x LB described above and the use of richer media may increase filtration times and/or elevate protein contaminant levels and adversely affect downstream applications.

### Bacterial Cultures Growth

Bacterial cultures must be started the day before the intended run of the robot.

Inoculate *E. coli* host into 1 mL aliquots of 2X LB plus antibiotic in sterile 96 deep well blocks (2.2 mL capacity). Cover blocks with the lids provided and secure in incubator. Incubate at 37 °C at 320 rpm for 20–24 hours.

## Reagents Preparation

- Add RNase (total contents of tube) to Solution 1, mix thoroughly, and store at 2 °C to 8 °C. (All other solutions should be stored at 15 °C to 30 °C.)
- Bring Solution 2 to room temperature before using in order to dissolve detergent that may have precipitated due to lower temperatures during shipping.
- Screw cap on Solution 2 tightly immediately after use in order to avoid destabilization that may occur on exposure to air.

CAUTION: Avoid skin contact with Solution 2 to prevent irritation.

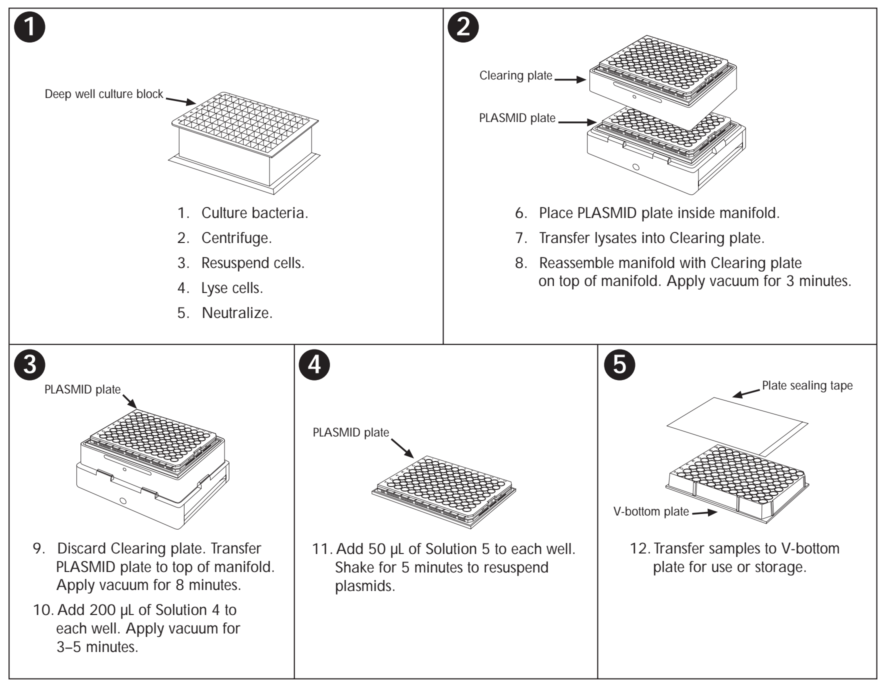
# Qiagen® Robot (9600 or 3000) Deck Setup

The protocol for using the robotics system to purify plasmid DNA starts with the bacterial cell pellet obtained after the culture has been grown and centrifuged, and the supernatant discarded. Set up the robot as follows:

1. Place the PLASMID plate inside the Millipore vacuum manifold.
2. Replace top ring.
3. Place Lysate Clearing plate on the top ring of the manifold.

The robot is now ready for processing the bacterial cultures started the day before.

# Overview of Procedure



## Standard Protocol

1. Centrifuge the deep well blocks containing the bacterial cultures at 1500 × g for 5–7 minutes. After centrifugation, immediately decant culture supernatant to a container for proper disposal. Invert and tap the plates firmly on several layers of paper towels to remove residual culture supernatant.

   NOTE: Failure to remove media will add undesired volume to lysate.

2. Place deep well block on the front left plate shaker position of the Qiagen robot deck. Resuspend pellets by adding 150 µL of Solution 1 to each well then mixing on the plate shaker until cells are totally resuspended.

   NOTE: Thorough resuspension of cells is critical for successful lysis.

3. Add 150 µL of Solution 2 to each well and mix immediately and vigorously on the plate shaker.

4. Add 150 µL of Solution 3 to each well and mix immediately and vigorously on the plate shaker. At this point, the bacterial lysate is ready for transfer to the Lysate Clearing plate.

5. Transfer 200 µL of lysate from the deep well block to the Lysate Clearing plate on top of the Millipore manifold. Apply vacuum at 270 mbar for 3 minutes.

   NOTE: For processing of entire lysate volume, see “Alternative Protocol” below.

6. Discard the Clearing plate and place the PLASMID plate on top of the empty manifold. Apply vacuum at 815 mbar for 8 minutes or until wells are empty. Direct filtrate to waste.

   NOTE: Filtration time is sample, temperature, and pressure dependent. The filters will appear shiny even after the wells are empty.

7. Add 200 µL of Solution 4 to each well of the PLASMID plate. Apply vacuum at 815 mbar for 3–5 minutes, or until wells are empty. Direct filtrate to waste.

8. Move the PLASMID plate to front right shaker position. Place V-bottom collection plate on back right shaker position.

9. Recover plasmid by adding 50 µL of Solution 5 to each well of the PLASMID plate. Shake for 5 minutes.

10. Pipette retained plasmid from the wells of the PLASMID plate into the V-bottom plate for storage. Use the sealing tape to seal wells of the V-bottom storage plate. Alternatively, samples may be stored in sealed PLASMID plates for several weeks in the refrigerator.

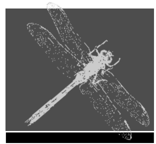
## Alternative Protocol

An alternative protocol may be used to maximize plasmid DNA yields from the same bacterial cultures described above. This protocol makes use of the full lysate generated from the entire 1 mL culture and plasmid DNA yields may reach up to 10 µg per well. However, filtration times may have to be extended for optimal performance, and there may be a decrease in the uniformity of the yields across the plate. See Table 2 in the "DNA Yields" section for typical yields obtained with this alternative protocol.

The following two modifications need to be made to the standard protocol:

- Use 100 µL (instead of 150 µL) of each of Solutions 2, 3, and 4 in steps 2, 3, and 4 of the standard protocol, and
- Load the entire bacterial lysate into the Clearing plate (instead of only 200 µL) in step 5 of the standard protocol.

For all other steps, proceed as described in the standard protocol.



# Product Performance

The devices and protocols included in this kit are designed to generate high quality plasmid DNA for automated platforms such as the Qiagen 9600 and 3000 robotics systems.

In the manual protocols, after the centrifugation of cultures, each 96 well block can typically be processed in less than 15 minutes to produce a bacterial lysate. The time required for clarification of the lysate is typically 1–3 minutes, while the filtration of the cleared lysate through the PLASMID plate and the ensuing wash step are generally 5 minutes and 3 minutes, respectively, when following the standard protocol. The total time required for the combined lysis and purification steps is typically less than 25 minutes. The processing time for more than one plate depends on the number of available vacuum manifolds.

For details on automated protocols, please refer to the publications shipped with the instrument. Also, log onto Millipore's web site at www.millipore.com/automation for information and products to help you set up and operate Qiagen robotics systems with the Montage Plasmid Miniprep$_{96}$ Kit. Contact Millipore if you would like our automation specialists to work with you to optimize your Qiagen automated platform for use with Montage Plasmid Miniprep$_{96}$ Kit.

# DNA Yields

The DNA sample yields are dependent upon several factors, including the type of plasmid employed[2], the type of media used, and the final cell densities. Typical yields achieved using our protocols are shown in Table 2, below. The agarose gel analysis in Figure 1 demonstrates the purity and reproducibility of the plasmid DNA samples prepared using the Montage Plasmid $Miniprep_{96}$ kit.

*Table 2. Typical yields of plasmid DNA using Montage Plasmid $Miniprep_{96}$ kits.*

| Host Strain | Plasmid | Protocol | Yield (µg) |
|---|---|---|---|
| XL1-blue | pUC19 | Standard | 2.6 ± 0.5 |
| | | Alternative | 5.1 ± 1.0 |
| | pLH2* | Standard | 3.9 ± 0.4 |
| | | Alternative | 8.2 ± 1.0 |
| JM109 | pUC19 | Standard | 2.7 ± 0.3 |
| | | Alternative | 5.7 ± 0.7 |
| | pLH2* | Standard | 3.9 ± 0.4 |
| | | Alternative | 8.0 ± 1.1 |

*The plasmid pLH2 is derived from pUC19. The 2.0 Kb fragment of HindIII digested λ phage was cloned into the HindIII site of pUC19, resulting in a 4.7 Kb plasmid.

*Figure 1. Agarose gel showing plasmid DNA purified using the Montage Plasmid Miniprep$_{96}$ Kit.*

M pUC19 pLH2 M

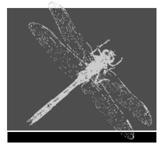
## DNA Sequence Data

When using fluorescent dye terminator chemistry for sequencing of plasmid DNA, the purity of the plasmids is a critical determinant of the quality and utility of the DNA sequence data, especially when attempting to miniaturize reactions on automated platforms. Table 3 demonstrates that plasmids prepared using this kit consistently yield high quality sequence data, as judged by PHRED analysis [5,6].

*Table 3. Sequence data from plasmids prepared with Montage Plasmid Miniprep$_{96}$ Kit.*

| Host Strain | Plasmid | Avg Read Length n=24 (>98% accuracy) | Avg PHRED 20 bases n=24 |
|---|---|---|---|
| XL1-blue | PUC19 | 707 | 618 |
| | PLH2 | 658 | 623 |
| JM109 | PUC19 | 706 | 602 |
| | PLH2 | 695 | 543 |

*All plasmid DNA samples were prepared with the Standard Protocol. They were sequenced using 1/16th BigDye® sequencing reactions (Applied Biosystems) and were run on a MegaBACE® capillary array sequencer (Molecular Dynamics). 160ng of pLH2 and 100ng of pUC19 were used in the respective reactions. Reactions were cleaned up using Montage SEQ$_{96}$ plates.



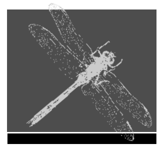
# Troubleshooting

This section outlines how to troubleshoot problems you may encounter when using the manual protocols for the Montage Plasmid Miniprep$_{96}$ Kit.

| Problem | Possible Causes | Suggestions |
|---|---|---|
| Low plasmid yields | Inadequate resuspension of cell pellets | Ensure that cells are completely resuspended in Solution 1 prior to addition of Solution 2. Failure to do so will result in reduced plasmid yields. |
| | No antibiotic added to media | Ensure that antibiotics are added at every stage of bacterial culturing. |
| | Inappropriate culturing times | Adhere to culturing conditions prescribed in the protocols. |
| | Proliferation of non-transformed cells | Always inoculate cultures from freshly picked colonies grown on a selective plate. |
| | Low copy number plasmid used | Use high copy number plasmids such as pUC, pBluescript, and pGEM. |

# Troubleshooting, continued

| Problem | Possible Causes | Suggestions |
|---|---|---|
| Low plasmid yields | Overdrying PLASMID plate | Stop vacuum when wells appear empty. |
| Plates separating | No underdrain support grid | Use Millipore manifold or equivalent with support grid. |
| Wells not filtering uniformly | Airlock due to bubble in the well | Agitate the lysate until bubble surfaces. |
| Nicking or denaturation of plasmid DNA; poor plasmid quality. | Excessive incubation at the alkaline lysis step (Solution 2). | Do not exceed the incubation time specified in the protocol and avoid excessive shaking or vortexing. |



# References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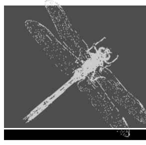
6. Ewing B, Hillier L, Wendl MC, Green P. (1998) “Base-calling of automated sequencer traces using phred. I. Accuracy assessment.” Genome Res.8(3): 175-85.



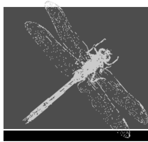
# Ordering Information

This section lists catalogue numbers for the Montage Plasmid Miniprep$_{96}$ Kit. See "Technical Assistance" for information about contacting Millipore. You can also buy Millipore products on-line at www.millipore.com/purecommerce.

| Product | Catalogue No. | Qty/Pk |
|---|---|---|
| **Kits** | | |
| *Montage Plasmid Miniprep$_{96}$ Kit suitable for Qiagen 3000 and 9600 robotics systems:* plates, culture blocks, and reagents for 4 × 96 samples | LSKP Q96 04 | 4/pk |
| *Montage Plasmid Miniprep$_{96}$ Kit suitable for Qiagen 3000 and 9600 robotics systems:* plates, culture blocks, and reagents for 24 × 96 samples | LSKP Q96 24 | 24/pk |
| **Reagents** | | |
| Solution 1, cell resuspension solution, 1,500 mL | LSKCR1500 | 1/pk |
| Solution 1, cell resuspension solution, 500 mL | LSKCRS500 | 1/pk |
| Solution 2, cell lysis solution, 1,500 mL | LSKCL1500 | 1/pk |
| Solution 2, cell lysis solution, 500 mL | LSKCLS500 | 1/pk |
| Solution 3, neutralization solution, 1,500 mL | LSKNS1500 | 1/pk |
| Solution 3, neutralization solution, 500 mL | LSKNS0500 | 1/pk |

# Ordering Information, continued

| Product | Catalogue No. | Qty/Pk |
|---|---|---|
| Solution 4, wash solution, 1,500 mL | LSKNF1500 | 1/pk |
| Solution 4, wash solution, 500 mL | LSKNF0500 | 1/pk |
| Solution 5, storage solution, 500 mL | LSKCTB500 | 1/pk |
| RNase A, 30mg, (in 50% glycerol) | LSKPMRN30 | 1/pk |
| **Accessories** | | |
| V-bottom plates | LSKV BP1 00 | 100/pk |
| Cell culture blocks with lids, 96 wells, 2.2 mL | LSKC CB0 50 | 50/pk |
| Adhesive plate sealing tape | LSKA ST1 00 | 100/pk |
| Millipore filtration manifold | MAVM 096 0R | 1/pk |
| Vacuum pressure pump, 115V/60Hz | XX55 000 00 | 1/pk |
| Vacuum pressure pump, 220V/50Hz | XX55 220 50 | 1/pk |
| Vacuum pressure pump, 100V/50 or 60Hz | XX55 100 00 | 1/pk |
| Manifold adapter and tubing assembly | Contact Millipore Technical Service | |
| Software program for plasmid prep application | Contact Millipore Technical Service | |



# Technical Assistance

For more information, contact the Millipore office nearest you. In the U.S., call 1-800-MILLIPORE (1-800-645-5476). Outside the U.S., see your Millipore catalogue for the phone number of the office nearest you or go to our web site at www.millipore.com/offices for up-to-date worldwide contact information. You can also visit the tech service page on our web site at http://www.millipore.com/techservice.

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