

One Plate

Plasmid Purification™

User Manual



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www.NeXprep.com

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KIT CONTENTS

Kits (Cat no.)	4 PLATE (2046)	20 PLATE (2351)	40 PLATE (2186)
Solution A	150 mL	750 mL	1,500 mL
Solution B*	200	1,000	2,000,
Solution C*	40	200	400
Solution E	40	200	400
RNase A**	40 mg	200 mg	400 mg
NeXplate	4	20	40
Collection Plates	4	20	40
User Manual	1	1	1
Kits (Cat no.)	50 Column (xxxx)	100 Column (xxxx)	250 Column (xxxx)
Solution A	20	40	100
Solution B*	25	50	110
Solution C*	5	10	25
Solution E	5	10	25
RNase A**	5 mg	10	25
Column	50	100	250
User Manual	1	1	1

*Provided as a concentrate. Add isopropyl alcohol as described in Appendices A through F based on kit type.

**Provided as a lyophilized powder.

STORAGE

All components, except RNase A, of the NeXprep Plasmid Purification Kit can be stored at room

temperature (~25°C). RNase A provided as dried powder should be stored at -20°C. After adding RNase A, Solution B is stable for six months when stored at 2-8°C. When stored under these conditions the components can be kept until the expiration date without showing any reduction in performance.

TECHNICAL ASSISTANCE

At DeWalch Life Technologies we are dedicated to providing outstanding service for all of our customers. For all questions and comments regarding the DeWalch NeXprep Plasmid DNA purification kits or other DeWalch products call 800-880-8993 x230.

SAFETY INFORMATION

Exercise universal precautions during the performance of this procedure. Technical staff should wear a lab coat, gloves, and safety glasses. Technical staff must be aware of the material safety data for all chemicals and biological agents used in this procedure. Report all incidents to the Laboratory Director.

Solution A:

Contains guanidine Isothiocyanate and reacts violently when combined with products containing bleach.



Guanidine Isothiocyanate:

Harmful if swallowed or inhaled and in contact with skin. Skin, eye and respiratory irritant.

Risk phrases :

R20 R21 R22 R32 R36 R37 R38.

Contact DeWalch Life Technologies at 800-880-8993 for additional information including Material Safety Data Sheets for this product.

INTRODUCTION AND OVERVIEW

The DeWalch NeXprep was developed to meet the demand for a better, and plasmid preparation kit. This kit is streamlined so that a 96 well plate can be processed in approximately thirty minutes providing purified DNA for a multitude of uses including sequencing and analysis of high copy number plasmids.

Overview of the Procedure (for example, using a NeXplate)

- Grow bacterial cells in the NeXplate;
- Add **Solution A** to lyse cells and bind DNA to the matrix;
- Pierce the NeXplate to enable drainage;
- Wash with **Solution B** twice and with **Solution C** once;
- Dry the binding matrix;
- Add **Solution E** and elute the DNA into a collection plate;
- For comparison of DeWalch NeXprep with traditional methods see the flow chart (next page).

REAGENTS AND EQUIPMENT NEEDED

Reagents/Materials Supplied:

- **Solution A** (lysis buffer).
- **Solution B** (wash buffer I): Using a he kit supplies this buffer in a concentrate form and should be mixed with isopropyl alcohol and RNase A provided as instructed prior to use.

- **Solution C** (wash buffer II): The kit supplies this buffer in a concentrate form and should be mixed with isopropyl alcohol as instructed prior to use.
- **Solution E** (elution buffer).
- **NeXplate**: These 96 well format deep-well blocks are provided to perform the steps for bacterial growth and plasmid purification all in a single plate (see **Figure 1**).

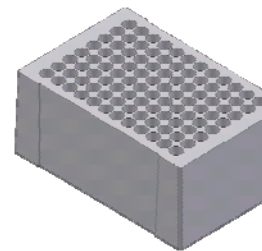
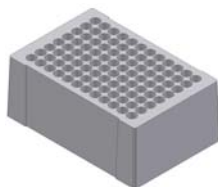


Figure 1 - NeXplate



- **Mini Spin Column**: The Mini Column with receiving tube (**Figure 2**) for the Spin Column Procedure of plasmid purification.

Figure 2 – Mini Spin Column with receiving tube.



- **Collection Plate**: There are two types of collection plate available. One type of plate facilitates elution via vacuum (see **Figure 3**). The other type (see **Figure 4**) facilitates elution of nucleic acid via

centrifugation. Either type can be supplied per customer request.

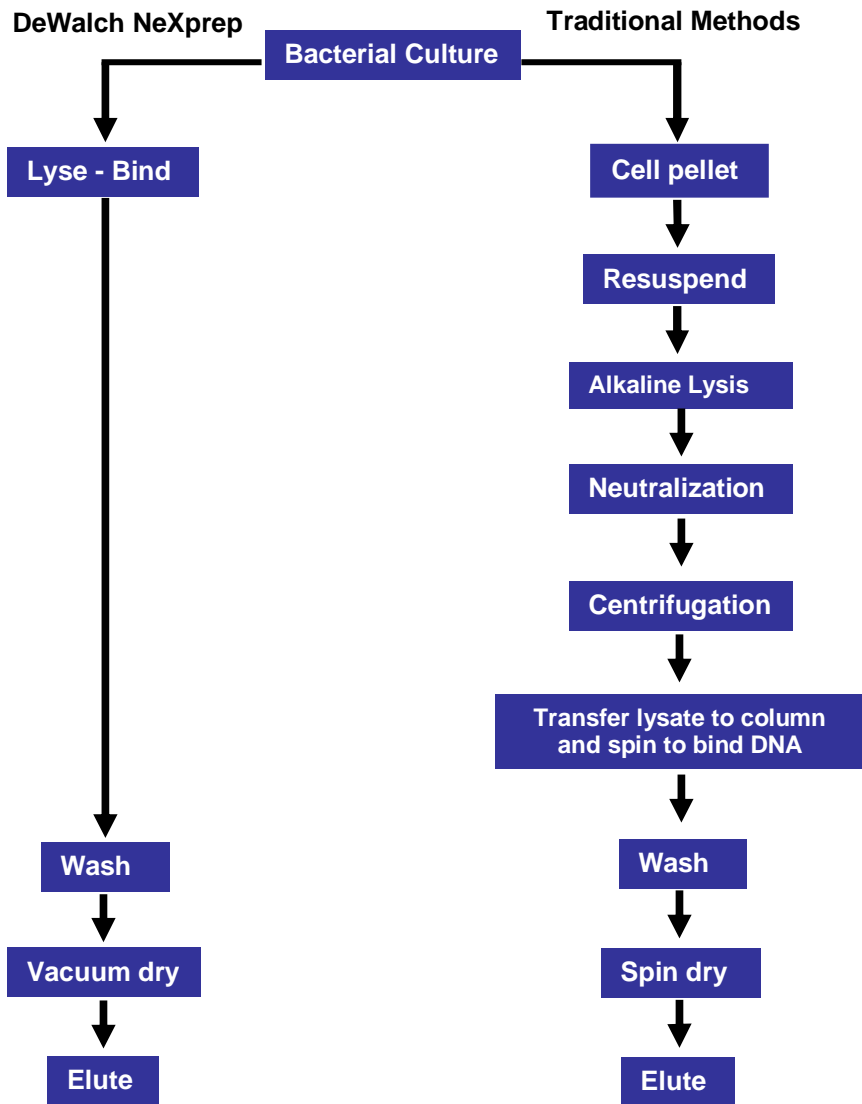
Figure 3 – Collection Plate (vacuum style)



Figure 4 - Collection plate (Centrifuge Style)



- **NeXprep User Manual:** One manual is supplied per kit. Manual also contains a quick reference protocol.



Comparison of DeWalch NeXprep with traditional methods

Reagents/Materials Required but not Supplied in the Kits

- Bacterial growth medium
- Isopropyl alcohol
- Molecular grade water

Equipment Needed

- **DeWalch Piercer** – (see Figure 5)
(Purchase from DeWalch Technologies 96 Well - Cat No. 2111, 8 well - Cat No. 2189).

Figure 5 - DeWalch piercer (Cat No. 2111)



- **DeWalch Vacuum Manifold** - (see Figure 6)
(Purchase from DeWalch Technologies Single System - Cat No. 2048, Quad System – Cat No. 1566).

Figure 6 - DeWalch vacuum manifold (Cat No. 2048)



- **Incubator** - the plates must be firmly anchored in a temperature controlled incubator typically set at 37°C, with an orbital radius of 10 mm and capable of sustained shaking at 350 to 420 rpm.

- **Plate Centrifuge** (optional) - 96-well plate centrifuge that can accommodate a 3-inch tall plate and can be set to 14000 x g.
- **Vacuum Source** - Vacuum pump or in house vacuum with a range of 5 –10 inch Hg, 2 cfm capacity.
- **Multichannel Pipettor** - delivers 200, 400 and 120 μ l volumes.
- **Flat top vortexer.**

QUALITY CONTROL

DeWalch Life Technologies is committed to providing clients with a quality product. All reagents, consumables and equipment associated with the NeXprep purification system are tested on a lot to lot basis utilizing set standards to insure constant, reproducible results.

TECHNICAL NOTES

Growing Cells

Growing *E. coli* cells harboring plasmid of interest is a key step in achieving high, consistent yields with the NeXprep plasmid preparation system. *E. coli* can grow effectively in the presence of the DNA binding matrix packed in the DeWalch NeXplate.

Keys to achieving high bacterial growth:

- **Quality of the inoculum:** Using fresh bacterial transformants in a standard cloning host strain such as DH5, XL1 Blue, etc. or quality frozen glycerol stocks is recommended.
- **Growth Medium:** 2X YT medium is recommended. Using other growth media, such as LB, might result in lower plasmid yield. Appropriate

antibiotic for the plasmid / host combination should be added.

- **Incubation:** Incubate for 16 to 18 hours at 37°C with a constant agitation (340 rpm) using single colony or freshly preserved glycerol stocks (1-2 µl per well). Incubate period might increase to 22 - 24 hrs if using aged glycerol stocks. Using cold growth medium directly from refrigeration can alter the incubation time.
- **Cell culture density:** Measure culture density by optical density (OD). First vortex the plate, allow the binding matrix to settle and then transfer a small portion (5-10 µl) from each culture to the corresponding well of a cell reading plate, mix with 90-95 µl of water and measure the OD at a 600 nm wavelength.

Harvesting Cells (optional, to freeze the cell pellets for later plasmid purification)

Centrifuge the NeXplate for 5 minutes at 1,400 x *g*. Do not exceed 2,500 x *g*. Carefully, decant the growth media after centrifugation. Plates may be processed immediately or stored at -20°C or below for later purification. However, it might result in lower plasmid yield when aged samples are used for plasmid preparation. Proper thawing of frozen plates prior to use is also an important factor in the yield and integrity of the plasmids. Plates should be thawed at room temperature for at least 15 minutes and no more than 1 hour, and should not be stacked when thawing.

Sequencing

Sequencing of the plasmid DNA prepared using the NeXprep is facilitated by preheating the template for 5 minutes at 96°C.

PROCEDURE

Prior to Starting Procedure

1. **Opening the NeXplate.** Extra caution should be taken when opening the NeXplate. **Hold the NeXplate in the upright position and tap it on a flat surface prior to removing the seal. Retain seal for the overnight incubation.**
2. **Prepare overnight cultures** by placing 0.5 mL of growth medium into each well of the NeXplate. Inoculate each well with an individual colony or 1-2 μ l of glycerol stocks and place the seal over the top of the NeXplate. **Incubate for 16 to 18 hours at 37°C with vigorous shaking at 340 rpm.**
3. **Prepare the wash buffers** by adding RNase A and Isopropyl alcohol as describes in **Appendices A through F.**
4. **Set up the vacuum manifold and piercer.**

Plasmid DNA Purification Using a NeXplate (using bacterial culture as a starting material)

1. Add 300 μ l of **Solution A** to each well and shaking vigorously for 2 minutes. Incubate at room temperature for 3 minute. **Use caution as the lysis buffer contains chaotropic salts.**
2. Pierce the bottom of the NeXplate with the DeWalch piercer. Place the NeXplate in the piercer making sure the nozzles of the plate are aligned with the piercing pin array. Pull the lever over to the down position and press. The NeXplate is now pierced. Return the lever to the upper position and remove the pierced NeXplate. Place the plate on the

- DeWalch vacuum manifold. **DO NOT apply vacuum yet.**
3. Add 400 μ l of **Solution B** to each well without mixing. Incubate at room temperature for 1 minute.
 4. Evacuate lysis and wash buffer I using the vacuum manifold. Evacuate until all of the liquid has been removed from the wells.
 5. Add an additional 400 μ l of **Solution B**. Incubate at room temperature for 2 minutes. Apply vacuum until all the liquid has been removed from the NeXplate.
 6. Add 400 μ l of **Solution C** to each well. Evacuate Solution C using the vacuum manifold.
 7. Remove the NeXplate from the vacuum manifold and using a dry folded paper towel pat the bottom of the plate to remove excess alcohol clinging to the bottom. **Repeat patting and applying vacuum until little to no excess alcohol is left clinging to the plate.**
 8. Continue applying vacuum for 4-8 minutes as described in the following table (Table 1) until the binding matrix in all wells is completely dry. **The wells must be completely dry (but not over-dried) prior to moving to the next step. The binding matrix should appear dry and light tan in color. There should be little to no alcohol present on the bottom of the plate when patted on a paper towel.**

Table 1: Recommended vacuum pressures and time (min.) for complete dry (but not over dry) of the plate

No.	Inches of Mercury (in Hg)*	Min Required
1	15-20	4

2	12-15	6
3	6-8	8

*Vacuum pressure (inches of Mercury) when each well of the plate was filled with Buffer C.

Elution Option 1 (by Vacuum)

- Place the collection plate underneath the NeXplate and then place the NeXplate/Collection plate combination on the vacuum manifold. Make sure both plates fit tightly and are securely on the manifold.
- Elute plasmid DNA by adding 80 μ l of **Solution E** to each well of the NeXplate and incubating for 3 minutes at room temperature.
- Collect purified plasmid DNA by increasing vacuum pressure gradually and reaching final vacuum pressure 5 in Hg for approximately 20 seconds.
- Use the centrifuge perform a quick spin to pull all of the elutant to the bottom of the collection plate.
- Nucleic acid is ready for further processing or storage.

Elution Option 2 (by Centrifugation)

- Place the collection plate underneath the NeXplate. Make sure both plates fit tightly.
- Elute plasmid DNA by adding 80 μ l of **Solution E** to each well of the plate and incubating for 3 minutes at room temperature.
- Collect purified plasmid DNA by centrifuging at 1,400 x g for 2 minutes.

12. Nucleic acid is ready for further processing or storage.

Always preheat NeXprep purified plasmid DNA for 5 minutes @ 96°C to relieve supercoiling prior to sequencing. NeXprep DNA contains little or no relaxed DNA and resists primer annealing if not relaxed by heating

Plasmid DNA Purification Using a NeXplate (Optional, using bacterial pellet as the starting materials)

1. Proper thawing of frozen plates prior to use is also an important factor in the yield and integrity of the plasmids. Plates should be thawed at room temperature for at least 15 minutes and no more than 1 hour, and should not be stacked when thawing.
2. Lyse cells by the addition of 250 µl of **Solution A** to each well and vortex vigorously for 30 seconds. Incubate at room temperature for 1 minute. **Use caution as the lysis buffer contains chaotropic salts.**
3. Pierce the bottom of the NeXplate with the DeWalch piercer. Place the NeXplate in the piercer making sure the nozzles of the plate are aligned with the piercing pin array. Pull the lever over to the down position and press. The NeXplate is now pierced. Return the lever to the upper position and remove the pierced NeXplate. Place the plate on the DeWalch vacuum manifold. **DO NOT apply vacuum yet.**
4. Add 400 µl of **Solution B** to each well without mixing. Incubate at room temperature for 1 minute.

5. Evacuate lysis and wash buffer I using the vacuum manifold. Evacuate until all of the liquid has been removed from the wells.
6. Add an additional 400 µl of **Solution B**. Incubate at room temperature for 2 minutes. Apply vacuum until all the liquid has been removed from the NeXplate.
7. Add 400 µl of **Solution C** to each well. Evacuate Solution C using the vacuum manifold.
8. Remove the NeXplate from the vacuum manifold and using a dry folded paper towel pat the bottom of the plate to remove excess alcohol clinging to the bottom. **Repeat patting and applying vacuum until little to no excess alcohol is left clinging to the plate.**
9. Continue applying vacuum for 4-8 minutes as described in the following table (Table 1) until the binding matrix in all wells is completely dry. **The wells must be completely dry (but not over-dried) prior to moving to the next step. The binding matrix should appear dry and light tan in color. There should be little to no alcohol present on the bottom of the plate when patted on a paper towel.**

Table 1: Recommended vacuum pressures and time (min.) for complete dry (but not over dry) of the plate

No.	Inches of Mercury (in Hg)*	Min Required
1	15-20	4
2	12-15	6
3	6-8	8

*Vacuum pressure (inches of Mercury) when each well of the plate was filled with Buffer C.

Elution Option 1 (by Vacuum)

10. Place the collection plate underneath the NeXplate and then place the NeXplate/Collection plate combination on the vacuum manifold. Make sure both plates fit tightly and are securely on the manifold.
11. Elute plasmid DNA by adding 100 μ l of **Solution E** to each well of the NeXplate and incubating for 3 minutes at room temperature.
12. Collect purified plasmid DNA by increasing vacuum pressure gradually and reaching final vacuum pressure 5 in Hg for approximately 2 minutes.
13. Using the centrifuge perform a quick spin to pull all of the elutant to the bottom of the collection plate.
14. Nucleic acid is ready for further processing or storage.

Elution Option 2 (by Centrifugation)

10. Place the collection plate underneath the NeXplate. Make sure both plates fit tightly.
11. Elute plasmid DNA by adding 120 μ l of Solution E to each well of the plate and incubating for 3 minutes at room temperature.
12. Collect purified plasmid DNA by centrifuging at 1,400 x *g* for 2 minutes.
13. Nucleic acid is ready for further processing or storage.

Plasmid Purification Using a Spin Mini Column

1. Grow an overnight culture by placing 0.5 mL of growth medium into each well of a 96-well plate (or each individual test tube). Inoculate and grow bacterial culture as described in pages 10-11.
2. Add 300 μ l of **Solution A** to each well (tube) and shaking vigorously for 2 minutes.
3. Incubate at room temperature for 3 minutes. **Use caution as the Solution A contains chaotropic salt.**
4. Transfer the entirely mixture from each well (tube) into the Mini column that has been placed in a 2-ml collection tube (see Fig. 2).
5. Centrifuge for 1 min at $\geq 6,500$ xg (or at the maximum speed of a benchtop microcentrifuge). Separate the column from its collection tube, discard the flow-through waste, reassemble the spin column with its collection tube.
6. Add 400 μ l of **Solution B**, incubate for 2 min. at room temperature.
7. Centrifuge for 1 minute at $\geq 6,500$ xg , and the discard the collection tube and flow-through waste.
8. Place the mini column in a new collection tube, add 400 μ l of **Solution C**, and centrifuge for 3 minutes at $\geq 6,500$ xg to dry the spin column.

Ensure that the spin column is completely dry and no residual alcohol is present. Centrifuge for another 1 minute at $\geq 6,500$ xg if needed.

Discard the collection tube and flow-through waste.

9. Place the mini column in a sterile 1.5-ml microcentrifuge tube, and pipet 60 μ l of **Solution E** directly onto the center of spin column.
10. Incubate the column for 2 minutes at room temperature, and centrifuge for 1 minute at $\geq 6,500$ xg.
11. Repeat elution once as described above (steps 9 and 10, optional), which could increase total yield of purified DNA by at least 25%.

Always preheat NeXprep purified plasmid DNA for 5 minutes @ 96°C to relieve supercoiling prior to sequencing. NeXprep DNA contains little or no relaxed DNA and resists primer annealing if not relaxed by heating.

PRODUCT PERFORMANCE

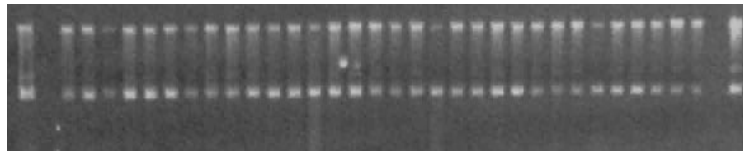


Figure 6 Agarose gel (1%) of uncut puc19 prepared by Standard (left) or No Spin NeXprep Plasmid Purification Kits.

Chromas 1.45 File: S32019.DEW-2-M13R.ab1 Sequence Name: S32019.DEW-2-M13R Run ended: Mar 13, 2003

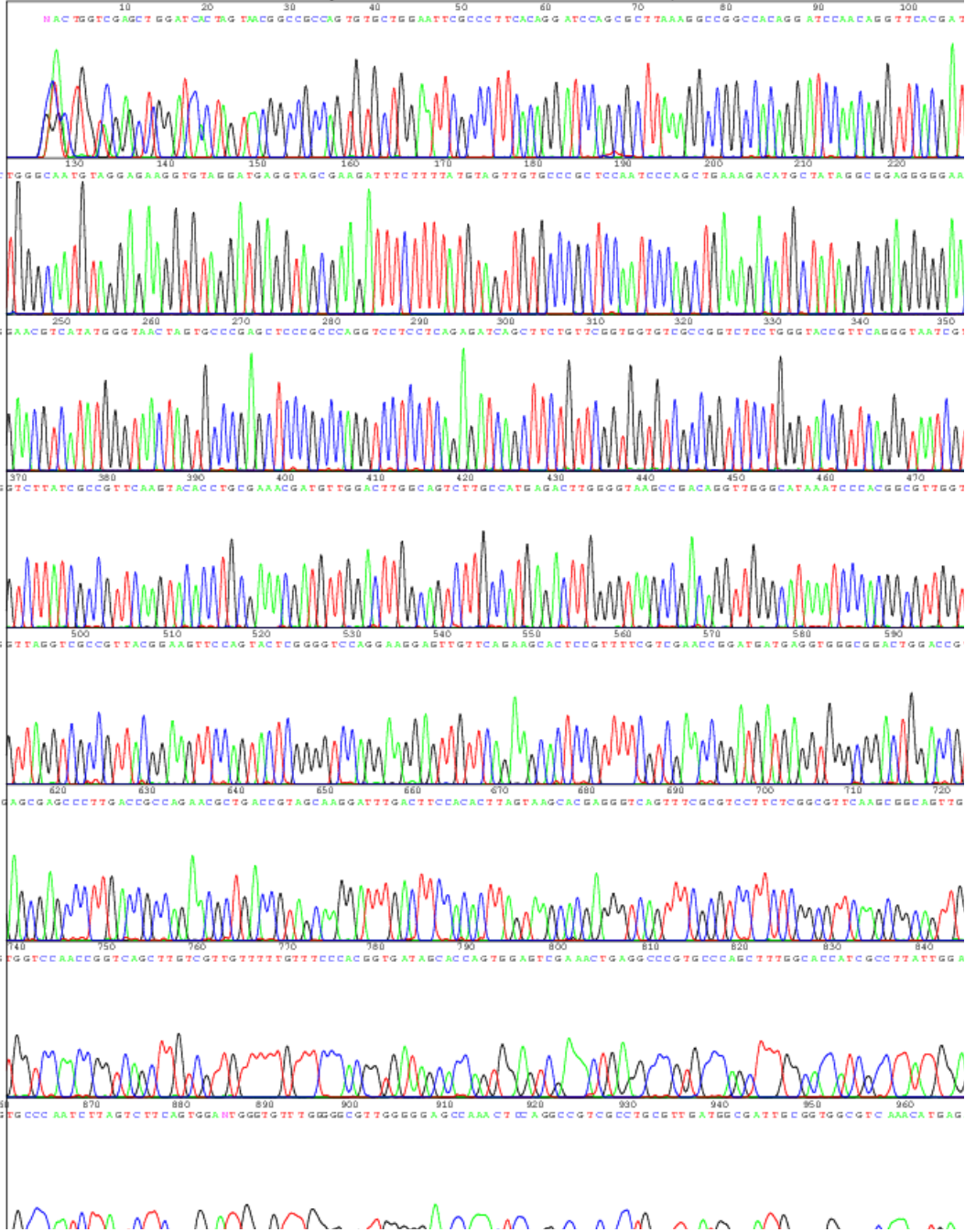


Figure 7 - Sequencing with ABI 3730 of puc19 prepared by NeXprep.

TROUBLESHOOTING

Symptoms	Possible Causes	Corrective Actions
Filter/Drip Director Clogging	Breaking the filter membrane during inoculation allowing binding media to clog the drip directors	Make sure not to disturb the binding media and filter during inoculation process. Bottom of the well should appear white after processing.
	Low or inconsistent vacuum	Check pump, traps, and ensure valves are open
	Centrifugation force too high to force the DNA binding matrix to clog the drip director	Centrifuge the plate for 5 min at 1,400 x <i>g</i> .
Slow draining and drying	Low vacuum	Increase vacuum Free constricted tubing (do not use thin wall tubing)
	Cell pellets too old	Place the drained cell pellets (plate) on ice for a few hours, or frozen at -20°C or -80°C for a short period (a few weeks).

Low yields overall	Insufficient agitation of cultures during.	Increase agitation to a speed in the range between
Symptoms (continued)	Possible Causes	Corrective Actions
	incubation	340 and 420 rpm.
	Old or weak bacterial inoculation	Use fresh transformants or remake glycerol stocks
	Growth media	Use richer media such as 2x YT or TB
	Wrong amount of glycerol stock	Optimize the amount added
Low yields Overall (continued)	Insufficient drying	Use a stronger vacuum pump or increase drying time
	Low copy number plasmid	Increase the concentration of plasmid by evaporating and resuspending in less volume
Spotty Yields	Cells are too old	Use newly transformed cells
Degraded Plasmid DNA	Cells were stored at wrong temperature	Keep cells stored at -20 °C
RNA Present in the gel	Insufficient buffer B wash	Ensure buffer B wash and incubation is sufficient
	Insufficient RNase digestion time	Incubate plasmid sample for 20 min at 37°C or for 1 hr at

		room temperature.
	RNase has lost activity	Contact RNase manufacture
Symptoms (continued)	Possible Causes	Corrective Actions
Low or No Sequencing Signal Strength	Preheat the template for 5 minutes at 96°C prior to sequencing	Critical step for the highly supercoiled NeXprep DNA
Low or no sequencing signal strength (continued)	Improper amount of template DNA	Try optimizing with different amounts of DNA within the range recommended by sequencing kit manufacturer
	Excess alcohol in the final elute	Increase the drying time or precipitate the DNA
Dirty Sequence	RNA present or low concentration of DNA	Use agarose gel electrophoresis to identify problem
	Mixed clone(s), contaminated culture	Resolve library problems and prepare new DNA

Appendix A – 4 NeXprep Purification Kit

NeXprep 4 Block Purification Kit Isopropanol Fill

Solution	Concentrate	IPA Added	Total
B	180 mL	180 mL	360 mL
C	40 mL	140 mL	180 mL

Appendix B – 20 NeXprep Purification Kit

NeXprep 20 Block Purification Kit Isopropanol Fill

Solution	Concentrate	IPA Added	Total
B	900 mL	900 mL	1,800 mL
C	200 mL	800 mL	1,000 mL

Appendix C – 40 NeXprep Purification Kit

NeXprep 40 Block Purification Kit Isopropanol Fill

Solution	Concentrate	IPA Added	Total
B*	1,800 mL	1,800 mL	3,600 mL
C	400 mL	1,600 mL	2,000 mL

*Provided in two bottles of 1,000 mL

Appendix D –50 Column NeXprep Purification Kit

NeXprep 50 Spin Column Purification Kit Isopropanol Fill

Solution	Concentrate	IPA Added	Total
B*	25 mL	25 mL	50 mL
C	5 mL	20 mL	25 mL

Appendix E –100-Column NeXprep Purification Kit

NeXprep 100-Column Purification Kit Isopropanol Fill

Solution	Concentrate	IPA Added	Total
B*	50 mL	50 mL	100 mL
C	10 mL	40 mL	50 mL

Appendix F –250-Column NeXprep Purification Kit

NeXprep 250-Column Purification Kit Isopropanol Fill

Solution	Concentrate	IPA Added	Total
B*	125 mL	125 mL	250 mL
C	25 mL	100 mL	125 mL