BIO 101 Protocol

web

MINIPREP EXPRESS™

2000-000 17 preps
2000-200 1250 preps

- **No RNase Digestion**
- **No Slow Gravity Columns/No Dedicated Equipment/No Alcohol Concentration**
- **No Phenol/Chloroform Extractions**
- **Yields 1 - 5 μg of High Copy Number Vector DNA Per Prep**
- **DNA is Eluted in H_2O / TE and is Ready for Restriction Enzymes and PCR* Analysis**

Shipping & Storage:

The **Miniprep Express**™ is shipped and stored at ambient temperature.

"Check with BIO 101... We have you time in mind!"

BIO 101

Revision #: *2000-999-6H02P*

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MINIPREP EXPRESS™ Protocol

Kit Components

2000-000 (17 preps, sample size)

Name	Volume	Cat #
Miniprep Express Matrix™	7 ml	2000-000

2000-200 (1250 preps)

Name	Volume	Cat #
Miniprep Express Matrix™	520 ml	2000-200

User Supplied Solutions

80% ethanol.
Alkaline Lysis procedure: Solutions I, II and III (see page 5).
Boiling procedure: STET and Lysozyme (see page 5).

All references to user supplied reagents are italicized and bolded in the protocol for your convenience.

Introduction

The **Miniprep Express™** is the first truly cost effective plasmid miniprep. At **8 cents a prep**, it is the frequent cloners ideal tool for screening hundreds of recombinant clones in record time using restriction enzyme analysis and PCR*.

The **Miniprep Express™**, which consists only of the **Miniprep Express Matrix™****, is designed to complement the alkaline lysis[1,2] and boiling minipreps[3] procedures. Simply add **400 µl Miniprep Express Matrix™** to the crude plasmid prep, wash, air dry and elute the DNA in ***75 µl H_2O*** or ***TE***.

Plasmid DNA purified by the **Miniprep Express™** does not require the use of RNase since the majority of the bacterial RNA does not bind to the **Miniprep Express Matrix™** and is lost in the supernate. The residual RNA, if any, that copurifies with the plasmid DNA does not interfere with PCR*, restriction enzyme analysis and DNA visualization on ethidium bromide stained gels.

The **Miniprep Express™** allows the researcher to screen 30 - 60 minipreps in 1 - 2 hours without using any dedicated equipment. Once the desired clones are identified, BIO 101 offers a complete family of purification kits to isolate **GENECLEAN**® quality, 2x CsCl quality, plasmid DNA in any desirable scale: µg - mg amounts.

1. Molecular Cloning- A Laboratory Manual. 2nd edition. Sambrook, J., et al. eds. 1989. Cold Spring Harbor Lab. press. 1.25-1.28
2. Current Protocols in Molecular Biology. Ausubel, F.M. et al., eds. 1991. Wiely Interscience, New York. 1.6.1
3. Molecular Cloning- A Laboratory Manual. 2nd edition. Sambrook, J., et al. eds. 1989. Cold Spring Harbor Lab. press. 1.29 - 1.30
4. Current Protocols in Molecular Biology. Ausubel, F.M. et al., eds. 1991. Wiely Interscience, New York. 1.6.4

*PCR Process is owned by Hoffman LaRoche.

The **Miniprep Express Matrix™ contains guanidine thiocyanate, use with proper precaution.

User Supplied Solutions

Solution I

50 mM Glucose
25 mM Tris.Cl pH 8
10 mM EDTA pH 8

Solution II

0.2 M NaOH
1 % SDS

Solution III

60 ml 5 M potassium acetate
11.5 ml glacial acetic acid
28.5 ml H_2O

STET

0.1 M NaCl
10 mM Tris.Cl pH 8
1 mM EDTA pH 8
5 % Triton X-100

Lysozyme

10 mg/ml in 10 mM Tris pH 8

Miniprep Express™ Protocols

1. Alkaline Lysis[1,2]

a. Pellet 1 ml of overnight culture, remove and discard the supernate. Remove the medium by aspiration, or pour it off and remove the remaining drop with a pipette tip. (To make an aspirator, attach a disposable 200 µl pipette tip to a vacuum line with a trap in between).
b. Add ***100 µl Solution I*** and mix by vortexing until the cells are completely resuspended.
c. Add ***200 µl Solution II*** and invert few times to mix.
d. Add ***150 µl Solution III***, vortex 1 secon d and microfuge 2 minutes . *To prevent possible genomic DNA contamination when isolating low copy number vectors, vortex at a lower speed, or shake moderately a couple of times, until a uniform white precipitate is observed.*
e. Transfer supernate to a clean tube and continue with step 3.

2. Boiling Prep[3,4]

a. Pellet 1 ml of overnight culture, remove and discard the supernate. *See step 1a*.
b. Resuspend cells in ***300 µl STET*** and add ***25 µl Lysozyme***.
c. Incubate 2 - 5 minutes on ice and place 1 - 2 minutes in a boiling water bath.
d. Microfuge 5 minutes, transfer supernate to a new tube and continue with step 3.

3. Miniprep Express™

a. Add **400 µl Miniprep Express Matrix™** *(mix solution completely before each use)* and invert ~ 5 times to mix. *Plasmid DNA binds instantaneously to the* **Miniprep Express Matrix™**.
b. Microfuge 5 - 10 seconds at room temperature and decant supernate.
c. Add ***500 µl 80 % ethanol*** and resuspend the **Miniprep Express Matrix™**/DNA complex by gently stirring a couple of times with a 200 µl pipette tip. *Complete resuspension is not necessary for efficient washing.*
d. Spin 5 - 10 seconds and decant wash.
e. Pulse spin, remove last drop of ethanol with a pipette tip and air dry 5 minutes at room temperature. *Longer drying periods, even overnight, have no effect on the DNA quality and its recovery from matrix*.
f. Resuspend the dried Matrix/DNA complex in ***75 µl H_2O / TE*** by gently stirring with a pipette tip until a uniform suspension is obtained. *Complete resuspension ensures maximal DNA recovery*. Spin 1 minute and transfer supernate containing plasmid DNA to a new tube. *DNA is ready for restriction enzyme and PCR** analysis*.

Trouble Shooting Guide

1: Sub-Optimal/No Recovery of DNA

Note: Plasmid yield is a property of the copy number of the vector used, the cloned insert it contains, the growth conditions and the host cell

a) **Miniprep Express Matrix™** is not mixed prior to use resulting in no or little Matrix.

b) Failure to maintain appropriate antibiotic selection at all times during cell growth. Cells that have lost the plasmid often replicate at a faster rate than plasmid containing cells. Thus, although healthy vigorous growth is achieved, a sizable proportion of the cells would not contain the plasmid.

c) **Miniprep Express Matrix™**/DNA complex is not completely resuspended in ***H_2O*** or ***TE*** during the DNA elution step (Step 3f of protocol).

d) Check **Miniprep Express Matrix™** for crystals. If present, redissolve at 60°C before use. Crystals will lower the effective salt concentration resulting in suboptimal binding of plasmid DNA to the **Miniprep Express Matrix™**.

2: DNA Does Not Cut/No PCR

a) ***Ethanol*** is not completely removed from the **Miniprep Express Matrix™**/DNA complex.
Dry longer (step 3e of protocol).
Evaporate the already eluted DNA for 5 - 15 minutes at room temperature.

b) Wash a second time with ***80 % ethanol*** and adequately dry prior to elution.

3: Chromosomal DNA Contamination

a) Rough handling of the cells during lysis (i.e. vortexing and vigorous mixing) and/or prolonged lysis time.

4: RNA Contamintation

The **Miniprep Express Matrix™**, as used here, has a very low affinity for RNA. Thus the vast majority of RNA will not bind and is lost in the supernate. None-the-less a very small amount of RNA might be trapped in the **Miniprep Express Matrix™**/DNA complex.

To get rid of that RNA which can copurify with the plasmid DNA:

Alkaline lysis:	Add ***2µl 10 mg/ml RNaseA*** to ***100 µl Solution I*** or add ***1 µl 10 mg/ml RNaseA*** to the eluted DNA (step 3 f of protocol) and incubate 5 - 10 minutes at 37°C.
Boiling prep:	Add ***1 µl 10 mg/ml RNaseA*** to the eluted DNA (step 3f of protocol) and incubate 5 - 10 minutes at 37°C.

Subcloning Stratagies

1. Single Site Cloning

a. Cut vector with restriction enzyme and dephosphorylate with CIP*, BAP**, or SAP. *If restriction enzyme buffer is not compatible with that of BAP, CIP, or SAP cut with enzyme,* **GENECLEAN**® *and then dephosphorylate*.
b. Cut insert with the same restriction enzyme.
c. Run both vector and insert on a 0.5 - 2 % agarose gel and stain with ethidium bromide.
d. Using a razor blade or a scalpel, cut agarose bands corresponding to vector and insert, combine and **GENECLEAN**®.
e. Elute DNA from the **GLASSMILK**® in ***10 µl H_2O***, leave 5 - 15 minutes at room temperature to evaporate the residual ethanol if any and reduce the volume to < 7 µl. Add ***2 µl 10 X ligase buffer***, ***1 µl T4 DNA ligase*** and bring the volume to 10 µl with ***H_2O***. Ligate 1 - 3 hours at room temperature or overnight at 15°C and transform competent bacterial cells using either heat shock or electroporation.

2. Directional Cloning

a. Cut vector with two different restriction enzymes. *If restriction enzyme buffers are not compatible, cut with enzyme 1,* **GENECLEAN**® *and then cut with enzyme 2*.
b. Cut insert with the same restriction enzymes.
c. Run both vector and insert on a 0.5 - 2 % agarose gel and stain with ethidium bromide.
d. Using a razor blade or a scalpel, cut agarose bands corresponding to vector and insert, combine and **GENECLEAN**®.
e. Ligate, see step 1e.

3. Adding Linkers and Adaptors

a. Cut DNA with a restriction enzyme.
   blunt end: no further treatment necessary.
   3' protruding end: remove using T4 DNA polymerase.
   5' protruding end: fill with *klenow*.
b. **GENECLEAN®**, elute DNA in ***10 µl H_2O***, leave 5 - 15 minutes at room temperature to evaporate the residual ethanol if any and reduce the volume to < 7 µl. Add ***linkers /adaptors***, ***2 µl 10 X ligase buffer***, ***1 µl***

T4 DNA ligase and bring the volume to 10 µl with ***H_2O***. Ligate 1 - 3 hours at room temperature or overnight at 15°C.

c. **GENECLEAN®** and continue with desired application.

4. Multi-Fragment Cloning

a. Design the ligation strategy such that there is one way the various fragments can assemble to give the desired product and cut with the appropriate restriction enzymes. *If restriction enzyme buffers are not compatible*, **GENECLEAN®** *between restriction enzyme digestions.*

b. Run 0.5 - 2 % agarose gel and stain with ethidium bromide.

c. Using a razor blade or a scalpel, cut bands corresponding to the various fragments, combine and **GENECLEAN®**.

d. Ligate, see step 1e. (up to 5 different fragments can be ligated simultaneously to give the desired clone:

w————v + v————x + x———y + y————z + z ————w)

The **GENECLEAN®** procedure is a fast (< 20 minutes), efficient and reproducible way to purify DNA in solution and from agarose gels. It is a simple process which consists of selectively binding the DNA to a proprietary matrix: **GLASSMILK®**, washing and eluting the DNA in ***5 - 15 µl H_2O*** or ***TE***. The resulting DNA is free of restriction and modifying enzymes, salts and other contaminants. To get rid of residual ethanol that may be present in the eluted DNA, evaporate 5 - 15 minutes at room temperature. Alternatively, use the **GENECLEAN® SPIN™** kit which incorporate spin filter technology to speed up the purification process and efficiently remove residual ethanol if any. **GENECLEAN®** purification a fast alternative to phenol /chloroform extraction and alcohol precipitation. **GENECLEAN®** has served well the scientific community since 1986 and it continues to do so. For more information on the **GENECLEAN®** family of products call BIO 101 at 1-800-424-6101.

*CIP = Calf Intestinal Phosphatase.

**BAP = Bacterial Alkaline Phosphatase.

***SAP= Shrimp Alkaline Phosphatase.

Screening Stratagies

The **Miniprep Express Matrix™** is ideal for screening a large number of recombinant clones.

A. Prepare master plate and grow overnight cultures.

1. Place a master plate (LB/**CIRCLEGROW ®** + antibiotic) on the grid (see next page) and draw an arrow on the side of the plate corresponding to the mark on the grid.
2. Pick colonies from the transformation plates with sterile toothpicks or autoclaved pipette tips, inoculate the master plate (one colony per numbered sector) and place the toothpicks / pipette tips in numbered culture tubes containing liquid media + antibiotic.
3. Shake cultures overnight at 37°C and process the next day. Incubate master plate inverted at 37°C and grow overnight until colonies are observed. Seal plate with parafilm and store inverted at 4°C.

B. Miniprep Express Matrix™.

C. Identify recombinant clones by restriction enzyme analysis or PCR.

D. Locate bacteria harboring the recombinant vectors from the number assigned on the master plate. Prepare stocks for storage. Prepare GENECLEAN®-quality DNA in any desired scale.

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Plasmid Purification Kits

Miniprep Express Matrix™

Screen 30 - 60 preps in 1 - 2 hours using restriction enzyme digestion and/or PCR. No RNase digestion, No alcohol precipitation and No slow gravity columns. Simply add the **Miniprep Express Matrix™** to the crude plasmid prep, wash with 80 % ethanol and elute in 75 µl H_2O.

PREPS	YIELD*	CAT NO.
1250	1 - 5 µg	2000-200

RPM "MINIPREP"

Process 12 preps in 12 min to produce **GENECLEAN®** quality DNA which is eluted in H_2O or TE and is ready for use.

PREPS	YIELD*	CAT NO.
60	8 - 16 µg	2070-200
120	8 - 16 µg	2070-400
600	8 - 16 µg	2070-600

CIRCLEPREP SPIN MIDI

Process a midi prep in 30 - 40 min to produce **GENECLEAN®** quality DNA which is eluted in H_2O or TE and is ready for use**.**

PREPS	YIELD*	CAT NO.
25	50 - 150 µg	2005-200
75	50 - 150 µg	2005-400
200	50 - 150 µg	2005-600

RPM-AFS

Designed for high throughput template preparation for automated fluorescence sequencing. Process 12 preps in ~ 1 hour to produce **GENECLEAN®** quality DNA which is eluted in H_2O or TE and is ready for use**.**

PREPS	YIELD*	CAT NO.
50	20 -50 µg	2072-200
150	20 -50 µg	2072-400
2x150	20 -50 µg	2072-600

RPM®-1G "MAXIPREP"

Process a maxi prep in ~ 1 hour to produce **GENECLEAN®** quality DNA which is eluted in H_2O or TE and is ready for use**.**

PREPS	YIELD*	CAT NO.
20	0.5 - 1 mg	2077-200
40	0.5 - 1 mg	2077-400

RPM®-4G "MINI-MONSTERPREP"

Prep capacity: 10 mg DNA. Typical yields for high copy number vectors: 3 - 6 mg of **GENECLEAN®** quality DNA in ~ 2 hours.

PREPS	YIELD*	CAT NO.
12	up to 10 mg	2078-200

RPM®-12G "MONSTER PREP"

Prep capacity: 30 mg DNA. Typical yields for high copy number vectors: 10 - 15 mg of **GENECLEAN®** quality DNA in ~ 2.5 hours.

PREPS	YIELD*	CAT NO.
4	up to 30 mg	2079-200

****yield based on high copy number vectors.***

Notes

Product Use Limitation & Warranty

Unless otherwise indicated, this product is for research use only. Purchase of BIO 101 products does not grant rights to reproduce, modify, repackage the products or any derivative thereof to third parties. BIO 101 makes no warranty of any kind, expressed or implied, including merchantability or fitness for any particular purpose, except that the products sold will meet our specifications at the time of delivery. Buyer's exclusive remedy and BIO 101's sole liability hereunder shall be limited to, at our option, product credits, refund of the purchase price of, or the replacement of all material(s) that does not meet our specification. By acceptance of the product, Buyer indemnifies and holds BIO 101 harmless against, and assumes all liability for the consequence of its use or misuse by the Buyer, its employees or others, including, but not limited to, the cost of handling. Said refunds or replacement is conditioned on Buyer notifying BIO 101 within thirty (30) days of receipt of product. Failure of Buyer to give said notice within thirty (days) shall constitute a waiver by the Buyer of all claims hereunder with respect to said material(s).

General Information

BIO 101 is a pioneer in developing kits for molecular biology research. We introduced the **GENECLEAN®** Kits in 1986 and have since been manufacturing products to bring convenience into your research. Our goal is to make your life easier by simplifying the complexities of lab work.

Technical Support and Ordering Information

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