For Research Use Only

IBI SCIENTIFIC

INSTRUCTION MANUAL

96 Well Plasmid Kit

IB47153 (2 x 96 well plates/kit)

IB47154 (4 x 96 well plates/kit)

IB47155 (10 x 96 well plates/kit)

Introduction

The 96 Well Plasmid Kit was designed for high-throughput purification of plasmid DNA from 1-5 ml of cultured bacterial cells per well using an efficient 96 well filter plate and binding plate system. A modified alkaline lysis method and RNase treatment are used to obtain clear cell lysate with minimal genomic DNA and RNA contaminants. Typical yields are 16-20 µg for high-copy number plasmid or 3-5 µg for low-copy number plasmid from 4 ml of cultured bacterial cells. DNA phenol extraction or alcohol precipitation is not required and the entire procedure can be completed within 25 minutes. The purified plasmid DNA is ready for use in restriction enzyme digestion, ligation, PCR, and sequencing reactions.

Quality Control

The quality of the 96 Well Plasmid Kit is tested on a lot-to-lot basis by isolating plasmid DNA from a 4 ml overnight E. coli (DH5α) culture containing plasmid pBluescript (A600 > 3.7 U/ml). Following the purification process, a yield of more than 20 µg per well is obtained and the A260/A280 ratio is between 1.8-2.0. The purified plasmid DNA (1 µg) is used in EcoRI digestion, and analyzed by electrophoresis.

Kit Components

Catalogue Number	IB47153	IB47154	IB47155
P1 Buffer[1]	40 ml	80 ml	200 ml
P2 Buffer[2]	40 ml	80 ml	200 ml
P3 Buffer[3]	60 ml	120 ml	60 ml x 1 240 ml x 1
Wash Buffer[3] (Add Ethanol)	50 ml (200 ml)	50 ml x 2 (200 ml x 2)	50 ml x 4 (200 ml x 4)
Elution Buffer	30 ml	60 ml	120 ml
RNase A (50 mg/ml)	130 µl	260 µl	650 µl
Plasmid 96 Well Filter Plate	2	4	10
Plasmid 96 Well Binding Plate	2	4	10
96 Deep Well Plate[4]	2	2	2
0.35 ml Collection Plate	2	4	10
Adhesive Film	8	16	40

[1] Add provided RNase A to P1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. P1 Buffer and RNase A mixture should be stored at 2-8°C for up to 6 months.

[2] If precipitates have formed in P2 Buffer, warm the buffer in a 37°C water bath, followed by gentle shaking to dissolve.

[3] Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

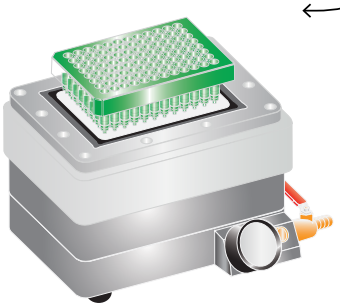
[4] Two 96 Deep Well Plates are provided in each kit. After use, rinse the plates with water then incubate in 0.4M HCl for 1 minute at room temperature. Wash the plates thoroughly with ddH_2O. The plates can be autoclaved after being washed and re-used.

IMPORTANT BEFORE USE! **During the procedure, always wear a lab coat, disposable gloves, and protective goggles.**

Quick Protocol Diagram

Harvest cultured bacterial cells followed by resuspension, lysis and neutralization

Cell debris filtration using the Plasmid 96 Well Filter Plate

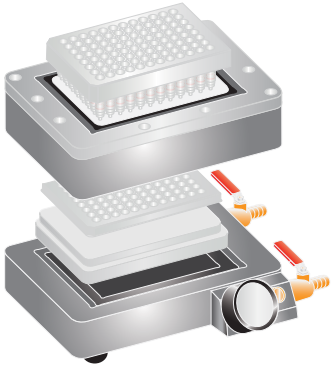


Optional cell debris filtration. DNA binding using the Plasmid 96 Well Binding Plate

DNA binding then wash using the Plasmid 96 Well Binding Plate

Wash using the Plasmid 96 Well Binding Plate

Elution of pure plasmid DNA into the 0.35 ml Collection Plate



Elution of pure plasmid DNA into the 0.35 ml Collection Plate

96 Well Plasmid Kit Protocol

IMPORTANT BEFORE USE!

1. Add provided RNase A to P1 Buffer then mix by shaking for a few seconds. Check the box on the bottle. P1 and RNase A mixture should be stored at 2-8°C for up to 6 months.
2. If precipitates have formed in P2 Buffer, warm the buffer in a 37°C water bath, followed by gentle shaking to dissolve.
3. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

Additional Requirements:
Absolute ethanol, microcentrifuge tubes (optional), microporous tape (optional), 96 Deep Well Plates

Cell Cultivation and Harvesting (For Vacuum and Centrifuge Protocol)

Grow Bacterial Cultures in Tubes or Flasks

Pick a single bacterial colony from a selective agar plate and inoculate the cell culture in 1-5 ml of LB medium containing appropriate antibiotics. Incubate at 37°C for 12-16 hours with 150-180 rpm shaking. Transfer 1.5 ml of cultured bacterial cells to a 1.5 ml microcentrifuge tube. Centrifuge at 14-16,000 x g for 1 minute at room temperature to form a cell pellet then discard the supernatant completely. Repeat the harvesting step as required for samples between 1-5 ml using the same 1.5 ml microcentrifuge tube.

Grow Bacterial Cultures in a 96 Deep Well Plate

Transfer 1.3 ml of fresh LB medium containing appropriate antibiotics into each well of a 96 Deep Well Plate then inoculate each well with a single bacterial colony. Seal the plate withmicroporous tape or Adhesive Film. When using non-porous adhesive tape, pierce 2-3 holes in the tape with a needle above each well to promote air exchange. Incubate at 37°C for 20-24 hours with 180-250 rpm shaking. Pellet the bacterial culture in the plate by centrifugation for 5 minutes at 2,000 x g. During centrifugation, the plate should be covered with Adhesive Film. Following centrifugation, remove the Adhesive Film then remove the supernatant in each well by quickly inverting the plate.

Plasmid DNA Vacuum Manifold Protocol

1. Vacuum Manifold Preparation

Place the waste tray on the manifold base then place the binding top plate on the manifold base. Place the Plasmid 96 Well Binding Plate in the binding top plate aperture. Place the filter top plate on the binding top plate. Place the Plasmid 96 Well Filter Plate in the filter top plate aperture then attach the vacuum manifold to a vacuum source.

2. Resuspension

Add 200 µl of P1 Buffer (make sure RNase A was added) to each well of the 96 Deep Well Plate or microcentrifuge tubes then resuspend the cell pellet by pipette. Continue to pipette until all traces of the cell pellet have been dissolved.
NOTE: Transfer the resuspended cell samples from microcentrifuge tubes to each well of a new 96 Deep Well Plate.

3. Lysis

Add 200 µl of P2 Buffer to each well of the 96 Deep Well Plate. Dry the top of the plate with paper towel then seal tightly with new Adhesive Film. Gently invert the plate 6-8 times then incubate at room temperature for 2 minutes.

4. Neutralization

Briefly centrifuge the 96 Deep Well Plate at 2,000 x g to collect any sample mixture remaining on the Adhesive Film. Allow the centrifuge to reach 2,000 x g prior to stopping. Remove the Adhesive Film from the 96 Deep Well Plate then add 300 µl of P3 Buffer to each well. Dry the top of the plate with paper towel then seal tightly with new Adhesive Film. Gently invert the plate 6-8 times.

5. Cell Debris Filtration

Briefly centrifuge the 96 Deep Well Plate at 2,000 x g to collect any sample mixture remaining on the Adhesive Film. Allow the centrifuge to reach 2,000 x g prior to stopping. Remove the Adhesive Film from the 96 Deep Well Plate. Transfer the lysate from each well (700 µl per well) to the wells of the Plasmid 96 Well Filter Plate (seal unused wells with adhesive film). Apply vacuum at 15 inches Hg until the lysate passes through completely (approximately 2-3 minutes) then switch off the vacuum.

6. DNA Binding

Remove the filter top plate and the Plasmid 96 Well Filter Plate. Seal unused wells of the Plasmid 96 Well Binding Plate with Adhesive Film. Apply vacuum at 15 inches Hg until the clear lysate passes through completely (approximately 10 seconds) then turn off the vacuum.

7. Wash

Optional Endonuclease Removal Wash Step Add 400 µl of Endonuclease Removal Buffer (4.2M guanidine hydrochloride/ 40% isopropanol, see page 11) to each well of the Plasmid 96 Well Binding Plate. Apply vacuum at 15 inches Hg until the buffer passes through completely (approx. 10 sec.) then turn off the vacuum.

NOTE: This optional wash step is recommended if using EndA+ bacterial strains such as E. coli HB 101, JM, or wild-type strains. However, plasmid DNA yield will be reduced by 20%.

For standard plasmid DNA purification

Add 1 ml of Wash Buffer (make sure ethanol was added) to each well of the Plasmid 96 Well Binding Plate. Apply vacuum at 15 inches Hg until Wash Buffer passes through completely. Continue to apply vacuum for an additional 10 minutes to dry the membrane then turn off the vacuum.

8. Elution

Remove the Plasmid 96 Well Binding Plate from the binding top plate aperture then blot the nozzles on a clean absorbent paper towel to remove residual ethanol. Remove the waste tray from the manifold base then place the collection plate spacer on the manifold base. Place a 0.35 ml collection plate on top of the collection plate spacer. Place the top binding plate back on the manifold base then place the Plasmid 96 Well Binding Plate back in the binding top plate aperture. Add 100 µl of Elution Buffer[1] , TE[2] or water[3] into the CENTER of each well of the Plasmid 96 Well Binding Plate. Let stand for at least 2 minutes to ensure the Elution Buffer, TE or water is absorbed by the membrane. Apply vacuum at 15 inches Hg for 5 minutes then turn off the vacuum. Seal the 0.35 ml Collection Plate with Adhesive Film and store the purified DNA at -20°C.

NOTE: The average eluate volume is 60 µl from 100 µl elution buffer volume.

[1] Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25°C) is added into the center of the well matrix and is completely absorbed.

[2] Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the well matrix and is completely absorbed.

[3] If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the center of the well matrix and is completely absorbed. DNA eluted in water should be stored at -20°C to avoid degradation.

Plasmid DNA Centrifuge Protocol (With Filter Plate)

1. Resuspension

Add 200 µl of P1 Buffer (make sure RNase A was added) to each well of the 96 Deep Well Plate or microcentrifuge tubes then resuspend the cell pellet by pipette. Continue to pipette until all traces of the cell pellet have been dissolved.

NOTE: Transfer the resuspended cell samples from microcentrifuge tubes to each well of a new 96 Deep Well Plate.

2. Lysis

Add 200 µl of P2 Buffer to each well of the 96 Deep Well Plate. Dry the top of the plate with paper towel then seal tightly with new Adhesive Film. Gently invert the plate 6-8 times then incubate at room temperature for 2 minutes.

3. Neutralization

Briefly centrifuge the 96 Deep Well Plate at 2,000 x g to collect any sample mixture remaining on the Adhesive Film. Allow the centrifuge to reach 2,000 x g prior to stopping. Remove the Adhesive Film from the 96 Deep Well Plate then add 300 µl of P3 Buffer to each well. Dry the top of the plate with paper towel then seal tightly with new Adhesive Film. Gently invert the plate 6-8 times.

4. Cell Debris Filtration

Briefly centrifuge the 96 Deep Well Plate at 2,000 x g to collect any sample mixture remaining on the Adhesive Film. Allow the centrifuge to reach 2,000 x g prior to stopping. Place the Plasmid 96 Well Filter Plate on a new 96 Deep Well Plate. Remove the Adhesive Film from the 96 Deep Well Plate containing the sample lysate. Transfer all sample lysate from each well (700 µl per well) to the wells of the Plasmid 96 Well Filter Plate. Centrifuge the Plasmid 96 Well Filter Plate and 96 Deep Well Plate together at 3,000 x g for 5 minutes then discard the Plasmid 96 Well Filter Plate.

5. DNA Binding

Place the Plasmid 96 Well Binding Plate on top of a new 96 Deep Well Plate. Transfer the cleared flow-through in each well of the 96 Deep Well Plate to each well of the Plasmid 96 Well Binding Plate. Centrifuge the Plasmid 96 Well Binding Plate and 96 Deep Well Plate together at 3,000 x g for 5 minutes. Discard the flow-through then place the Plasmid 96 Well Binding Plate back on the 96 Deep Well Plate.

6. Wash

Optional Endonuclease Removal Wash Step

Add 400 µl of Endonuclease Removal Buffer (4.2M guanidine hydrochloride/40% isopropanol, see page 11) to each well of the Plasmid 96 Well Binding Plate. Centrifuge the Plasmid 96 Well Binding Plate and 96 Deep Well Plate together at 3,000 x g for 5 minutes. Discard the flow-through then place the Plasmid 96 Well Binding Plate back on the 96 Deep Well Plate.

NOTE: This optional step is recommended if using EndA+ bacterial strains such as E. coli HB 101, JM, or wild-type strains. However, plasmid DNA yield will be reduced by 20%.

For standard plasmid DNA purification

Add 500 µl of Wash Buffer (make sure ethanol was added) to each well of the Plasmid 96 Well Binding Plate. Centrifuge the Plasmid 96 Well Binding Plate and 96 Deep Well Plate together at 3,000 x g for 5 minutes. Add another 500 µl of Wash Buffer (make sure ethanol was added) to each well of the Plasmid 96 Well Binding Plate. Centrifuge the Plasmid 96 Well Binding Plate and 96 Deep Well Plate together at 3,000 x g for 5 minutes. Discard the flow-through then place the Plasmid 96 Well Binding Plate back on the 96 Deep Well Plate. Centrifuge the Plasmid 96 Well Binding Plate and 96 Deep Well Plate together at 3,000 x g for 10 minutes to dry the membrane.

7. Elution

Remove the Plasmid 96 Well Binding Plate from the 96 Deep Well Plate then blot the nozzles on a clean absorbent paper towel to remove residual ethanol. Place the Plasmid 96 Well Binding Plate on a 0.35 ml collection plate.

Add 100 µl of Elution Buffer[1] , TE[2] or water[3] into the CENTER of each well of the Plasmid 96 Well Binding Plate. Let stand for at least 2 minutes to ensure the Elution Buffer, TE or water is absorbed by the membrane. Centrifuge the Plasmid 96 Well Binding Plate and 0.35 ml collection plate together at 3,000 x g for 5 minutes. Seal the 0.35 ml Collection Plate with Adhesive Film and store the purified DNA at -20°C.

NOTE: The average eluate volume is 60 µl from 100 µl elution buffer volume.

[1] Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25°C) is added into the center of the well matrix and is completely absorbed.

[2] Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the well matrix and is completely absorbed.

[3] If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification. Ensure that water is added into the center of the well matrix and is completely absorbed. DNA eluted in water should be stored at -20°C to avoid degradation.

Plasmid DNA Centrifuge Protocol (Without Filter Plate)

1. Resuspension

Add 200 µl of P1 Buffer (make sure RNase A was added) to each well of the 96 Deep Well Plate or microcentrifuge tubes then resuspend the cell pellet by pipette. Continue to pipette until all traces of the cell pellet have been dissolved.

NOTE: Transfer the resuspended cell samples from microcentrifuge tubes to each well of a new 96 Deep Well Plate.

2. Lysis

Add 200 µl of P2 Buffer to each well of the 96 Deep Well Plate. Dry the top of the plate with paper towel then seal tightly with new Adhesive Film. Gently invert the plate 6-8 times then incubate at room temperature for 2 minutes.

3. Neutralization

Briefly centrifuge the 96 Deep Well Plate at 2,000 x g to collect any sample mixture remaining on the Adhesive Film. Allow the centrifuge to reach 2,000 x g prior to stopping. Remove the Adhesive Film from the 96 Deep Well Plate then add 300 µl of P3 Buffer to each well. Dry the top of the plate with paper towel then seal tightly with new Adhesive Film. Gently invert the plate 6-8 times.

4. DNA Binding

Centrifuge the 96 Deep Well Plate at 5,000 x g for 10 minutes. Following centrifugation, a cell debris pellet and clear lysate/supernatant will be present. Place the Plasmid 96 Well Binding Plate on a new 96 Deep Well Plate. Remove the Adhesive Film from the 96 Deep Well Plate then transfer the clear supernatant to each well of the Plasmid 96 Well Binding Plate. Centrifuge the Plasmid 96 Well Binding Plate and 96 Deep Well Plate together at 3,000 x g for 5 minutes. Discard the flow-through then place the Plasmid 96 Well Binding Plate back on the 96 Deep Well Plate.

5. Wash

Optional Endonuclease Removal Wash Step

Add 400 µl of Endonuclease Removal Buffer (4.2M guanidine hydrochloride/40% isopropanol, see page 11) to each well of the Plasmid 96 Well Binding Plate. Centrifuge the Plasmid 96 Well Binding Plate and 96 Deep Well Plate together at 3,000 x g for 5 minutes. Discard the flow-through then place the Plasmid 96 Well Binding Plate back on the 96 Deep Well Plate.

NOTE: This optional wash step is recommended if using EndA+ bacterial strains such as E. coli HB 101, JM, or wild-type strains. However, plasmid DNA yield will be reduced by 20%.

For Standard Plasmid DNA Purification

Add 1 ml of Wash Buffer (make sure ethanol was added) to each well of the Plasmid 96 Well Binding Plate. Centrifuge the Plasmid 96 Well Binding Plate and 96 Deep Well Plate together at 3,000 x g for 5 minutes. Discard the flow-through then place

the Plasmid 96 Well Binding Plate back on the 96 Deep Well Plate. Centrifuge the Plasmid 96 Well Binding Plate and 96 Deep Well Plate together at 3,000 x g for 10 minutes to dry the membrane.

6. Elution

Remove the Plasmid 96 Well Binding Plate from the 96 Deep Well Plate then blot the nozzles on a clean absorbent paper towel to remove residual ethanol. Place the Plasmid 96 Well Binding Plate on a 0.35 ml collection plate. Add 100 µl of Elution Buffer[1], TE[2] or water[3] into the CENTER of each well of the Plasmid 96 Well Binding Plate. Let stand for at least 2 minutes to ensure the Elution Buffer, TE or water is absorbed by the membrane. Centrifuge the 96 Well Binding Plate and 0.35 ml collection plate together at 3,000 x g for 5 minutes. Seal the 0.35 ml Collection Plate with Adhesive Film and store the purified DNA at -20°C.
NOTE: The average eluate volume is 60 µl from 100 µl elution buffer volume.

[1] Ensure that Elution Buffer (10 mM Tris-HCl, pH8.5 at 25°C) is added into the center of the well matrix and is completely absorbed.

[2] Using TE (10 mM Tris-HCl, 1 mM EDTA, pH8.0) for elution is beneficial as EDTA preserves DNA for long term storage. However, EDTA will affect PCR and other sensitive downstream applications. Ensure that TE is added into the center of the well matrix and is completely absorbed.

[3] If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2can quickly cause acidification. Ensure that water is added into the center of the well matrix and is completely absorbed. DNA eluted in water should be stored at -20°C to avoid degradation.

Troubleshooting

Low Yield

Incomplete buffer and cell culture preparation.

Add provided RNase A to P1 Buffer then mix by shaking for a few seconds. Check the box on the bottle then store at 2-8°C for up to 6 months. If precipitates have formed in P2 Buffer, warm in a 37°C water bath followed by gentle shaking to dissolve. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle. Be sure and close the bottle tightly after each use to avoid ethanol evaporation. We recommend using a single freshly isolated E. coli colony to inoculate into 1.3 ml of LB medium. Solid and liquid medium should contain antibiotics. Do not use overgrown bacterial cultures (20-24 hours incubated in a 96 Deep Well Plate at 37°C with 180-240 rpm shaking).

Culture growth medium was not removed completely.

Following centrifugation in the harvesting step, use a narrow pipette tip to ensure the supernatant is completely removed.

Cell pellet was not resuspended completely.

Resuspend the cell pellet completely by vortex or pipette. Continue to vortex or pipette until all traces of the cell pellet have been dissolved.

Bacterial cells were not lysed completely.

Using 2 OD600 – 4 OD600 units of bacterial culture is recommended. Following P2 Buffer addition, gently invert the plate 6-8 times then incubate at room temperature for 2 minutes.

Incorrect DNA Elution step.

Ensure that Elution Buffer, TE or water is added into the center of the well matrix and is completely absorbed. If plasmid DNA are larger than 10 kb, use pre-heated Elution Buffer, TE, or water (60~70°C). If using water for elution, ensure the water pH is ≥8.0. ddH_2O should be fresh as ambient CO_2 can quickly cause acidification.

Eluted DNA Does Not Perform Well In Downstream Applications

Residual ethanol/salt contamination.
Following the Wash Step, dry the binding plate with additional vacuum for 10 minutes at 15 inch Hg or centrifuge at 3,000-5,000 x g for 10 minutes.

RNA contamination.
Add provided RNase A to P1 Buffer then mix by shaking for a few seconds. Check the box on the bottle then store at 2-8°C for up to 6 months.

Genomic DNA contamination.
Do not use overgrown bacterial cultures. Use only fresh cultures as they will contain less genomic DNA than old cultures. During P2 and P3 Buffer addition, mix gently to prevent genomic DNA shearing.

Nuclease contamination.
Following the DNA Binding step, add 400 µl of Endonuclease Removal Buffer into each well of the binding plate. Apply vacuum until the buffer passes through completely or centrifuge at 3,000 x g for minutes then proceed with Wash Buffer addition.

96 Well Plasmid Kit Functional Test Data

Table 1.
1. Yield and quality of plasmid DNA purified using the 96 Well Plasmid Kit. 5 µl aliquots of a 60 µl eluate of purified super coiled plasmid DNA from 1.5 ml and 4 ml overnight E. coli (DH5α) culture, containing a 3 kb plasmid pBluescript (OD600 = 3.7 U/ml) were EcoRI digestion and analyzed by electrophoresis on a 0.8% agarose gel.

E.coli Culture Volume	260/280	260/230	Average Yield
1.5 ml	1.90	2.10	6-9 µg
4.0 ml	1.90	2.10	16-20 µg

Sequencing Data

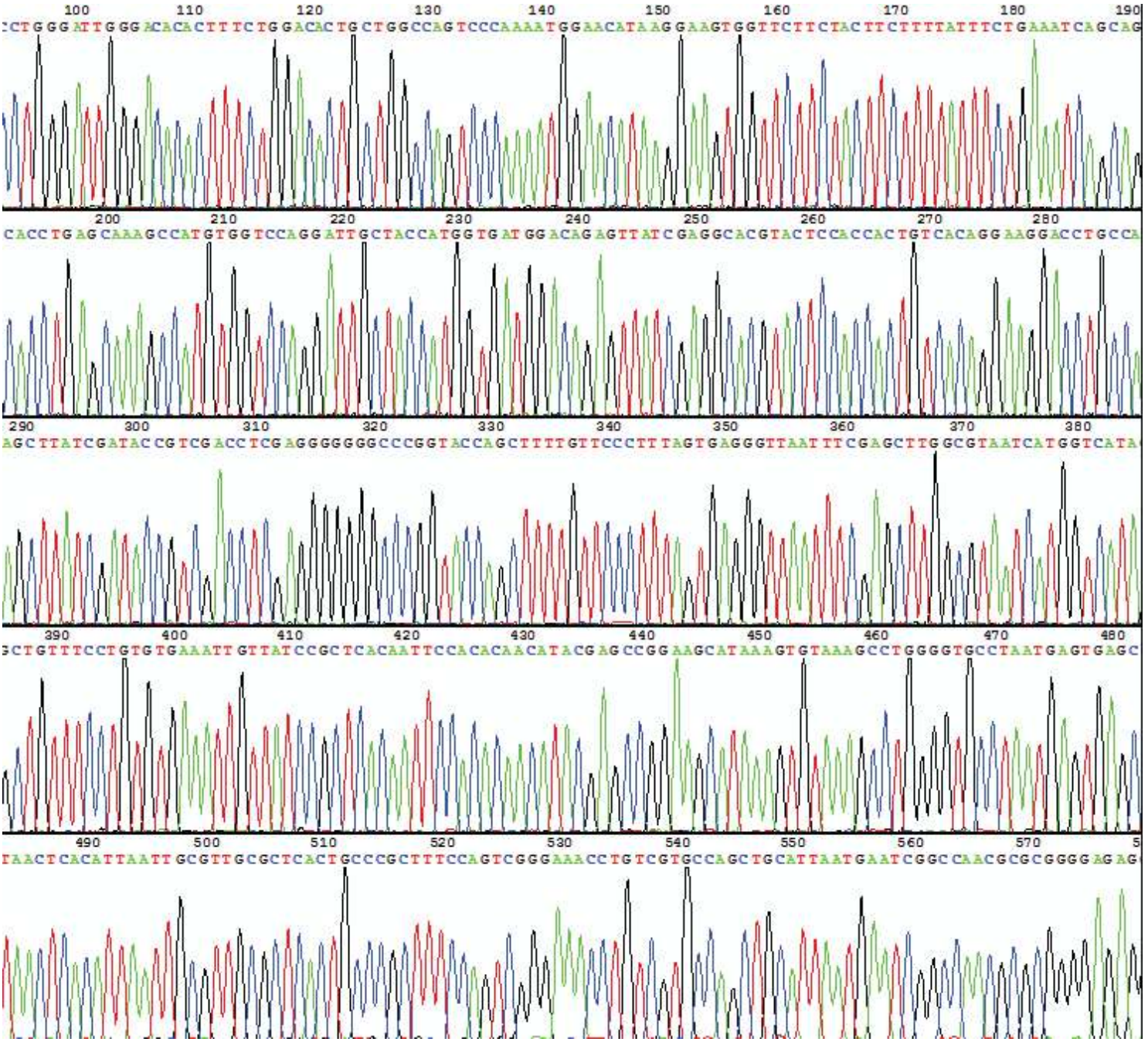


Figure 1.
Sequencing data of (pBluescript) plasmid DNA purified using the 96 Well Plasmid Kit.

Endonuclease Removal Buffer Preparation

Dissolve 20.1 g of guanidine hydrochloride in 15-18 ml of sterile ddH_2O using a 50 ml centrifuge tube. Warm the mixture to 37°C. Mixing thoroughly by vortex will speed up the process. Bring the mixture to a final volume of 30 ml with sterile ddH_2O. Add 20 ml of isopropanol then mix thoroughly. Store the Endonuclease Removal Buffer at room temperature. Endonuclease Removal Buffer can also be purchased directly from IBI.

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