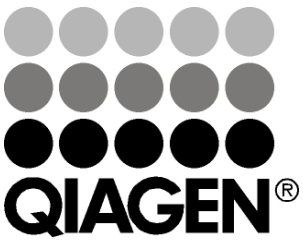
April 2012

# QIAprep® M13 Handbook

For purification of up to 10 µg single-stranded phage DNA




Sample & Assay Technologies

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# Contents

## Kit Contents

| **QIAprep Spin M13 Kit** | **(50)** |
|---|---|
| **Catalog no.** | **27704** |
| **Number of preps** | **50** |
| QIAprep Spin Columns | 50 |
| Buffer MP (M13 precipitation) | 5 ml |
| Buffer PB (M13 lysis and binding) | 2 x 100 ml |
| Buffer PE (concentrate) | 2 x 6 ml |
| Buffer EB | 15 ml |
| Collection Tubes (2 ml) | 50 |
| Quick-Start Protocol | 1 |

## Storage

The QIAprep Spin M13 Kit should be stored dry at room temperature (15–25°C). Buffers and components can be stored for up to 12 months without showing any reduction in performance, capacity, or quality of separation.

## Intended Use

The QIAprep Spin M13 Kit is intended for molecular biology applications. This product is not intended for the diagnosis, prevention, or treatment of a disease.

All due care and attention should be exercised in the handling of the products. We recommend all users of QIAGEN® products to adhere to the NIH guidelines that have been developed for recombinant DNA experiments, or to other applicable guidelines.

## Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDSs). These are available online in convenient and compact PDF format at www.qiagen.com/Support/MSDS.aspx where you can find, view, and print the MSDS for each QIAGEN kit and kit component.

### 24-hour emergency information

Emergency medical information in English, French, and German can be obtained 24 hours a day from:

Poison Information Center Mainz, Germany

Tel: +49-6131-19240

## Quality Control

In accordance with QIAGEN's ISO-certified Quality Management System, each lot of the QIAprep Spin M13 Kit is tested against predetermined specifications to ensure consistent product quality.

# Introduction

The QIAprep Spin M13 Kit provides the fastest and easiest way to perform up to 50 single-stranded M13 DNA minipreps. The pure single-stranded DNA obtained is highly suitable for standard radioactive and chemiluminescent sequencing, as well as for sensitive, automated fluorescent sequencing and site-directed mutagenesis.

The QIAprep Spin M13 Kit utilizes a unique silica-membrane technology under conditions that have been optimized to promote the binding of single-stranded DNA. The QIAprep Spin M13 miniprep procedure eliminates time-consuming phenol and chloroform extractions and alcohol precipitations, as well as the problems and inconvenience associated with loose resins and slurries. The QIAprep Spin M13 Kit accommodates all throughput requirements, and is designed for efficient sample processing using spin columns.

## The QIAprep M13 purification principle

The QIAprep M13 procedure is designed for purification of M13 ssDNA from liquid cultures of infected *E. coli* cells, using a unique silica-membrane technology. A general background to working with M13 bacteriophages is given in the Appendix (page 17). M13-infected bacterial cultures are first centrifuged extensively to remove bacterial cells, and culture supernatants are treated with Buffer MP (M13 precipitation buffer) to precipitate the phage particles. The samples are then applied to the QIAprep spin columns. Intact phage particles are retained on the specially adapted silica membrane. Upon addition of Buffer PB (M13 lysis and binding buffer), the phage particles are lysed and single-stranded DNA adsorbs to the membrane in the presence of high salt. Contaminants such as phage proteins pass through and are efficiently removed. After a brief wash step with Buffer PE, pure single-stranded DNA is eluted in 100 µl of Buffer EB or water. Further concentration and desalting are usually not required. All steps are performed without the use of phenol, chloroform, CsCl, and ethidium bromide, and without PEG or alcohol precipitation, saving time and eliminating unnecessary hazards, both to the user and to the environment.

## DNA yield

The QIAprep Spin M13 Kit allows the purification of up to 10 µg of single-stranded DNA from up to 3 ml of phage supernatant (Table 1), depending on the bacteriophage titer. Yields of single-stranded DNA obtained using the QIAprep Spin M13 Kit are highly reproducible when individual preparations are performed from the same M13 culture. However, please note that yields from different M13 clones may vary due to differences in phage replication rates and titer.

**Table 1. Yield of M13 ssDNA**

| Phage supernatant (ml) | Yield (µg) | Concentration (ng/µl) |
|---|---|---|
| 1 | 2.6 | 31 |
| 2 | 5.0 | 59 |
| 3 | 7.4 | 87 |
| 4* | 10.7 | 125 |

Yield of M13mp19 propagated in *E. coli* TG1 in LB broth for 6 hours, and purified with a QIAprep M13 Kit with elution in 100 µl water.

* Generally phage supernatant volumes of 1–3 ml are used for M13 ssDNA minipreps.

Please refer to the Appendix starting on page 17, which contains useful information concerning M13 propagation and parameters which can affect yields of single-stranded phage DNA.

The protocol is designed for the preparation of single-stranded M13 DNA from 1–3 ml *E. coli* culture grown in 2x YT or LB medium. Up to 3 µg of ssDNA can be expected per 1 ml phage supernatant depending on the particular phage clone. *E. coli* strains used for infection must contain the F' episome that drives pilus biosynthesis (e.g., JM101, JM109, TG1).

## QIAvac 24 Plus vacuum manifold

QIAprep spin columns can be processed using a vacuum manifold with luer connectors. The QIAvac 24 Plus vacuum manifold enables fast and efficient vacuum processing of QIAGEN spin columns in both clinical and laboratory research. Vacuum-driven liquid processing is an attractive alternative to centrifugation that minimizes the hands-on time needed for sample preparation.

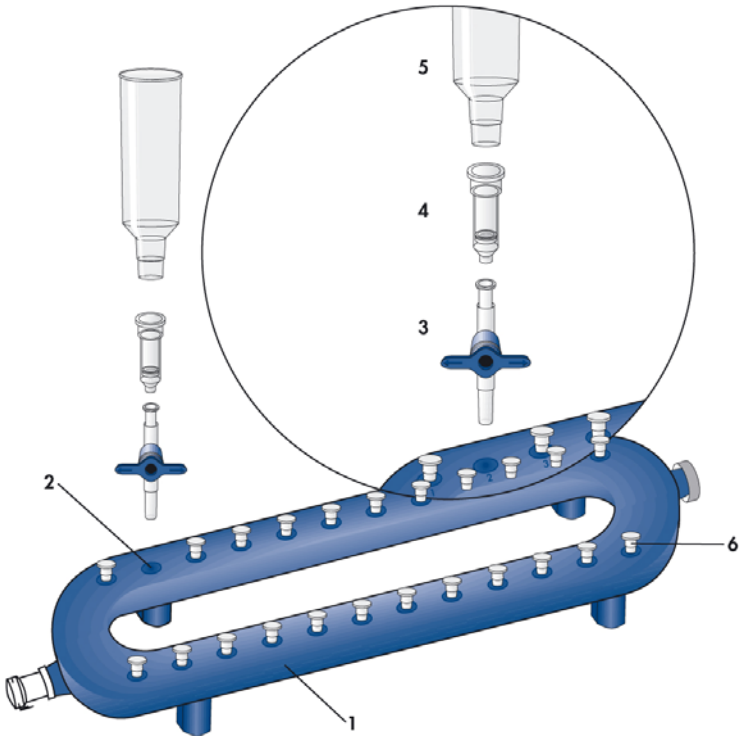
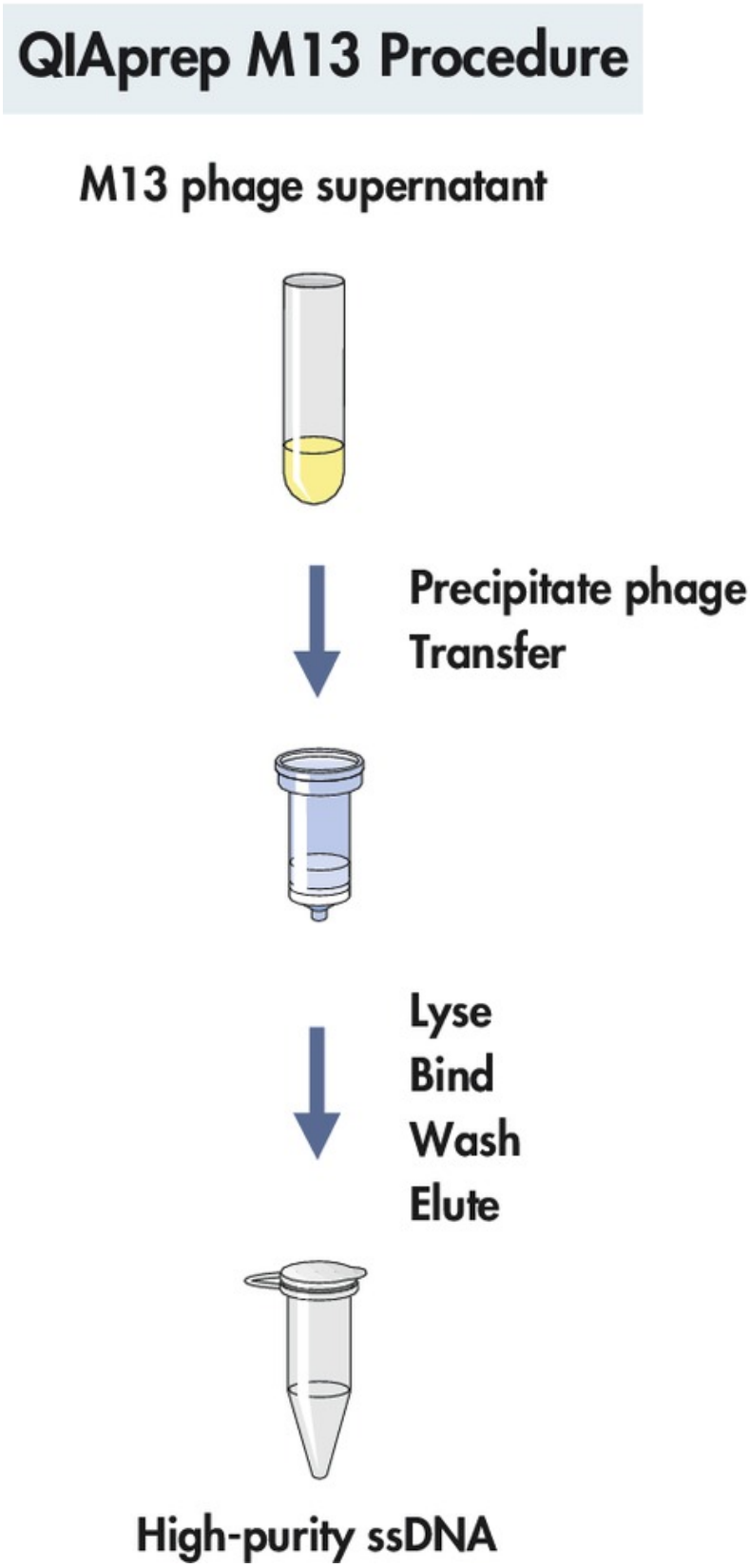



**Figure 1. Setting up the QIAvac 24 Plus with QIAprep columns using VacValves.**

1. QIAvac 24 Plus vacuum manifold
2. Luer slot of the QIAvac 24 Plus
3. VacValve (optional)*
4. QIAprep column
5. Tube extender
6. Luer slot closed with luer plug

* Must be purchased separately.




Samples can also be processed using a vacuum manifold with luer connectors (e.g., QIAvac 24 Plus, cat. no. 19413).

## Equipment and Reagents to Be Supplied by User

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

- Tabletop microcentrifuge or vacuum manifold with luer connectors (e.g., QIAvac 24 Plus, cat. no. 19413)
- Ethanol
- Reaction tubes
- Microcentrifuge tubes

# Protocol: Isolation of M13 DNA using the QIAprep Spin M13 Kit

This protocol is designed for preparation of single-stranded M13 DNA from 1–3 ml *E. coli* culture grown in 2x YT or LB medium, using spin columns in a microcentrifuge. Up to 3 µg of ssDNA can be expected per 1 ml phage supernatant depending on the particular phage clone. *E. coli* strains used for infection must contain the F' episome that drives pilus biosynthesis (e.g., JM101, JM109, TG1).

### Important points before starting

- Centrifugation of QIAprep spin columns is carried out in a conventional tabletop microcentrifuge at 8000 rpm (to reduce noise). If an adjustable speed microcentrifuge is unavailable, full speed centrifugation is acceptable. Lower speeds are acceptable as long as solutions are quantitatively transferred.
- QIAprep spin columns can also be processed using a vacuum manifold with luer connectors (e.g., QIAvac 24 Plus, cat. no. 19413). Vacuum processing can be used as an alternative to centrifugation in steps 6–11 of the protocol.

### Things to do before starting

- Add ethanol (96–100%) to Buffer PE (wash buffer) before use (see bottle label for volume).

### Procedure

1. **Grow an infected M13 culture.**

   Cultivation of M13-infected cultures should be performed at 37°C with constant agitation. Do not grow cultures infected with recombinant M13 bacteriophages for longer than 5–6 hours. Longer growth results in selection of deletion mutants and contamination of cultures with M13 RF, chromosomal DNA, and nucleases from lysed cells. For general information about M13 propagation, refer to the Appendix on page 17 or to molecular biology manuals such as *Current Protocols in Molecular Biology*, Ausubel, F.M. et al., eds (1991), Chapter 1.14.

2. **Centrifuge the bacterial culture at 5000 rpm for 15 min at room temperature (15–25°C).**

3. **Transfer supernatant containing M13 bacteriophage to a fresh reaction tube.**

   Be careful not to disturb the bacterial pellet. Any carryover of bacterial cells will result in contamination of the M13 precipitation with bacterial chromosomal DNA or double-stranded bacteriophage RF DNA.

4. **Optional: Repeat the centrifugation step.**

   A second centrifugation step may be necessary to ensure a clean supernatant fraction, and no bacterial cell carryover.

5. **Add 1/100 volume Buffer MP for M13 precipitation. (i.e., 10 µl per 1 ml phage supernatant.) Mix by vortexing and incubate at room temperature for at least 2 min.**

   During this step, bacteriophage particles are precipitated from the culture medium.

6. **Place a QIAprep spin column in a 2 ml microcentrifuge tube and apply 0.7 ml of the sample to the QIAprep spin column.**

   The bacteriophage supernatant must be loaded in successive 0.7 ml fractions due to the capacity of the QIAprep spin column.

7. **Centrifuge for 15 s at 8000 rpm and discard flow-through from the collection tube.**

   During this step, intact bacteriophage are retained on the QIAprep silica membrane.

8. **Repeat the loading and centrifugation (steps 6 and 7) until the entire sample has been loaded onto the QIAprep spin column.**

9. **To enable M13 lysis and binding, add 0.7 ml Buffer PB for M13 lysis and binding, to the QIAprep spin column and centrifuge for 15 s at 8000 rpm.**

   This step creates appropriate conditions for binding of the M13 DNA to the QIAprep silica membrane. Bacteriophage lysis begins.

10. **Add another 0.7 ml Buffer PB to the QIAprep spin column and incubate for 1 min at room temperature to lyse bacteriophages completely. Centrifuge for 15 s at 8000 rpm.**

    M13 single-stranded DNA is released from bacteriophage particles and adsorbed to the QIAprep silica membrane.

11. **Add 0.7 ml Buffer PE and centrifuge for 15 s at 8000 rpm.**

    Residual salt is removed in this step.

12. **Discard Buffer PE from the collection tube. Centrifuge the QIAprep spin column for 15 s at 8000 rpm to remove residual Buffer PE.**

    It is important to dry the QIAprep membrane with a quick microcentrifugation step. This prevents residual ethanol from being carried over into subsequent reactions.

13. **Place the QIAprep spin column in a clean 1.5 ml microcentrifuge tube. To elute DNA, add 100 µl Buffer EB (10 mM Tris·Cl, pH 8.5) to the center of the column membrane, incubate for 10 min, and centrifuge for 30 s at 8000 rpm.**

    Incubation of elution buffer in the QIAprep spin column significantly increases the recovery of single-stranded M13 DNA molecules, which adsorb tightly to the silica membrane. If yields are low or variable, recovery may be enhanced by preheating the elution buffer to 50°C.

    The DNA can also be eluted with water. When using water for elution, make sure that the pH is in the range 7.0–8.5. Elution efficiency is dependent on pH and the maximum elution efficiency is achieved within this range.

# Troubleshooting Guide

This troubleshooting guide may be helpful in solving any problems that may arise. For more information, see also the Frequently Asked Questions page at our Technical Support Center: www.qiagen.com/FAQ/FAQList.aspx. The scientists in QIAGEN Technical Services are always happy to answer any questions you may have about either the information and protocols in this handbook or sample and assay technologies (for contact information, see back cover or visit www.qiagen.com).

| | Comments and suggestions |
|---|---|
| **Low or no yield** | |
| a) General | Low yields and impure DNA may be caused by a number of different factors. Fractions can be saved from different steps during the procedure, and analyzed by gel electrophoresis to determine the stage where the problem occurred. Should the DNA be located in a particular fraction, it can generally be recovered by isopropanol precipitation. |
| b) Sequencing | Problems related to quality in fluorescent sequencing may need specialized troubleshooting with respect to the specific sequencing system used. |
| **Incorrect M13 propagation** | |
| a) Incorrect host strain | Make sure that the host strain carries the F' episome which is necessary for M13 infection, e.g., TG1, JM101, JM109. |
| b) Host strain pre-culture grown for too long | Stationary-phase cultures can lose the F' episome which is necessary for M13 infection. Do not grow pre-cultures into late stationary phase. Apply selection for F' episome if possible. |
| c) Incorrect M13 cultivation conditions | M13-infected cultures should be grown at 37°C with constant agitation. |
| **DNA passes through column in flow-through fraction** | |
| QIAprep membrane overloaded | Up to 3 ml M13 phage supernatant can be processed per spin column or well. Do not exceed this limit. |

| Comments and suggestions | |
|---|---|
| **DNA flows through with the wash fraction** | |
| Ethanol omitted from Buffer PE | Repeat procedure with correctly prepared Buffer PE. |
| **Little or no DNA in the eluate** | |
| a) Elution buffer incorrect | DNA is only eluted in the presence of low salt buffer, such as Buffer EB (10 mM Tris·Cl; pH 8.5) or water with a pH ≥ 7.0. Check pH and salt concentration of elution buffers prepared in the lab. |
| b) Elution buffer did not cover the QIAprep membrane completely | Ensure that the elution buffer is dispensed directly onto the center of the membrane and does not adhere to the sides of the column or well. |
| c) QIAprep membrane not incubated with elution buffer | After addition of elution buffer, incubate for 10 minutes before proceeding. This will ensure that the tightly adsorbed single-stranded DNA molecules are efficiently recovered from the QIAprep membrane. |
| d) Inefficient elution | Recovery of single-stranded DNA can be enhanced by preheating the elution buffer to 50°C before elution. |
| e) Residual ethanol on membrane | Ethanol can affect elution. Remove all traces of Buffer PE as indicated in the protocol. |
| **Low DNA quality** | |
| **DNA does not perform well** | |
| a) Eluate contains residual ethanol | Ethanol may have been carried over from the Buffer PE wash steps into the eluate. Ensure that the steps for removal of ethanol droplets are performed as carefully as recommended. Any residual ethanol can be removed from the DNA by vacuum drying or ethanol precipitation with no loss of DNA quality. |
| b) Eluate salt concentration too high | Check that wash step with Buffer PE was carried out correctly. |

| | Comments and suggestions |
|---|---|
| c) DNA poorly buffered | Elute DNA in Buffer EB (10 mM Tris·Cl, pH 8.5) to maintain pH during storage. If DNA is eluted in water, it must be stored at –20°C to prevent degradation which can occur in the absence of a buffering agent. |
| d) Possible deletion mutants | Some sequences are poorly maintained in M13 vectors. RF DNA may be purified by standard plasmid isolation methods, and checked for deletions by restriction analysis. Do not grow M13-infected cultures for more than 5–6 hours as this may result in selection of deletion mutants. |
| **Contamination with dsDNA** | |
| a) M13 propagated for too long | Ensure that M13-infected cultures are not grown for more than 5–6 hours, as this will result in contamination of phage supernatants with bacterial chromosomal and M13 RF DNA from lysed cells. |
| b) Bacterial pellet disturbed | When transferring the supernatant, make sure that the bacterial pellet is not disturbed. Centrifugation should be carried out twice to avoid any bacterial cell carryover. The second centrifugation step may be replaced by sterile filtration. |

# References

QIAGEN maintains a large, up-to-date online database of scientific publications utilizing QIAGEN products. Comprehensive search options allow you to find the articles you need, either by a simple keyword search or by specifying the application, research area, title, etc.

For a complete list of references, visit the QIAGEN Reference Database online at www.qiagen.com/RefDB/search.asp or contact QIAGEN Technical Services or your local distributor.

# Appendix A: Working with M13

On the following pages is a guide to factors that may influence the yield and quality of single-stranded phage DNA. A brief background to M13 biology is given, together with information on working with M13.

## M13 life cycle

M13 is a male-specific single-stranded filamentous bacteriophage of *E. coli*, that infects its host via the F-pilus (Figure 2, step 1). Upon entry into the cell, the phage particle is stripped of coat proteins, and its circular single stranded DNA molecule is converted into double-stranded replicative form (RF) (step 2). Replication of this form generates about 100 double-stranded copies, from which new single-stranded DNA (step 3) and phage proteins (step 4) are synthesized. The single-stranded DNA is packaged and phage particles are extruded from the cell in a nonlytic manner (step 5). Around 200 mature phages are produced per cell per generation. M13 does not cause cell lysis, but growth of infected cells is slowed down, resulting in turbid plaques on a lawn of uninfected cells.

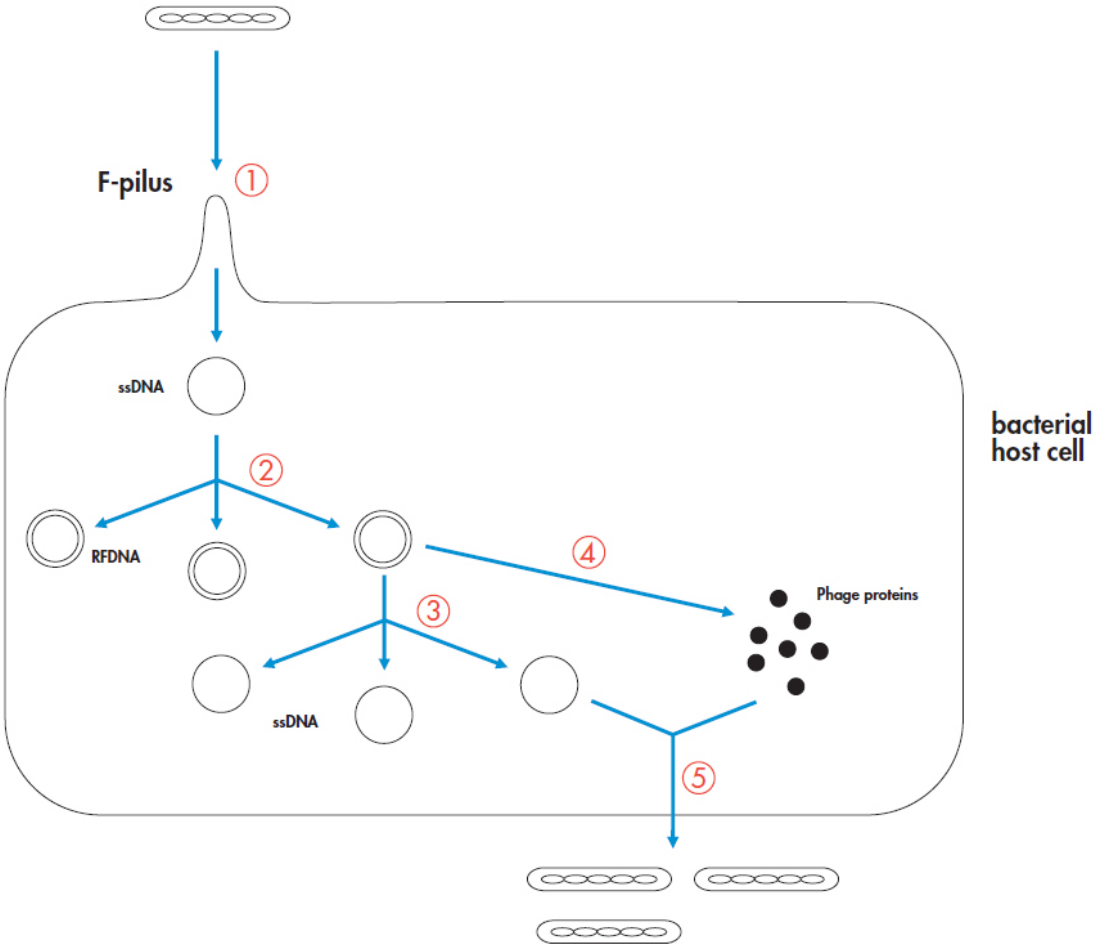



**Figure 2. M13 life cycle.**

## Isolation of M13 single-stranded and RF DNA

The different stages of the M13 life-cycle can be exploited for the isolation of both single-stranded and double-stranded (replicative form) DNA from liquid cultures of infected cells. Single-stranded DNA is isolated from phage particles in the culture supernatant, after centrifugation to remove *E. coli* cells, and can be used for sequencing and mutagenesis. The replicative form (RF) is isolated from the bacterial pellet by standard plasmid purification methods. RF M13 DNA can be used for cloning and for standard transformation of competent *E. coli* cells, from which single-stranded or further RF DNA can be isolated.

## M13 vectors and phagemids

A wide variety of M13 vectors are available, which have been adapted for many different research applications. Many M13 vectors contain polycloning sites, and since M13 is a filamentous phage, there are few structural restraints on insert size, although larger inserts tend to be unstable. An alternative tool are phagemids, which are vectors that combine features of plasmids and filamentous phages. They contain a plasmid origin of replication, a selectable antibiotic resistance gene, and the intergenic region (replication origin) of a filamentous bacteriophage. Phagemids can be propagated as plasmids, but when cells carrying phagemids are infected with the appropriate filamentous helper bacteriophage, the mode of DNA replication changes, and single-stranded DNA is synthesized and packaged into phage particles. Thus phagemids are convenient dual-purpose vectors, that offer the stability and handling of plasmids and avoid time-consuming subcloning into M13 vectors for sequencing, mutagenesis etc. The major disadvantage of phagemids is that yields of single-stranded DNA are lower and less reproducible than from recombinant M13 phages carrying the same insert.

## Selection of recombinants

Insertion of foreign DNA into M13 vectors results in inactivation of the ß-galactosidase marker. Recombinant M13 phages can easily be identified from a background of non-recombinant M13 phages by their color on media containing IPTG and X-gal — recombinant phages give rise to colorless plaques, while non-recombinant phages give rise to blue plaques.

## Host strain

Infection of *E. coli* with M13 requires a suitable host strain, such as TG1, JM101, JM109, or other strains of the JM100 series. M13 bacteriophage infects cells via the F-pilus, to which it adsorbs before penetrating the cell. Therefore, it is essential that host strains carry the F-pilus, which is encoded by the F' episome. Strains used for M13 work have a chromosomal deletion of the pro operon, rendering them deficient in proline biosynthesis. The pro operon is carried by the F' episome, and retention of the F' episome by M13 hosts is ensured by growth of the bacteria on glucose/minimal medium, for which biosynthesis of proline is necessary. It is important that prior to infection with M13, host strains are freshly streaked onto glucose/minimal medium plates to ensure that they still carry the F' episome. In other strains, e.g., XL1-Blue, tetracycline resistance is used as a selectable marker for the F' episome.

## Inoculation

Bacterial cultures for M13 infection should always be grown from a single colony picked from a freshly streaked glucose/minimal medium plate. Subculturing directly from glycerol stocks, agar stabs, and liquid cultures is poor microbiological practice, and may lead to loss of the F' episome. Host-strain cultures should only be grown for 6–8 hours prior to infection to avoid saturation, since stationary-phase cultures can lose the F' episome. M13phage plaques picked for infection should be well-isolated plaques from a freshly prepared plate. Incubation should be at 37°C with constant agitation. Cultures infected with recombinant M13 bacteriophages should not be grown for longer than 5–6 hours. Longer growth results in selection of deletion mutants and contamination of cultures with M13 RF and chromosomal DNA from lysed cells.

## Determination of DNA yield

Yields of M13 single-stranded DNA depend on the individual phage clone. Yields are best determined by measuring DNA concentration on a fluorimeter or spectrophotometer, followed by visual examination against a known standard on an agarose gel. Readings on a spectrophotometer are not always accurate, particularly if a single wavelength measurement is taken rather than a scan. Be sure to use a single-stranded standard for both instrument and agarose gel-based quantitation. The amount of fluorescent dye bound by single-stranded DNA is significantly less than that bound by double-stranded DNA.

# Ordering Information

| Product | Contents | Cat. no. |
|---|---|---|
| QIAprep Spin M13 Kit (50) | For 50 ssDNA preps: 50 QIAprep Spin Columns, Buffers, Collection Tubes (2 ml) | 27704 |
| **QIAvac Vacuum Manifolds** | | |
| QIAvac 24 Plus | Vacuum manifold for processing 1–24 spin columns: includes QIAvac 24 Plus Vacuum Manifold, Luer Plugs, Quick Couplings | 19413 |
| **Accessories** | | |
| Vacuum Regulator | For use with QIAvac manifolds | 19530 |
| 96-Well Microplates RB (24) | 96-well microplates with round-bottom wells plus lids, 24 per case, for use with QIAvac manifolds and the BioRobot® 9600 (no longer available) | 19581 |
| Tape Pads (5) | Adhesive tape sheets for sealing multiwell plates and blocks: 25 sheets per pad, 5 pads per pack | 19570 |
| AirPore Tape Sheets (50) | Microporous tape sheets for covering 96-well blocks: 50 sheets per pack | 19571 |

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## Notes

## Notes

**Limited License Agreement for QIAprep Spin M13 Kit**

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