

QIAGEN® Lambda Handbook

For preparation of up to 300 µg lambda DNA

February 1999



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Table of Contents

Kit Contents	4
Storage Conditions	4
Technical Assistance	5
Product Use Limitations	5
Product Warranty and Satisfaction Guarantee	5
The QIAGEN Lambda Principle	6
■ Protocol for Mini, Midi, and Maxi Lambda DNA Preparations	8
Important notes for buffers	8
Recommended amount of starting material	8
Preparation of starting material	10
Procedure	10
Agarose Gel Analysis of the Procedure	13
Troubleshooting Guide	15
Appendix A: Titering Lambda Phage	20
Titering phage lysates and stocks	20
Typical titers	20
Expected yields	20
Appendix B: Composition of Buffers	21
References	21
Ordering Information	22

Kit Contents

QIAGEN® Lambda Kit Catalog No.	Starter Kit 12549	Mini (25) 12523	Midi (25) 12543	Maxi (10) 12562
QIAGEN-tip 20	5	25	—	—
QIAGEN-tip 100	3	—	25	—
QIAGEN-tip 500	2	—	—	10
Buffer L1	1.5 ml	1.5 ml	3 ml	5 ml
Buffer L2	2 x 80 ml	1 x 65 ml	1 x 210 ml, 1 x 65 ml	2 x 210 ml, 2 x 65 ml
Buffer L3	1 x 50 ml	1 x 50 ml	1 x 110 ml	1 x 110 ml
Buffer L4	1 x 50 ml	1 x 50 ml	1 x 110 ml	1 x 110 ml
Buffer L5	1 x 50 ml	1 x 50 ml	1 x 110 ml	1 x 110 ml
Buffer QBT	1 x 50 ml	1 x 50 ml	1 x 110 ml	1 x 110 ml
Buffer QC	1 x 120 ml	1 x 75 ml	1 x 190 ml, 1 x 85 ml	2 x 165 ml
Buffer QF	1 x 50 ml	1 x 50 ml	1 x 170 ml	1 x 170 ml
RNase A + DNase I (combined)	30 mg + 9 mg	30 mg + 9 mg	60 mg + 18 mg	100 mg + 30 mg
Handbook	1	1	1	1

Storage Conditions

QIAGEN-tips should be stored dry and at room temperature (15–25°C). They can be stored for at least 2 years without showing any reduction in performance, capacity, or quality of separation.

QIAGEN Lambda Kits should be stored at room temperature. After addition of RNase A + DNase I, Buffer L1 should be stored at 2–8°C and is stable for six months. Other buffers can be stored for two years at room temperature.

Technical Assistance

At QIAGEN we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of QIAGEN products. If you have any questions or experience any difficulties regarding QIAGEN Lambda Kits or QIAGEN products in general, please do not hesitate to contact us.

QIAGEN customers are a major source of information regarding advanced or specialized uses of our products. This information is helpful to other scientists as well as to the researchers at QIAGEN. We therefore encourage you to contact us if you have any suggestions about product performance or new applications and techniques.

For technical assistance and more information please call one of the QIAGEN Technical Service Departments or local distributors listed on the last page.

Product Use Limitations

QIAGEN Lambda Kits are developed, designed, and sold for research purposes only. They are not to be used for human diagnostic or drug purposes or to be administered to humans unless expressly cleared for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. All due care and attention should be exercised in the handling of many of the materials described in this text.

Product Warranty and Satisfaction Guarantee

QIAGEN guarantees the performance of all products in the manner described in our product literature. The purchaser must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, QIAGEN will replace it free of charge or refund the purchase price. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a QIAGEN product does not meet your expectations, simply call your local Technical Service Department. We will credit your account or exchange the product — as you wish.

A copy of QIAGEN terms and conditions can be obtained on request, and is also provided on the back of our invoices. If you have questions about product specifications or performance, please call QIAGEN Technical Services or your local distributor.

The QIAGEN Lambda Principle

The QIAGEN lambda procedure (see flow chart opposite) is based on an optimized PEG precipitation step, followed by anion-exchange chromatography on a QIAGEN-tip. Lambda DNA is bound to QIAGEN Anion-Exchange Resin under appropriate low-salt and pH conditions, and impurities such as proteins, carbohydrates, and metabolites are removed by a medium-salt wash. Pure lambda DNA is then eluted in high-salt buffer, and subsequently concentrated and desalted by isopropanol precipitation. The isolated DNA is free of protein and RNA and can be used for all downstream applications, including automated or manual sequencing, PCR or other enzymatic reactions, and in vitro packaging.

The QIAGEN lambda procedure can be used to isolate DNA from:

Liquid cultures — in which bacteriophages are propagated in bacteria grown in liquid medium.

Plate lysates — in which bacteriophages are propagated in bacteria grown in soft agarose.

QIAGEN Lambda Kits are available in three kit sizes for preparation of 12 to 300 µg DNA.

- QIAGEN Lambda Mini Kits utilize QIAGEN-tip 20 for preparation of up to 12 µg DNA from up to 10 ml culture/plate lysate.
- QIAGEN Lambda Midi Kits utilize QIAGEN-tip 100 for preparation of up to 60 µg DNA from up to 50 ml culture/plate lysate.
- QIAGEN Lambda Maxi Kits utilize QIAGEN-tip 500 for preparation of up to 300 µg DNA from up to 250 ml culture/plate lysate.

The actual amount of lambda DNA that can be isolated depends on the type of vector, size of foreign DNA fragment inserted, individual *E. coli* host strain, inoculation efficiency, and culture medium. See Appendix A on page 20 for hints on titrating phage in the lysate and calculating the expected yield for your system. For general information on propagating lambda in liquid culture or on plates, please refer to current molecular biology manuals [1, 2, 3].

QIAGEN Lambda Procedure

Phage supernatant
(from liquid culture
or plate lysate)



Precipitate and
lyse phage particles



Bind DNA
Phage DNA



Wash



Elute

Phage DNA



Isopropanol precipitate



Ultrapure lambda DNA

Protocol for Mini, Midi, and Maxi Lambda DNA Preparations

This protocol is for purification of lambda DNA from liquid cultures or plate lysates. Volumes of buffers to be used for the different scales of preparation are color-coded as follows:

Green denotes volumes for a Mini (QIAGEN-tip 20) preparation

Blue denotes volumes for a Midi (QIAGEN-tip 100) preparation

Red denotes volumes for a Maxi (QIAGEN-tip 500) preparation

If you are a new user, it may be helpful to monitor the procedure by agarose gel analysis. Remove fractions at the steps indicated in the protocol and analyze them as described on page 13. If you experience any difficulties with QIAGEN lambda preparations, please refer to the detailed troubleshooting section of this handbook on pages 15–19 or call QIAGEN Technical Services.

Important notes for buffers

- Before using the kit for the first time, dissolve the lyophilized RNase A + DNase I (supplied in the kit) in Buffer L1. This buffer should then be stored at 2–8°C and can be used for 6 months.
- Pre-chill Buffer L2 to 4°C
- Check Buffer L4 for SDS precipitation due to low storage temperatures. If necessary, dissolve the SDS by warming to 37°C.

Recommended amount of starting material

Liquid cultures

Recommended starting volumes of liquid lysate are given in Table 1.

Table 1. Recommended starting volume of liquid lysate

Preparation size	Volume of liquid lysate	Expected yield
Mini preparation	10 ml	Up to 12 µg
Midi preparation	50 ml	Up to 60 µg
Maxi preparation	250 ml	Up to 300 µg

Plates

Plate lysates generally yield higher titers than liquid cultures, therefore less lysate is needed for the purification. Table 2 gives the recommended number of 90-mm plates or 140-mm plates for preparation of the plate lysate, and the volume of plate lysate obtained following step 1B of the protocol.

Table 2. Recommended starting number of plates for plate lysates

Preparation size	Number of 90-mm plates	Number of 140-mm plates	Expected yield
Mini preparation	1 plate (6 ml lysate)	0.5 plates (6 ml lysate)	Up to 12 μ g
Midi preparation	4 plates (24 ml lysate)	2 plates (24 ml lysate)	Up to 60 μ g
Maxi preparation	20 plates (120 ml lysate)	10 plates (120 ml lysate)	Up to 300 μ g

Notes on amount of starting material

- The recommended amounts of starting material given in Tables 1 and 2 are based on typical titers for liquid and plate lysates. The optimal starting volume to obtain the maximum yield for your system will depend on the actual phage titer. Ideally the phage titer in the lysate should be determined, and from this the approximate expected yield calculated (see Appendix A, page 20). This will enable the starting volume of lysate to be better matched to the capacity of the QIAGEN-tip.
- When the starting volume of lysate is <10 ml for a Mini preparation, <50 ml for a Midi preparation, or <250 ml for a Maxi preparation, volumes of Buffers L1 to L5 should be reduced proportionally to maintain correct lysis conditions (volumes of Buffers QGT, QC, and QF should not be changed).

Preparation of starting material

1 A. Liquid cultures:

- i) Propagate lambda phage in liquid cultures according to current molecular biology manuals (1, 2, 3), or according to the instructions of the vector supplier.
- ii) Add chloroform to the culture (2% v/v) and incubate at 37°C for a further 15 min to enhance lysis efficiency. Centrifuge at $>10,000 \times g$ for 10 min to remove bacterial debris, and retain the supernatant.

1 B. Plates:

- i) Propagate lambda phage on agarose plates according to current molecular biology manuals (1, 2, 3), or according to the instructions of the vector supplier.

Note: Lambda plates (top and bottom layers) must be prepared with electrophoresis-grade agarose. Preparations from agar plates contain polyanionic contaminants that can inhibit enzymatic reactions.

- ii) Overlay plates with 5 ml (90-mm plates) or 10 ml (140-mm plates) SM (Suspension Medium: for composition see Appendix B, page 21). Incubate with shaking at room temperature for several hours or at 4°C overnight, and transfer the liquid to a clean tube.
- iii) Wash plates with additional 1 ml (90-mm plates) or 2 ml (140-mm plates) SM and add the washings to the tube.
- iv) Add chloroform (2 % v/v), vortex or shake vigorously, centrifuge at $>10,000 \times g$ for 10 min to remove residual agarose, and retain the supernatant.

Procedure

2. Add 30 μ l, 100 μ l, or 400 μ l of Buffer L1 per 10 ml, 50 ml, or 250 ml supernatant. Incubate at 37°C for 30 min.

If the starting volume of lysate used was less than 10 ml (Mini), 50 ml (Midj), or 250 ml (Maxi), reduce the volume of Buffer L1 proportionally.

Ensure that RNase A + DNase I have been added to Buffer L1. During this step bacterial RNA and chromosomal DNA are digested.

- Remove a 500- μ l, 500- μ l, or 500- μ l aliquot of the sample (fraction 1) and save for an analytical gel (see page 13).

3. Add 2 ml, 10 ml, or 50 ml of ice-cold Buffer L2, mix gently, and incubate on ice for 60 min.

If the starting volume of lysate used was less than 10 ml (Mini), 50 ml (Midj), or 250 ml (Maxi), reduce the volume of Buffer L2 proportionally. Buffer L2 contains PEG for precipitation of phage particles. The ice incubation promotes formation of the precipitate.

4. Centrifuge at $>10,000 \times g$ for 10 min and discard the supernatant.

After centrifugation and removal of the supernatant, the tube should be placed upside down for 1 min to allow the residual liquid to drain. **IMPORTANT:** Do not wipe out the inside of the tube.

5. Resuspend the pellet in 1 ml, 3 ml, or 9 ml of Buffer L3 by pipetting up and down.

If the starting volume of lysate used was less than 10 ml (Mini), 50 ml (Midi), or 250 ml (Maxi), reduce the volume of Buffer L3 proportionally.

The phage precipitate formed after PEG precipitation carried out in the previous steps is difficult to see, as it is clear and distributed over the wall of the tube. Buffer L3 should therefore be pipetted several times over the wall to ensure complete resuspension of the pellet.

6. Add 1 ml, 3 ml, or 9 ml of Buffer L4 and mix gently. Incubate at 70°C for 10 min, 20 min, or 20 min, then cool on ice.

If the starting volume of lysate used was less than 10 ml (Mini), 50 ml (Midi), or 250 ml (Maxi), reduce the volume of Buffer L4 proportionally.

After addition of Buffer L4, the solution should be mixed gently but thoroughly by inverting the tube 4–6 times. Do not vortex as this can result in shearing of the lambda DNA. Buffer L4 contains SDS which denatures phage proteins and releases the DNA.

7. Add 1 ml, 3 ml, or 9 ml of Buffer L5, mix immediately but gently by inverting the tube 4–6 times. Centrifuge at 4°C for 30 min at $\geq 15,000 \times g$. Transfer supernatant promptly to a fresh tube.

If the starting volume of lysate used was less than 10 ml (Mini), 50 ml (Midi), or 250 ml (Maxi), reduce the volume of Buffer L5 proportionally.

After addition of Buffer L5, the solution becomes cloudy and viscous. Thorough mixing is essential to avoid localized precipitation. Do not vortex as this can result in shearing of the lambda DNA. The precipitate contains phage proteins complexed with potassium dodecyl sulfate.

8. Centrifuge the supernatant again at 4°C for 10 min at $\geq 15,000 \times g$ to obtain a particle-free cleared lysate (non-turbid).

This second, shorter centrifugation is required to completely avoid applying suspended or particulate material to the column. Suspended material (causing the sample to appear turbid) can clog the column and reduce gravity flow.

- Remove a 150- μ l, 90- μ l, or 55- μ l aliquot of the cleared lysate (fraction 2) and save for an analytical gel (see page 13).

9. **Equilibrate a QIAGEN-tip 20, -tip 100, or -tip 500 by applying 1 ml, 4 ml, or 10 ml of Buffer QBT, and allow the column to empty by gravity flow.**

Flow of buffer will begin automatically due to the presence of detergent in the equilibration buffer. Allow the QIAGEN-tip to drain completely. QIAGEN-tips can be left unattended, since the flow of buffer will stop when the meniscus reaches the top of the column packing.

10. **Apply the supernatant from step 8 to the QIAGEN-tip and allow it to enter the resin by gravity flow.**

IMPORTANT: The supernatant must be loaded onto the QIAGEN-tip promptly. If it is left too long and becomes cloudy it must be re-centrifuged (as in step 8) before loading to prevent clogging of the QIAGEN-tip and reduced yield.

- Remove a 150- μ l, 90- μ l, or 55- μ l aliquot of the flow-through (fraction 3) and save for an analytical gel (see page 13).

11. **Wash the QIAGEN-tip 20, -tip 100, or -tip 500 with 2 ml, 10 ml, or 30 ml of Buffer QC.**

Allow Buffer QC to move through the column by gravity flow.

- Remove a 100- μ l, 100- μ l, or 60- μ l aliquot of the wash flow-through (fraction 4) and save for an analytical gel (see page 13).

12. **Elute DNA with 1.5 ml, 5 ml, or 15 ml of Buffer QF into a clean collection tube.**

Use of polycarbonate centrifuge tubes for collection is not recommended as polycarbonate is not resistant to the alcohol used in subsequent steps.

- Remove a 75- μ l, 50- μ l, or 30- μ l aliquot of the eluate (fraction 5) and save for an analytical gel (see page 13).

13. **Precipitate the DNA by adding 1 ml, 3.5 ml, or 10.5 ml of room-temperature isopropanol to the eluted DNA. Mix and centrifuge at $\geq 15,000 \times g$ for 30 min at 4°C. Carefully decant the supernatant.**

All solutions should be at room temperature to minimize salt precipitation although centrifugation is carried out at 4°C to prevent overheating of the sample. Isopropanol pellets have a glassy appearance and may be more difficult to see than the fluffy, salt-containing pellets that result from ethanol precipitation. Marking the outside of the tube allows the pellet to be more easily located. Isopropanol pellets are also more loosely attached to the side of the tube, and care should be taken when removing the supernatant.

14. **Wash DNA pellet with 1 ml, 2 ml, or 5 ml of room-temperature 70% ethanol, and centrifuge at $\geq 15,000 \times g$ for 10 min. Carefully decant the supernatant without disturbing the pellet.**

The 70% ethanol removes residual salt and replaces isopropanol with the more volatile ethanol, making the DNA easier to redissolve. A optional second wash with room-temperature 70% ethanol may be carried out to further reduce the amount of residual salt.

- 15. Air-dry the pellet for 5–10 min, and redissolve the DNA in a suitable volume of buffer (e.g., TE, pH 8.0, or 10 mM Tris-Cl, pH 8.5). Do not use a vacuum evaporator for drying.**

Redissolve the DNA by rinsing the walls to recover all the DNA, especially if glass tubes have been used. Pipetting the DNA up and down to promote resuspension may cause shearing and should be avoided. Overdrying the pellet will make the DNA difficult to redissolve. DNA dissolves best under alkaline conditions; it does not easily dissolve in de-ionized water or acidic buffers.

Agarose Gel Analysis of the Procedure

Lambda yields and DNA quality can be analyzed by agarose gel electrophoresis. Poor yields and quality can be caused by a number of different factors. To determine at what stage of the procedure any problem occurred, save fractions from different steps of the lambda procedure (see below and Table 3) and analyze them by agarose gel electrophoresis.

Preparation of samples

Starting material (fraction 1): remove an aliquot (as indicated in Table 3) following addition of Buffer L1 (step 2 of the protocol), and extract it with phenol/chloroform. The phenol will lyse the phage and free the lambda DNA. Precipitate the lambda DNA with 1.25 M sodium acetate and 0.8 volumes of isopropanol, and resuspend in 10 μ l TE, pH 8.0.

Cleared lysate (fraction 2): remove an aliquot (as indicated in Table 3) of the cleared lysate after PEG precipitation (step 8 of the protocol). Precipitate with 1 volume isopropanol and resuspend in 10 μ l TE, pH 8.0.

Flow-through, wash, and eluate (fractions 3, 4, and 5, respectively): remove aliquots (as indicated in Table 3) of the flow-through, wash, and eluate (steps 10, 11, and 12 of the protocol, respectively). Precipitate each with 1 volume isopropanol and resuspend in 10 μ l TE, pH 8.0.

Agarose gel analysis

Run 2 μ l of each sample on a 0.8% agarose gel alongside a known amount of DNA (20–200 ng), and evaluate the amount of DNA in each fraction. The starting material and the cleared lysate after PEG precipitation (fractions 1 and 2) should contain approximately equal amounts of DNA as they represent approximately equal volumes of the original culture. The flow-through (fraction 3) should contain only traces of DNA (2–5%). The wash fraction (fraction 4) should not contain any DNA, since the DNA will remain bound during the washing. The eluate (fraction 5) should be checked to see that all the DNA has eluted from the column.

If a problem has occurred in a particular step of the protocol, turn to the hints in the relevant section of the troubleshooting guide on pages 15–19. If the problem remains unresolved, or if you have any further questions, please call QIAGEN Technical Services.

Table 3. Analytical samples for the lambda procedure

Fraction	Protocol step	Mini prep	Midi prep	Maxi prep
1	Starting material (step 2)	500 µl	500 µl	500 µl
2	Cleared lysate (step 8)	150 µl	90 µl	55 µl
3	Flow-through (step 10)	150 µl	90 µl	55 µl
4	Wash (step 11)	100 µl	100 µl	60 µl
5	Eluate (step 12)	75 µl	50 µl	30 µl
(% of preparation represented by each fraction volume)		5%	1%	0.2%

Troubleshooting Guide

The following troubleshooting guide may be helpful in resolving any problems that may arise. The guide is divided into two sections, the first dealing with factors that may cause low or no yield, and the second dealing with poor DNA quality. DNA yield and quality can be analyzed by agarose gel electrophoresis. Fractions can be saved from different steps of the purification procedure and analyzed as described on page 13, to determine at what stage any problem occurred. These fractions are indicated in the guide to help you locate the relevant troubleshooting section. If you have any further questions, please call QIAGEN Technical Services.

Comments and suggestions

Low or No Yield

No lambda DNA in starting material (fraction 1)

- | | |
|--------------------------------------|--|
| a) Ineffective propagation of lambda | Many problems in isolating lambda DNA are due to inefficient propagation of the bacteriophage. Some hints for propagation in liquid culture/agarose plates are given below. For more detailed information refer to current molecular biology manuals (1, 2, 3). |
| b) Insufficient starting titer | Ineffective lysis can often be attributed to low phage titer used for infection. Titer phage stocks prior to infection (see Appendix A, page 20). For optimum infection, adjust the multiplicity of infection or the length of incubation. For example, with strains of lambda that grow poorly, increase the lambda inoculum.

For propagation of lambda in liquid culture, start with phage titers between 5×10^5 and 5×10^8 pfu per ml bacterial inoculum. For propagation of lambda on plates, use around 10^5 pfu for a 90-mm plate (1, 2). |
| c) Poor adsorption of lambda phage | Synthesis of the lambda receptor <i>lamB</i> is induced by maltose and repressed by glucose. Host bacteria to be infected with lambda should be grown in medium containing 0.2% maltose. Addition of Mg^{2+} ions (10 mM $MgSO_4$) to the medium also aids adsorption. |
| d) Incorrect propagation temperature | Lambda should be propagated at 37°C. Although adsorption occurs at both room temperature and 37°C, penetration and subsequent steps of the lytic cycle do not occur efficiently at room temperature. |

Comments and suggestions

- e) Insufficient aeration of liquid cultures When propagating lambda in liquid medium, aerate cultures thoroughly. Fill flasks no more than 1/5 full and shake vigorously during growth.
- f) Incubation time too long In liquid culture, lysis should occur after 6–8 hours. Harvest soon after lysis to prevent re-attachment of released phage to receptors in the cell debris or growth of lysis-resistant survivors.
- Agarose plates for preparing plate lysates should be incubated for 6–8 hours or until plaques just touch one another.

No lambda DNA in cleared lysate (fraction 2)

- a) Inefficient phage precipitation Chill Buffer L2 to 4°C before use. Incubate phage with Buffer L2 for at least 60 min on ice to allow phage particles to form a precipitate.
- b) Phage lysis buffer conditions were incorrect Check Buffer L4 for SDS precipitation resulting from low storage temperatures and dissolve the SDS by warming at 37°C.
- c) Incomplete phage lysis Proteinase K treatment during phage lysis may increase the yield and quality of DNA, by allowing complete digestion of phage head proteins and eliminating residual traces of DNase I.
- Perform a proteinase K digestion as follows: after addition of Buffer L4 (step 6) add proteinase K at a final concentration of 0.1–0.2 mg/ml. Incubate at 55°C for 30–60 min, and continue the protocol by incubating the sample at 70°C for 10 min.

DNA in flow-through fraction (fraction 3)

- a) Column was overloaded with DNA Check the starting volume and yield against the capacity of the respective QIAGEN-tip. Reduce the volume if necessary.
- b) Inappropriate salt or pH conditions in buffers Ensure that any buffers prepared in the laboratory were prepared correctly (see page 21). Recover DNA by precipitation, and purify on a new QIAGEN-tip.

Comments and suggestions

- | | |
|---------------------------|---|
| c) Column flow was uneven | Ensure that the QIAGEN-tips are stored at room temperature. If they are stored under cold, damp conditions for prolonged periods of time, the resin may clump. This problem can be overcome by shaking the column before use. |
|---------------------------|---|

DNA in wash fraction (fraction 4)

- | | |
|-----------------------------------|--|
| a) Column was overloaded with DNA | Check the starting volume and expected yield against the capacity of the respective QIAGEN-tip. Titer the phage and reduce the starting volume if necessary. |
| b) Wash buffer was incorrect | Check pH and salt concentration of Buffer QC. Recover DNA by precipitation, and purify on a new QIAGEN-tip. |

No DNA in eluate (fraction 5)

- | | |
|--|---|
| a) No lambda DNA in starting material | See appropriate section above. |
| b) DNA passed through in the flow-through or wash fraction | See previous section. |
| c) Elution buffer was incorrect | Check pH and salt concentration of Elution Buffer QF. Recover DNA by eluting with fresh buffer. |

Little or no DNA upon precipitation

- | | |
|-------------------------------|--|
| a) DNA failed to precipitate | Ensure that the precipitate is centrifuged at $\geq 15,000 \times g$ for 30 min. Recover DNA by centrifuging longer at higher speeds. Try another isopropanol batch. |
| b) DNA pellet was lost | Isopropanol pellets are glassy and may be difficult to see. Mark the tube at the expected location of the pellet before centrifugation. Isopropanol pellets may also be loosely attached to the side of the tube, so pour supernatant off gently. |
| c) DNA was poorly redissolved | Check that DNA is completely redissolved. Be sure to wash any DNA off the walls, particularly if glass tubes and a fixed-angle rotor are used. Up to half of the total DNA may be smeared on the walls. Alternatively, a swinging bucket rotor can be used to ensure that the pellet is located at the bottom of the tube. |

DNA not fully redissolved

- | | |
|-----------------------------------|---|
| a) Pellet was overdried | Air-dry the DNA pellet. Do not use a vacuum evaporator. High-molecular-weight DNA is difficult to redissolve when overdried. Recover DNA by warming the solution slightly, and allowing more time for redissolving. Increase buffer volume if necessary. |
| b) Residual isopropanol in pellet | Ensure that pellets are washed with 70% ethanol to remove traces of isopropanol. Recover DNA by warming the solution slightly, and allowing more time for redissolving. Increase buffer volume if necessary. |
| c) Too much salt in pellet | Ensure that isopropanol is at room temperature for precipitation (use of cold isopropanol will result in precipitation of salt), and that the pellet is washed twice with room-temperature 70% ethanol. Recover DNA by increasing the volume of buffer used for redissolving. |
| d) Buffer pH was too low | Ensure that the pH of the buffer used for redissolving is ≥ 8.0 , since DNA does not dissolve well in de-ionized water or acidic buffers. |
| e) Resuspension volume too low | Increase resuspension volume if the solution above the pellet is highly viscous. |

Poor DNA Quality**Bacterial genomic DNA or RNA in eluate**

- | | |
|---|--|
| a) Lambda propagation too long | Harvest infected cells soon after lysis to avoid contamination of phage samples with bacterial RNA or chromosomal DNA from lysed cells. |
| b) RNase A/DNase I digestion ineffective | If Buffer L1 is more than 6 months old add more RNase A + DNase I. Ensure that lambda cultures were grown in the presence of 10 mM MgSO_4 . Mg^{2+} is a cofactor for DNase I and is necessary for optimal activity. |
| c) RNase A/DNase I digestion insufficient | If the phage was harvested on the previous day and the lysate stored overnight at 4°C, ensure that the lysate is at 37°C for the 30-min digestion (step 2), especially for large lysate volumes. |

Comments and suggestions

- d) Bacterial pellet disturbed When transferring the phage supernatant, make sure that the bacterial pellet is not disturbed. Centrifuge twice to avoid any bacterial cell carryover.

DNA is nicked/sheared/degraded

- a) Lambda was propagated in the absence of Mg^{2+} ions Grow lambda cultures in the presence of 10 mM $MgSO_4$. Mg^{2+} ions keep phage particles intact and prevent lambda DNA from degradation during step 2.
- b) Residual DNase I activity Ensure that supernatant is completely removed following PEG precipitation (step 4), to ensure that no traces of DNase I remain. Drain the centrifuge tube upside down for at least 1 min. If necessary rinse the phage pellet with the same volume of cold Buffer L2 used in step 3.
- c) DNA was poorly buffered Redissolve DNA in TE, pH 8.0, to inhibit nuclease activity and maintain stable pH during storage.
- d) Shearing during redissolving Redissolve DNA gently, without vortexing or vigorous pipetting. Avoid using small pipet tips.

DNA does not perform well

- a) Too much salt Ensure that isopropanol is at room temperature for precipitation, and that the pellet is washed twice with room-temperature 70% ethanol. Re-precipitate the DNA to remove the salt.
- b) Residual ethanol Drain pellet well and air-dry until all residual liquid has evaporated. However, do not heat or vacuum-dry pellets.
- c) Agar or poor-grade agarose used for preparation of plate lysates Use electrophoresis-grade agarose for plate lysates. Lambda preparations from agar plates contain polyanionic contaminants that inhibit enzymatic reactions.

Appendix A: Titering Lambda Phage

Titering phage lysates and stocks

Phage lysates and stocks can be titered by plating serial dilutions onto semi-solid medium. The resulting plaques are each derived from infection of a single bacterium by a single phage particle. Daughter progeny released after infection go on to infect neighboring bacteria and release another generation of phage particles. After many generations, areas of lysed bacteria originating from one phage particle appear as clear plaques on the bacterial lawn. The number of plaques on the plate corresponds to the number of phage particles in the starting solution.

To titer a phage lysate or stock, make 10 to 100-fold serial dilutions of the stock in SM (Suspension Medium, for composition, see Appendix B on the next page) and plate out according to standard protocols (1, 2). Incubate at 37°C for 8–12 hours or until plaques are big enough to count.

Typical titers

Phage stocks prepared by incubating a single plaque in 1 ml SM + chloroform (1, 2) contain around 10^6 to 10^7 pfu/ml.

Plate lysates typically contain 10^{10} to 10^{11} pfu/ml.

Lysates from liquid cultures generally have lower and more variable phage titers of 5×10^9 to 3×10^{10} pfu/ml).

Expected yields

The total amount of lambda DNA that can be isolated depends on the type of vector, size of foreign DNA fragment inserted, individual *E. coli* host strain, inoculation efficiency, and culture medium.

The expected yield of lambda DNA can be calculated from the titer of the lysate and the volume used for the preparation, assuming that a single phage particle contains $\sim 5 \times 10^{-11}$ µg of DNA. For example, a lysate with titer of 1×10^{10} will yield 0.5 µg of DNA per ml of lysate. If the phage titer is very high the starting volume may need to be reduced to prevent overloading of the system. The maximum capacity of QIAGEN Lambda Kits is 12 µg for Mini, 60 µg for Midi, and 300 µg for Maxi.

Appendix B: Composition of Buffers

Buffer	Composition	Storage
Buffer L1	300 mM NaCl; 100 mM Tris-Cl, pH 7.5; 10 mM EDTA; 0.2 mg/ml BSA; 20 mg/ml RNase A; 6 mg/ml DNase I	4°C
Buffer L2	30% polyethylene glycol (PEG 6000); 3 M NaCl	room temp. or 4°C
Buffer L3	100 mM NaCl; 100 mM Tris-Cl, pH 7.5; 25 mM EDTA	room temp.
Buffer L4	4% sodium dodecyl sulfate (SDS)	room temp.
Buffer L5	3 M potassium acetate, pH 5.5	room temp.
Buffer QBT (Equilibration Buffer)	750 mM NaCl; 50 mM MOPS, pH 7.0; 15% isopropanol; 0.15% Triton® X-100	room temp.
Buffer QC (Wash Buffer)	1.0 M NaCl, 50 mM MOPS, pH 7.0; 15% isopropanol	room temp.
Buffer QF (Elution Buffer)	1.25 M NaCl, 50 mM Tris-Cl, pH 8.5; 15% isopropanol	room temp.
TE	10 mM Tris-Cl, pH 8.0; 1 mM EDTA	room temp.
SM (Suspension Medium)	50 mM Tris-Cl, pH 7.5; 0.1 M NaCl; 8 mM MgSO ₄ ; 0.01% gelatin	room temp.

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3. Glover, D.M., and Hames, B.D., eds. (1995) *DNA Cloning Volume I: Core Techniques (Practical Approach, No 148)*, 2nd Edition. Irl Pr.

Ordering Information

Product	Contents	Cat. No.
QIAGEN Lambda Starter Kit	5 QIAGEN-tip 20, 3 QIAGEN-tip 100, 2 QIAGEN-tip 500, Reagents, Buffers	12549
QIAGEN Lambda Mini Kit (25)	25 QIAGEN-tip 20, Reagents, Buffers	12523
QIAGEN Lambda Midi Kit (25)	25 QIAGEN-tip 100, Reagents, Buffers	12543
QIAGEN Lambda Maxi Kit (10)	10 QIAGEN-tip 500, Reagents, Buffers	12562
Accessories		
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