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GENOMICS

Montage Plasmid Miniprep$_{HTS}$ Kit User Guide

Notice

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Contents

Introduction

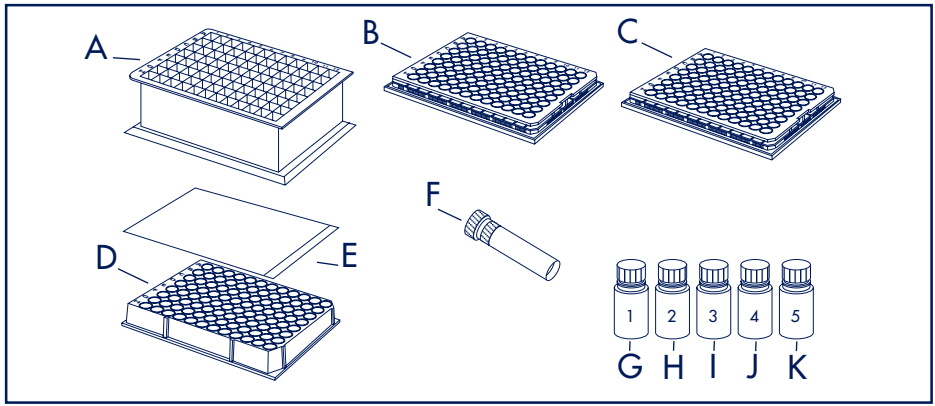
The Montage Plasmid Miniprep$_{HTS}$ Kit provides all of the reagents and materials necessary to purify plasmid or bacterial artificial chromosome (BAC) DNA using a simple protocol that eliminates lengthy bind/elute methods and centrifugation. Employing unique separation technology, Millipore has developed a line of easy-to-use DNA miniprep kits that yield DNA suitable for the most sensitive downstream applications. In addition, this 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 DNA is retained by Millipore's proprietary size-exclusion membrane while proteins and contaminants are filtered through to waste.

Current genome sequencing strategies require the cloning of large fragments of genomic DNA into F factor-based BAC vectors. One obstacle when adapting this strategy to a high throughput format is the low copy number of these vectors. Because there are only 1–2 copies of each BAC maintained per cell, the theoretical yield of BAC DNA from a given volume of culture is significantly lower than that for plasmid DNA. In addition, the large variability in insert size contributes proportionally to the variability in BAC DNA yield. Following the recommendations provided in this protocol to optimize bacterial cell culturing conditions will result in a sufficient yield of high quality DNA for desired downstream applications.

Automation with MultiScreen$_{HTS}$ 96-Well PLASMID Plates

Millipore has enhanced the MultiScreen$_{HTS}$ PLASMID plate's compatibility with automated systems by making it possible to collect purified samples from the top side of the plates and by eliminating lengthy centrifugation steps. Automation with the PLASMID plate can be achieved using a wide range of robotics systems. Some systems may require manifold adapters and/or software alterations to facilitate compatibility with MultiScreen$_{HTS}$ PLASMID plates. For protocols for robotics systems, please contact Millipore Technical Service.

Kit Components



Letter	Part	Function
A	96-well culture block	Growth of host bacteria
B	MultiScreen$_{HTS}$ PLASMID plate	Purification of plasmid or BAC DNA
C	MultiScreen$_{HTS}$ CLEARING plate	Clearing of bacterial lysate
D	V-bottom storage plate	Storage of DNA samples
E	Sealing tape	Sealing of DNA samples
F	RNase A	Required additive for Solution 1

Kit Components, continued

Letter	Part	Function
G	Solution 1	Cell resuspension
H	Solution 2	Cell lysis
I	Solution 3	Neutralization
J	Solution 4	Wash
K	Solution 5	Resuspension/storage of DNA
Not shown	Foil seal	Sealing plates during growth (must be pierced)

Additional Equipment Required

- Pipettor
- MultiScreen$_{HTS}$ vacuum manifold (Millipore Cat. No. MSVM HTS 00, or equivalent)
- Vacuum pump or uniform vacuum source (Millipore Cat. No. WP61 115 60, or equivalent)
- Centrifuge (for deep well culture block)
- Plate shaker
- Incubator shaker
- 96 well blocks for preculturing BAC clones (Millipore Cat. No. LSKC CB0 50)
- Foil piercing tool (e.g., Scienceware® Bel-Blotter™ tool with 96 23-gauge 3/4" needles)

Precautions

- MultiScreen 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.
- MultiScreen 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 MultiScreen polystyrene plate. Separation will result in plate failure and well leakage.**

Note on Plate Shaker Speed

In the following protocols, all plate shaking steps after growth of the bacterial cells were performed with a Lab-Line® titer plate shaker (Lab-Line Instruments, Model No. 4625) at setting 6, estimated at 800 rpm. Because speed settings in different shakers may correspond to different speeds, determine which setting on your shaker offers the optimal speed before performing the protocol.

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).

Preparation for Plasmid and BAC DNA Miniprep

The Montage Plasmid Miniprep$_{HTS}$ Kit can be used to purify both plasmid and BAC DNA. The following sections will describe how to culture the bacteria using the correct media and antibiotics and then will provide guidelines for purifying both plasmid and BAC DNA.

The procedures for purifying plasmid or BAC DNA consist of two phases: First, the bacterial host must be cultured; then the DNA must be isolated from the host. Appropriate culturing conditions are key to maximizing the yield of DNA obtained. Therefore, it is absolutely essential that the cells be grown according to these instructions.

Culturing the Bacteria

Before proceeding with the protocol for purifying DNA, the bacterial host strain must be chosen and grown in the appropriate media.

Host Strains for DNA Miniprep

Choosing a bacterial host strain is a critical part of plasmid or BAC 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[1]. 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 plasmids or BACs for applications such as fluorescent dye terminator sequencing. Millipore's Montage Plasmid $Miniprep_{HTS}$ Kit can be used to purify DNA from both $EndA^+$ and $EndA^-$ strains. The table below lists some common host strains.

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

$EndA^-$	$EndA^+$
DH1	BL21(DE3)
DH5α	CJ236
JM109	HB101
SRB	JM101
XL1-Blue	Q358
XLO	TB1

Culture Media and Antibiotics

Cultures grown for plasmid or BAC 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[2], because many plasmids and BACs 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.

Procedure

1. Culture the bacterial host prior to purification of the DNA in 2x Luria-Bertani (Miller) broth. Use of other media (i.e., 2xYT or TB) is not recommended as it will result in reduced yield and/or clogging of the purification plates.

   Formulation for 1 Liter (L) of 2x LB (Miller) media:

Tryptone	20g
Yeast Extract	10g
NaCl	10g

   Mix media components in a final volume of 1 liter (L) using Milli-Q® grade water and sterilize by filtration using a Stericup® filter cup or autoclaving prior to use.
2. Inoculate *E. coli* host into 1 mL aliquots (for plasmid) or 1.5 mL aliquots (for BAC) of 2x LB (Miller) plus antibiotic (e.g., 12.5 µg/mL chloramphenicol for BACs) in the provided sterile 96-well culturing blocks.
3. Cover the blocks with the aluminum foil tape and puncture 3–4 holes in each well to insure appropriate aeration. Secure the block in a shaking incubator and incubate at 37 °C and 320 rpm for 20–24 hours (optimal speed will vary depending on the geometry of the incubator). The final OD650 reading should be in the range of 3–5.

Protocol Guidelines

Before beginning the protocols for purifying the plasmid or BAC DNA, review the following:

- Add RNase A (total contents of tube) to Solution 1 and mix thoroughly prior to use. If not using the solution immediately, 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.

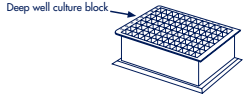
  CAUTION: Avoid skin contact with Solution 2 to prevent irritation.

The protocol for purifying plasmid DNA continues on page 10.

The protocol for purifying BAC DNA continues on page 14.

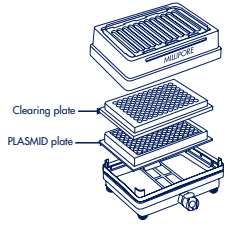
Overview of Procedure for Plasmid DNA Miniprep

1



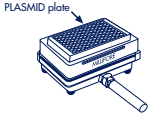
1. Culture bacteria.
2. Centrifuge.
3. Resuspend cells.
4. Lyse cells.
5. Neutralize.

2



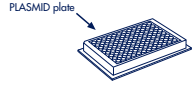
6. Place PLASMID plate inside manifold.
7. Transfer lysates into Clearing plate.
8. Reassemble manifold with Clearing plate on top of PLASMID plate. Apply vacuum for 5 minutes.

3



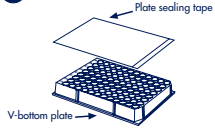
9. Discard Clearing plate. Transfer PLASMID plate to top of manifold. Apply vacuum for 5–7 minutes.
10. Add 200 µL of Solution 4 to each well. Apply vacuum for 3–5 minutes.

4



11. Add 50 µL of Solution 5 to each well. Shake for 5 minutes to resuspend plasmids.

5



12. Transfer samples into V-bottom plate for use or storage.

Protocols 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[3]. All of the necessary reagents are provided with this kit, including the final storage buffer (Solution 5).

Two protocols exist for plasmid miniprep using MultiScreen$_{HTS}$ PLASMID Plates: the partial lysate protocol and the full lysate protocol. The partial lysate procedure is faster but the full lysate procedure delivers more plasmid DNA.

High copy number plasmids such as pUC, pBluescript®, and pGEM[4] are recommended for use with this kit in order to achieve optimal plasmid yields.

Partial Lysate Protocol

1. Centrifuge deep well blocks 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. Resuspend pellets by adding 150 µL of Solution 1 to each well then mixing on a plate shaker, vortexing, or pipetting until cells are totally resuspended.

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

Partial Lysate Protocol, continued

3. Add 150 µL of Solution 2 to each well. Mix immediately and vigorously with a plate shaker for 1 minute. Incubate for an additional 2 minutes at room temperature.

   NOTE: Total lysis time should not exceed 5 minutes.

4. Add 150 µL of Solution 3 to each well. Mix immediately and thoroughly with a plate shaker for 2 minutes. At this point, the bacterial lysate is ready for transfer to the MultiScreen$_{HTS}$ CLEARING plate.
5. Place the MultiScreen$_{HTS}$ PLASMID plate inside the vacuum manifold.
6. Pipette 200 µL of lysate from the bottom of each deep well, and dispense into the corresponding well of the MultiScreen$_{HTS}$ CLEARING plate. (For processing of entire lysate volume, see "Full Lysate Protocol" below.)
7. Place the MultiScreen$_{HTS}$ CLEARING plate on top of the MultiScreen$_{HTS}$ PLASMID plate. Adjust the vacuum to 8 inches of Hg (0.27 bar - 203 torr.). Apply the vacuum for 5 minutes, drawing the lysate through the clearing plate into the MultiScreen$_{HTS}$ PLASMID plate. Discard the MultiScreen$_{HTS}$ CLEARING plate.
8. Place the MultiScreen$_{HTS}$ PLASMID plate on top of the manifold collar. Apply vacuum at 24 inches of Hg (812.7 millibar - 609.6 torr.) for 5–7 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.

Partial Lysate Protocol, continued

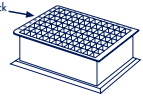
9. Add 200 µL of Solution 4 to each well of the MultiScreen$_{HTS}$ PLASMID plate. Apply vacuum at 24 inches of Hg (812.7 millibar - 609.6 torr.) for 3–5 minutes, or until wells are empty. Direct filtrate to waste.
10. Recover plasmid by adding 50 µL of Solution 5 to each well of the MultiScreen$_{HTS}$ PLASMID plate. Shake for 5 minutes on a plate shaker. Alternatively, add Solution 5 to the wells then let the plate sit for 30 minutes. The samples can then be recovered without shaking.
11. Pipette retained plasmid from the wells of the MultiScreen$_{HTS}$ 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 MultiScreen$_{HTS}$ PLASMID plates for several weeks in the refrigerator.

Full Lysate Protocol

An alternative protocol may be used to maximize plasmid yields from the same bacterial cultures described above. Simply use 100 µL each of the solutions in steps 2, 3, and 4. Instead of loading only 200 µL of the bacterial lysate into the clearing plate as described in step 6 of the partial lysate protocol above, load the entire lysate volume into the clearing plate and proceed with steps 7–12 as usual. The filtration times for this alternate protocol will be greater, but the increase in plasmid yield is significant.

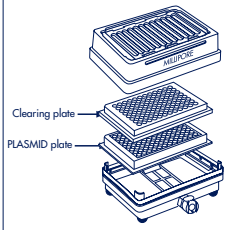
Overview of Procedure for BAC DNA Miniprep

1



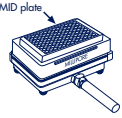
1. Culture bacteria.
2. Centrifuge.
3. Freeze cell pellets.
4. Thaw at room temperature for 15 mins.
5. Resuspend cells.
6. Lyse cells.
7. Neutralize.
8. Pipette up and down three times.

2



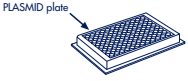
9. Place PLASMID plate inside manifold.
10. Transfer lysates into Clearing plate.
11. Reassemble manifold with Clearing plate on top of PLASMID plate. Apply vacuum at 8 inches Hg for 5 minutes or until wells are empty.

3



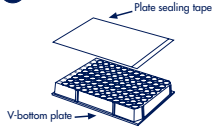
12. Discard Clearing plate. Transfer PLASMID plate to top of manifold. Apply vacuum at 24 inches Hg for 6–10 minutes or until wells are empty.
13. Add 200 µL of Solution 4 to each well. Apply vacuum at 24 inches Hg for 4–6 minutes or until wells are empty.

4



14. Add 35 µL of Solution 5 to each well. Shake for 10 minutes to resuspend BAC DNA.

5



15. Transfer samples into V-bottom plate for storage.

Protocol for BAC DNA Miniprep

Since their development, bacterial artificial chromosomes have been used to manipulate large fragments of genomic DNA[5] and are the vehicles of choice for the cloning and sequencing of large regions of genomic DNA. Like plasmid DNA, BAC DNA is replicated in an *E. coli* host. However, the low copy number of the BAC vectors (1–2 copies per cell) greatly reduces the yield per unit of culture volume. The procedure for BAC DNA purification is designed to optimize DNA yields, and includes a modified version of a common alkaline lysis method used for isolation of plasmid DNA from bacteria[3].

1. Centrifuge deep well blocks at 1500 × g for 10 minutes. After centrifugation, immediately decant culture supernatant to a container for proper disposal. Invert and tap the plates firmly on absorbent paper/pads to remove residual culture supernatant.

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

2. Freeze the plates containing the pellets at -20 °C for 1 hour (if desired the samples can be frozen for up 24 hours prior to processing).

   NOTE: Freezing the pellets improves resuspension and yield of BAC DNA.

3. Allow the pellets to thaw at room temperature for 15 minutes.

4. Resuspend pellets by adding 100 µL of Solution 1 (containing RNase A) to each well then mixing on a plate shaker for 5 minutes until cells are completely resuspended. If the cells are not completely resuspended, increase the shaking time as needed. Alternatively, resuspension may be achieved by vortexing or pipetting.

   NOTE: Thorough resuspension of cells is critical for successful lysis. No pellets should be visible at the bottom of the wells.

5. Add 100 µL of Solution 2 to each well without mixing or shaking. Incubate at room temperature for 5 minutes.

   NOTE: Mixing after the addition of Solution 2 may decrease yield.

6. Add 100 µL of Solution 3 to each well. Mix immediately for 2 minutes using a plate shaker.
7. Pipette the entire volume of lysate up and down three times to break up any large clumps of flocculent.
8. Place the MultiScreen$_{HTS}$ PLASMID plate inside the vacuum manifold for filtrate collection.
9. Pipette the entire lysate volume from the bottom of each deep well and dispense into the corresponding well of the MultiScreen$_{HTS}$ CLEARING plate (labeled “CLEARING”).
10. Place the MultiScreen$_{HTS}$ CLEARING plate on top of the MultiScreen$_{HTS}$ PLASMID plate. Adjust the vacuum to 8 inches of Hg (270 millibar/200 torr). Draw the lysate through the clearing plate into the PLASMID plate by applying vacuum for 5 minutes or until the wells are empty. See the manifold user guide for details. Discard the MultiScreen$_{HTS}$ CLEARING plate.

11. Place the MultiScreen$_{HTS}$ PLASMID plate containing clarified lysates on top of the empty manifold. Apply vacuum at 24 inches of Hg (810 millibar/610 torr) until wells are empty. Direct filtrate to waste. When filtration is complete, switch off vacuum.

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

12. Add 200 µL of Solution 4 to each well of the MultiScreen$_{HTS}$ PLASMID plate. Apply vacuum at 24 inches of Hg (810 millibar/610 torr) until wells are empty. Direct filtrate to waste. When filtration is complete, switch off vacuum.

13. Resuspend BAC DNA samples by adding 35 µL of Solution 5 to the wells of the MultiScreen$_{HTS}$ PLASMID plate. After adding Solution 5 to the wells, shake for 10 minutes on a plate shaker.

14. Pipette retained BAC DNA from the wells of the MultiScreen$_{HTS}$ PLASMID plate into the V-bottom plate for storage. The recovery volume can be maximized by tilting the MultiScreen$_{HTS}$ PLASMID plate before collecting the sample. Use the sealing tape to seal wells of the V-bottom storage plate.

Procedure for Sequencing BAC DNA

In order to simplify sample processing, we have optimized protocols for direct end sequencing[6] of BAC DNA samples resuspended in Solution 5. The sequencing protocol described below uses a fixed volume of dissolved BAC DNA (10 µL), accommodating a range of BAC DNA yields that we have observed when processing 96-clone BAC library plates (human chromosome 22 library, Invitrogen/Research Genetics). Although each BAC clone has its own optimal window for the amount of BAC template used in cycle sequencing reactions, the sample volume we prescribe has produced the highest number of Phred 20 bases[7,8] and the lowest variation in scores across a 96 well plate in titration experiments.

Reaction Set-up

Assemble a 1/4x BigDye® Terminator DNA sequencing reaction as outlined below. As with any DNA sequencing protocol, optimal primer design is essential for high quality DNA sequence results.

	1/4x Reaction
BAC DNA	10 µL
BigDye Terminator v3.1	2 µL
ABI 5X Sequencing Buffer	3 µL
10 µM Primer (10 pmoles)	1 µL
Milli-Q water	4 µL
Total volume	20 µL

NOTE: Although other sequencing reaction scales may provide satisfactory sequencing results, users should balance any potential cost savings resulting from reaction miniaturization against the shorter read lengths and lower pass rates typically obtained with increased miniaturization.

Cycling Conditions

Following addition of all components to the sequencing reaction, mix well and spin briefly to insure that all of the liquid is at the bottom of the wells. Appropriate sealing of the wells is essential to minimize volumetric loss during thermal cycling. Cycle reaction according to the following program (100 cycles should be used to maximize the production of sequencing products):

1. 95 °C for 5 minutes
2. 95 °C for 30 seconds
3. 50 °C for 10 seconds
4. 60 °C for 4 minutes
5. Repeat steps 2–4 for a total of 100 cycles
6. Hold at 4 °C

NOTE: The annealing temperature was optimized for use with a T7 primer (5'-TAA TAC GAC TCA CTA TAG GG-3') and the vector pBACe3.6. Optimization of annealing temperature may be required when other vectors/primer combinations are employed.

Troubleshooting

This section outlines how to troubleshoot problems you may encounter when performing plasmid DNA Miniprep or BAC DNA Miniprep.

Plasmid DNA Miniprep Issues

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.

Plasmid DNA Miniprep Issues, continued

Problem	Possible Causes	Suggestions
Low plasmid yields	Low copy number plasmid used	Use high copy number plasmids such as pUC, pBluescript, and pGEM.
	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.
Slow filtration	Use of weak or house vacuum	Use a portable vacuum pump that produces at least 24 inches of Hg (812.7 millibar - 609.6 torr.) vacuum strength
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.

BAC DNA Miniprep Issues

This section outlines how to troubleshoot problems you may encounter when performing BAC DNA Miniprep.

Problem	Possible Causes	Suggestions
Low BAC DNA yields	Inadequate growth conditions	Ensure that bacterial cells are grown with adequate aeration and for **at least** 20 hours.
		Check cell density. OD_{650} should be between 3 and 5.
	Use of media other than 2xLB	Use of other cell culture media will result in low yields.
	Failure to freeze bacterial pellets prior to resuspension	Freezing of cell pellets improves cell resuspension efficiency.
	Inadequate resuspension of cell pellets	Ensure that cells are completely resuspended in Solution 1 prior to addition of Solution 2.
	No antibiotic added	Ensure that antibiotics are added at to media every stage of bacterial culturing.

BAC DNA Miniprep Issues, continued

Problem	Possible Causes	Suggestions
Low BAC DNA Yields	Over drying of BAC plate	Stop vacuum when wells appear empty.
	Failure to mix neutralized lysate	Mixing of neutralized lysate prior to filtration is essential for optimal yield of BAC DNA.
Variable BAC DNA yields	Highly variable BAC DNA inserts size (50–300 Kb) and low copy number result in variable yield of DNA mass (6-fold range)	Molar concentrations of BAC DNA will be equivalent and adequate for downstream applications.
Chromosomal DNA contamination	Excessive shaking or overincubation after addition of Solution 2	Do not exceed the incubation time specified in the protocol and avoid excessive shaking or vortexing. Minimal contamination will not interfere with DNA sequencing.

BAC DNA Miniprep Issues, continued

Problem	Possible Causes	Suggestions
No sequence from BAC clone.	Not enough BAC DNA in sequencing reaction	Ensure that enough material has been added to sequencing reaction (>100 ng).
	Use of sequencing chemistries that are not adequate for BAC end sequencing	Use BigDye terminator chemistry.
	BAC end is difficult to sequence.	Redesign primers or use alternative sequencing method for difficult regions.

References

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8. Ewing B, Hillier L, Wendl MC, Green P. Base-calling of automated sequencer traces using phred. I. Accuracy assessment. Genome Res. 1998;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.

Kits

Product	Catalogue No.	Qty/Pk
Montage Plasmid Miniprep$_{96}$ Kit: plates, culture blocks, and reagents for 96 samples	LSKP 096 01	1/pk
Montage Plasmid Miniprep$_{96}$ Kit: plates, culture blocks, and reagents for 4 × 96 samples	LSKP 096 04	4/pk
Montage Plasmid Miniprep$_{96}$ Kit: plates, culture blocks, and reagents for 24 × 96 samples	LSKP 096 24	24/pk

Ordering Information, continued

Kit Components

Product	Catalogue No.	Qty/Pk
Solution 1, cell resuspension solution, 500 mL	LSKC RS5 00	1/pk
Solution 2, cell lysis solution, 500 mL	LSKC LS5 00	1/pk
Solution 3, neutralization solution, 500 mL	LSKN S05 00	1/pk
Solution 4, wash solution, 500 mL	LSKN F05 00	1/pk
Solution 5, storage buffer, 500 mL	LSKC TB5 00	1/pk
RNase A, 30mg (in 50% glycerol)	LSKP MRN 30	1/pk
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

Ordering Information, continued

Accessories

Product	Catalogue No.	Qty/Pk
MultiScreen$_{HTS}$ vacuum manifold	MSVM HTS 00	1/pk
Stericup filter cup	SCGV U01 RE	12/pk
Chemical duty pump, 115V/60Hz	WP61 115 60	1/pk
Chemical duty pump, 220V/50Hz	WP61 220 50	1/pk
Chemical duty pump, 100V/50 or 60Hz	WP61 100 60	1/pk
Tubing, silicone, 3/16" I.D., 1.4 m	XX71 000 04	1/pk
Vacuum filtering flask, 1L	XX10 047 05	1/pk
Stopper, No. 8, perforated, silicone	XX20 047 18	5/pk
Millex®-FG$_{50}$ filter unit	SLFG 050 10	10/pk

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 www.millipore.com/techservice.

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