



E.Z.N.A.[®] Endo-free Plasmid DNA Mini Kit I

D6948-00B	5 preps
D6948-01B	50 preps
D6948-02B	200 preps

E.Z.N.A.[®] Endo-free Plasmid DNA Mini Kit II

D6950-00B	5 preps
D6950-01B	50 preps
D6950-02B	200 preps

May 2013

For research use only. Not intended for diagnostic testing.

E.Z.N.A.® Endo-Free Plasmid DNA Mini Kit I
E.Z.N.A.® Endo-Free Plasmid DNA Mini Kit II

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Manual Revision: May 2013



Introduction and Overview

The E.Z.N.A.® Endo-Free Plasmid DNA Mini Kits combine the power of HiBind® technology with the Omega Bio-tek's innovative ETR technology to deliver high-quality plasmid DNA with low endotoxins levels for use in eukaryotic transfections and *in vitro* experiments.

Endotoxins are lipopolysaccharides (LPS) found in the outer cell membrane of gram-negative bacteria such as *E. coli*. One *E. coli* cell contains around 2 million LPS molecules, each having hydrophobic, hydrophilic, and charged regions. Bacteria release small quantities of endotoxins during growth, and large quantities at death. At the time of lysis during plasmid purification, endotoxins are shed into the lysate. The chemical and physical properties that endotoxin molecules possess lead to their co-purification with plasmid DNA by behaving similarly on the surface of silica and anion-exchange resins. The E.Z.N.A.® Endo-Free Plasmid System uses a specially formulated buffer that prevents endotoxin molecules from binding to the surface of the HiBind® matrix. For the best interpretation of results, it is crucial the purified plasmid DNA be free of endotoxins. Endotoxin contamination lowers transfection efficiencies for endotoxin sensitive cell lines. For gene therapy, endotoxin contamination should be of major concern since endotoxins have the potential to cause fever, endotoxic shock syndrome, and interfere with *in vitro* transfection into immune cells.

New in this Edition:

- HB Buffer has been replaced by HBC Buffer. Isopropanol is required and supplied by the user.
- Equilibration Buffer is no longer included with this kit. An optional Column Equilibration Protocol has been added to the protocol for your convenience.
- Equilibration Buffer is replaced with 3M NaOH provided by the user.

Kit Contents

Endo-Free Plasmid Mini Kit I	D6948-00B	D6948-01B	D6948-02B
Purifications	5	50	200
HiBind® DNA Mini Columns	5	50	200
2 mL Collection Tubes	5	50	200
Solution I	5 mL	20 mL	60 mL
Solution II	5 mL	20 mL	60 mL
N3 Buffer	2.5 mL	10 mL	30 mL
ETR Binding Buffer	5 mL	40 mL	160 mL
ETR Wash Buffer	5 mL	30 mL	120 mL
HBC Buffer	4 mL	25 mL	80 mL
DNA Wash Buffer	1.5 mL	15 mL	3 x 25 mL
RNase A	Pre-added	100 µL	300 µL
Endotoxin-free Elution Buffer	1.2 mL	10 mL	30 mL
User Manual	✓	✓	✓

Endo-Free Plasmid Mini Kit II	D6950-00B	D6950-01B	D6950-02B
Purifications	5	50	200
HiBind® DNA Mini Columns II	5	50	200
2 mL Collection Tubes	5	50	200
Solution I	5 mL	30 mL	120 mL
Solution II	5 mL	30 mL	120 mL
N3 Buffer	2.5 mL	15 mL	60 mL
ETR Binding Buffer	10 mL	80 mL	2 x 160 mL
ETR Wash Buffer	5 mL	30 mL	120 mL
HBC Buffer	4 mL	25 mL	80 mL
DNA Wash Buffer	1.5 mL	15 mL	3 x 25 mL
RNase A	Pre-added	100 µL	400 µL
Endotoxin-free Elution Buffer	1.5 mL	15 mL	40 mL
User Manual	✓	✓	✓

Note: ETR Binding Buffer and HBC Buffer contain chaotropic salts. Use gloves and protective eye wear when handling these solutions.

Preparing Reagents

1. Add the vial of RNase A to the bottle of Solution I and store at 2-8°C. (50 and 200 prep size only).
2. Dilute DNA Wash Buffer with 100% ethanol as follows and store at room temperature.

Kit	100% Ethanol to be Added
D6948-00B	6 mL
D6948-01B	60 mL
D6948-02B	100 mL
Kit	100% Ethanol to be Added
D6950-00B	6 mL
D6950-01B	60 mL
D6950-02B	100 mL

3. Dilute HBC Buffer with isopropanol as follows and store at room temperature.

Kit	Isopropanol to be Added
D6948-00B	1.6 mL
D6948-01B	10 mL
D6948-02B	32 mL
Kit	Isopropanol to be Added
D6950-00B	1.6 mL
D6950-01B	10 mL
D6950-02B	32 mL

4. Check Solution II for precipitates before use. Redissolve any precipitates by warming to 37°C.

Storage and Stability

All of the E.Z.N.A.® Endo-Free Plasmid DNA Mini Kit components are guaranteed for at least 24 months from the date of purchase when stored as follows. Solution I (once RNase A is added) should be stored at 2-8°C. All other materials should be stored at room temperature. Solution II must be tightly capped when not in use.

Recommended Settings

Growth and Culture of Bacteria

Bacterial Strain Selection

It is strongly recommended that an end A negative strain of *E. coli* be used for routine plasmid isolation. Examples of such strains include DH5 α [™], DH1, and C600. These host strains yield high-quality DNA with E.Z.N.A.[®] Plasmid DNA Mini Kit Protocols. XL1-Blue, although a slower growing strain is also recommended due to its yield of high-quality DNA.

Host strains derivatives from HB101 such as TG1 and the JM100 series release large amounts of carbohydrates during lysis, which may inhibit enzyme activity when not completely removed. Some strains may also lower DNA quality due to having high levels of endonuclease activity, and therefore are not recommended (i.e. JM101, JM110, HB101). One may reduce the amount of culture volume or double the volumes of Solution I, Solution II, and Solution III, if problems are encountered with strains such as TG1 and Top10F.

Inoculation

Bacterial cultures for plasmid preparations should always be grown from a single colony picked from a freshly streaked plate. Subculturing directly from glycerol stock or liquid cultures may lead to uneven yields or plasmid loss. Optimal results are obtained by using one single isolated colony from a freshly transformed or freshly streaked plate to inoculate an appropriate volume of starter culture containing the appropriate antibiotic, and then incubated for 12-16 at 37°C with vigorous shaking (~300 rpm; shaking incubator).

Note: Aeration is very important. The culture volume should not exceed 1/4 the volume of the container.

Culture Media

The E.Z.N.A.[®] Endo-Free Plasmid DNA Mini Kit is specially designed for use with cultures grown in Luria Bertani (LB) medium. Richer broths such as TB (Terrific Broth) or 2 x YT lead to high cell densities that can overload the purification system, and therefore are not recommended. If rich media has to be used, growth times have to be optimized, and the recommended culture volumes must be reduced to match the capacity of the HiBind[®] DNA Mini Column.

Note: As culture ages, DNA yield may begin to decrease due to cell death and lysis within the culture.

Recommended Settings

Culture Volume and Cell Density

Do Not Exceed Maximum Recommended Culture Volumes

For optimal plasmid yields, the starting culture volume should be based on culture cell density. A bacterial density between 2.0 and 3.0 at OD₆₀₀ is recommended. When using nutrient-rich media, care should be taken to ensure that the cell density does not exceed an OD₆₀₀ of 3.0. Using a high-density culture outside of the recommended OD range may overload the purification system.

Yield and Quality of DNA

Determine the absorbance of an appropriate dilution (20- to 50-fold) of the sample at 260 nm and then at 280 nm. The DNA concentration is calculated as follows:

$$\text{DNA concentration} = \text{Absorbance 260} \times 50 \times (\text{Dilution Factor}) \mu\text{g/mL}$$

A ratio greater than 1.8 indicates greater than 90% nucleic acid. Alternatively, quantity (as well as quality) sometimes can be determined best by agarose gel/ethidium bromide electrophoresis by comparison to DNA samples of known concentrations. Typically, the majority of the DNA eluted is in monomeric supercoil form, though concatemers may also be present.

Low Copy-Number Plasmid and Cosmid DNA Purification

Low copy number plasmids generally give 0.1-1 μg DNA per mL overnight culture. For the isolation of plasmid DNA from low copy-number plasmids (0.1-1 $\mu\text{g/mL}$ culture) or low mini copy-number plasmid (1-2 $\mu\text{g/mL}$ culture) bacteria, use the following modified protocol.

1. Double the volume of starting culture from that of high copy number plasmids. Use up to 10 mL for Mini Kit I and 20-30 mL for Mini Kit II. Pellet the bacterial cells by centrifugation.
2. Perform resuspension, lysis and N3 Buffer steps by using double volumes of Solution I, Solution II, N3 Buffer, and ETR Binding Buffer. Additional buffer for Solution I, Solution II, N3 Buffer, and ETR Binding Buffer can be purchased separately.
3. Continue with each step of the standard protocol by following the wash, drying and elution steps. There is no need to increase the volumes of HBC Buffer, ETR Wash Buffer, DNA Wash Buffer, or Endotoxin-free Elution Buffer.

E.Z.N.A.[®] Endo-Free Plasmid DNA Mini Kit Protocols

E.Z.N.A.[®] Endo-Free Plasmid DNA Mini Kit I Protocol - Spin Protocol

All centrifugation should be performed at room temperature unless otherwise noted. For low copy number plasmids refer to Page 6. This protocol is designed to isolate plasmid DNA from *E. coli* grown in an overnight 1-5 mL LB culture.

Materials and Equipment to be Supplied by User:

- 100% ethanol
- Isopropanol
- Microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Ice bucket
- Culture tubes
- Optional: sterile deionized water
- Optional: water bath or incubator capable of 70°C
- Optional: 3M NaOH

Before Starting:

- Chill N3 Buffer on ice
- Heat Elution Buffer to 70°C if plasmid DNA is >10 kb
- Prepare DNA Wash Buffer, HBC Buffer, and Solution I according to the instructions in the Preparing Reagents section on Page 4

1. Isolate a single colony from a freshly streaked selective plate, and inoculate a culture of 1- 5 mL LB medium containing the appropriate selective antibiotic. Incubate for ~12-16 hr at 37°C with vigorous shaking (~300 rpm). Use a 10-20 mL culture tube or a flask with a volume of at least 4 times the volume of the culture. It is strongly recommended that *E. coli* be used for routine plasmid isolation. An OD₆₀₀ reading between 2.0 and 3.0 is an indication that bacterial cells are at an optimal density for harvesting, and plasmid DNA isolation
2. Centrifuge at 10,000 x *g* for 1 minute at room temperature.
3. Decant or aspirate and discard the culture media.

E.Z.N.A.[®] Endo-Free Plasmid DNA Mini Kit Protocols

4. Add 250 μ L Solution I/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields.

Note: RNase A must be added to Solution I before use. Please see the instructions in the Preparing Reagents section on Page 4.

5. Transfer suspension into a new 1.5 mL microcentrifuge tube (not provided).
6. Add 250 μ L Solution II. Invert and gently rotate the tube several times to obtain a clear lysate. A 2-3 minute incubation may be necessary.

Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution II tightly capped when not in use to avoid acidification from CO₂ in the air.

7. Add 125 μ L ice cold N3 Buffer. Gently invert several times until a flocculent white precipitate forms.
8. Centrifuge at maximum speed ($\geq 13,000 \times g$) for 10 minutes. A compact white pellet will form. Promptly proceed to the next step.
9. Transfer the cleared lysate to a new 1.5 mL microcentrifuge tube. Measure the volume of the cleared lysate transferred.
10. Add 1 volume ETR Binding Buffer. Invert the tube 10 times to mix thoroughly.

Note: If you transferred 500 μ L cleared lysate, then add 500 μ L ETR Binding Buffer.

11. Insert a HiBind[®] DNA Mini Column into a 2 mL Collection Tube.

Optional Protocol for Column Equilibration:

1. Add 100 μ L 3M NaOH to the HiBind[®] DNA Mini Column.
2. Centrifuge at maximum speed for 30-60 seconds.
3. Discard the filtrate and reuse the collection tube.

E.Z.N.A.[®] Endo-Free Plasmid DNA Mini Kit Protocols

12. Transfer 700 μ L cleared supernatant from Step 10 by CAREFULLY aspirating it into the HiBind[®] DNA Mini Column. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind[®] DNA Mini Column.
13. Centrifuge at maximum speed for 1 minute.
14. Discard the filtrate and reuse the collection tube.
15. Repeat Steps 12-14 until all of the cleared lysate has been transferred to the column.
16. Add 500 μ L ETR Wash Buffer.
17. Centrifuge at maximum speed for 1 minute.
18. Discard the filtrate and reuse the collection tube.
19. Add 500 μ L HBC Buffer.

Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 4 for instructions.
20. Centrifuge at maximum speed for 1 minute.
21. Discard the filtrate and reuse collection tube.
22. Add 700 μ L DNA Wash Buffer.

Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.
23. Centrifuge at maximum speed for 1 minute.
24. Discard the filtrate and reuse the collection tube.

E.Z.N.A.® Endo-Free Plasmid DNA Mini Kit Protocols

25. Repeat Steps 22-24 for a second DNA Wash Buffer wash step.
26. Centrifuge the empty HiBind® DNA Mini Column for 2 minutes at maximum speed to dry the column matrix.

Note: It is important to dry the HiBind® DNA Mini Column matrix before elution. Residual ethanol may interfere with downstream applications.

27. Transfer the HiBind® DNA Mini Column to a clean 1.5 mL microcentrifuge tube.
28. Add 30-100 μ L Elution Buffer or sterile deionized water directly to the center of the column membrane.

Note: The efficiency of eluting DNA from the HiBind® DNA Mini Column is dependent on pH. If using sterile deionized water, make sure that the pH is around 8.5.

29. Let sit at room temperature for 1 minute.
30. Centrifuge at maximum speed for 1 minute.

Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

31. Store DNA at -20°C.

E.Z.N.A.[®] Endo-Free Plasmid DNA Mini Kit Protocols

E.Z.N.A.[®] Endo-Free Plasmid DNA Mini Kit II Protocol - Spin Protocol

For the isolation of 40-70 µg of high copy plasmid DNA from a 10-15 mL culture. All centrifugation should be performed at room temperature unless otherwise noted. For low copy number plasmids refer to Page 6. This protocol is designed to isolate plasmid DNA from *E. coli* grown in an overnight 1-5 mL LB culture.

Materials and Equipment to be Supplied by User:

- 100% ethanol
- Isopropanol
- Centrifuge capable of at least 5,000 x *g* with swing buckets
- Microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Ice bucket
- Culture tubes
- Optional: sterile deionized water
- Optional: water bath or incubator capable of 70°C
- Optional: 3M NaOH

Before Starting:

- Chill N3 Buffer on ice
- Heat Elution Buffer to 70°C if plasmid DNA is >10 kb
- Prepare DNA Wash Buffer, HBC Buffer, and Solution I according to the instructions in the Preparing Reagents section on Page 4

1. Isolate a single colony from a freshly streaked selective plate, and inoculate a culture of 10- 15 mL LB medium containing the appropriate selective antibiotic. Incubate for ~12-16 hr at 37°C with vigorous shaking (~300 rpm). Use a 50 mL culture tube or a flask with a volume of at least 4 times the volume of the culture. It is strongly recommended that *E. coli* be used for routine plasmid isolation. An OD₆₀₀ reading between 2.0 and 3.0 is an indication that bacterial cells are at an optimal density for harvesting, and plasmid DNA isolation
2. Centrifugation at 5,000 x *g* for 10 minutes at room temperature.
3. Decant or aspirate the medium and discard.

E.Z.N.A.[®] Endo-Free Plasmid DNA Mini Kit Protocols

4. Add 500 μL Solution I/RNase A. Vortex or pipet up and down to mix thoroughly. Complete resuspension of cell pellet is vital for obtaining good yields.

Note: RNase A must be added to Solution I before use. Please see the instructions in the Preparing Reagents section on Page 4.

5. Transfer suspension into a new 2 mL microcentrifuge tube.
6. Add 500 μL Solution II. Invert and gently rotate the tube several times to obtain a clear lysate. A 2-3 minute incubation may be necessary.

Note: Avoid vigorous mixing as this will shear chromosomal DNA and lower plasmid purity. Do not allow the lysis reaction to proceed more than 5 minutes. Store Solution II tightly capped when not in use to avoid acidification from CO_2 in the air.

7. Add 250 μL ice cold N3 Buffer. Gently invert several times until a flocculent white precipitate forms.
8. Centrifuge at maximum speed ($\geq 13,000 \times g$) for 10 minutes. A compact white pellet will form. Promptly proceed to the next step.
9. Transfer the cleared lysate to a new 1.5 mL microcentrifuge tube. Measure the volume of the cleared lysate transferred.
10. Add 1 volume ETR Binding Buffer. Invert the tube 10 times to mix thoroughly.

Note: If you transferred 500 μL cleared lysate, then add 500 μL ETR Binding Buffer.

11. Insert a HiBind[®] DNA Mini Column II into a 2 mL Collection Tube.

Optional Protocol for Column Equilibration:

1. Add 100 μL 3M NaOH to the HiBind[®] DNA Mini Column.
2. Centrifuge at maximum speed for 30-60 seconds.
3. Discard the filtrate and reuse the collection tube.

E.Z.N.A.[®] Endo-Free Plasmid DNA Mini Kit Protocols

12. Transfer 700 μ L cleared supernatant from Step 10 by CAREFULLY aspirating it into the HiBind[®] DNA Mini Column II. Be careful not to disturb the pellet and that no cellular debris is transferred to the HiBind[®] DNA Mini Column II.
13. Centrifuge at maximum speed for 1 minute.
14. Discard the filtrate and reuse the collection tube.
15. Repeat Steps 12-14 until all of the cleared lysate has been transferred to the column.
16. Add 500 μ L ETR Wash Buffer.
17. Centrifuge at maximum speed for 1 minute.
18. Discard the filtrate and reuse the collection tube.
19. Add 500 μ L HBC Buffer.

Note: HBC Buffer must be diluted with isopropanol before use. Please see Page 4 for instructions.
20. Centrifuge at maximum speed for 1 minute.
21. Discard the filtrate and reuse collection tube.
22. Add 700 μ L DNA Wash Buffer.

Note: DNA Wash Buffer must be diluted with 100% ethanol prior to use. Please see Page 4 for instructions.
23. Centrifuge at maximum speed for 1 minute.
24. Discard the filtrate and reuse the collection tube.

E.Z.N.A.® Endo-Free Plasmid DNA Mini Kit Protocols

25. Repeat Steps 22-24 for a second DNA Wash Buffer wash step.
26. Centrifuge the empty HiBind® DNA Mini Column II for 2 minutes at maximum speed to dry the column matrix.

Note: It is important to dry the HiBind® DNA Mini Column II matrix before elution. Residual ethanol may interfere with downstream applications.

27. Transfer the HiBind® DNA Mini Column II to a clean 1.5 mL microcentrifuge tube.
28. Add 60-100 μ L Elution Buffer or sterile deionized water directly to the center of the column membrane.

Note: The efficiency of eluting DNA from the HiBind® DNA Mini Column II is dependent on pH. If using sterile deionized water, make sure that the pH is around 8.5.

29. Let sit at room temperature for 1 minute.
30. Centrifuge at maximum speed for 1 minute.

Note: This represents approximately 70% of bound DNA. An optional second elution will yield any residual DNA, though at a lower concentration.

31. Store DNA at -20°C.

E.Z.N.A.® Endo-Free Plasmid DNA Mini Kit Protocols

E.Z.N.A.® Endo-Free Plasmid DNA Mini Kit Protocol - Purification of plasmid DNA prepared by other methods

Materials and Equipment to be Supplied by User:

- 100% ethanol
- Isopropanol
- Microcentrifuge capable of at least 13,000 x *g*
- Nuclease-free 1.5 mL or 2 mL microcentrifuge tubes
- Ice bucket
- Optional: sterile deionized water
- Optional: water bath or incubator capable of 70°C

Before Starting:

- Chill N3 Buffer on ice
 - Heat Elution Buffer to 70°C if plasmid DNA is >10 kb
 - Prepare DNA Wash Buffer according to the instructions in the Preparing Reagents section on Page 4
1. Bring the volume of plasmid DNA to 300 μ L with nuclease-free water.
 2. Add 1 volume ETR Binding Buffer. Vortex to mix thoroughly.
 3. Transfer the sample to the HiBind® DNA Mini Column.
 4. Centrifuge at maximum speed for 1 minute.
 5. Continue to Steps 22-31 on Pages 9-10.

Troubleshooting Guide

Please use this guide to troubleshoot any problems that may arise. For further assistance, please contact the technical support staff, toll free, at **800-832-8896**.

Possible Problems and Suggestions

Low DNA yields	
Poor cell lysis	Reduce the initial volume of culture or increase the lysis time while monitoring the lysis visually.
	Cells may not have been dispersed adequately prior to the addition of Solution II. Vortex to completely resuspend the cells.
	Increase Solution II incubation time to obtain a clear lysate.
	Solution II, if not tightly closed, may need to be replaced.
Culture is overgrown or not fresh	Do not incubate cultures for more than 16 hours at 37°C. Storage of cultures for extended periods prior to plasmid isolation is detrimental.
Low elution efficiency	The pH of Elution Buffer or water must be pH 8.0-9.0.
Low copy number plasmid used	Such plasmids may yield as little as 0.1 µg DNA from a 1 mL overnight culture. Double the culture volume and follow the low copy number plasmid protocol.
Alkaline lysis is prolonged	Reduce the lysis time (Solution II) to 3 minutes or until the suspended cells form a clear viscous solution.
Too many or too few cells were used	Confirm the cell density by measuring OD ₆₀₀ . To calculate the volume of culture to use, take the desired cell mass and divide by the absorbance of the overnight culture at 600 nm.
No DNA eluted	
DNA Wash Buffer not diluted with ethanol	Prepare DNA Wash Buffer according to instructions on Page 4.
HBC Buffer not diluted with isopropanol	Prepare HBC Buffer according to instructions on Page 4.

Troubleshooting Guide

High molecular weight DNA contamination of product	
Over mixing of cell lysate upon addition of Solution II	Do not vortex or mix aggressively after adding Solution II.
Culture overgrown	Overgrown cultures contain lysed cells and degraded DNA. Do not grow cell longer than 16 hours.
Plasmid DNA floats out of well while loading agarose gel	
Ethanol was not completely removed from column following wash steps	Centrifuge column as instructed to dry the column before elution.
Absorbance of purified DNA does not accurately reflect quantity of the plasmid (A_{260}/A_{280} ratio is high or low)	
DNA Wash Buffer is diluted with ethanol containing impurities	Check the absorbance of the ethanol between 250 nm and 300 nm. Do not use ethanol with high absorbance. Traces of impurities may remain on the binding column after washing and contribute to the absorbance in the final product.
Plasmid DNA is contaminated with RNA; RNase A treatment is insufficient	Confirm that the RNase A Solution was added to Solution I prior to first use. The RNase A solution may degrade due to high temperatures (>65 °C) or prolonged storage (> 6 months at room temperature).
Background reading is high due to silica fine particulates	Spin the DNA sample at maximum speed for 1 minute; use the supernatant to repeat the absorbance readings.
Purification is incomplete due to column overloading	Reduce the initial volume of culture.
Plasmid DNA is contaminated with chromosomal DNA	Do not use cultures that have grown for more than 24 hours or are in the cell death phase. Do not vortex or vigorously shake the cells during the lysis reaction or N3 procedure.

Ordering Information

The following components are available for purchase separately.
(Call Toll Free at 1-800-832-8896)

Product	Part Number
Solution I (250 mL)	PS001
Solution II (250 mL)	PS002
DNA Wash Buffer (40 mL)	PDR044

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PCR is a patented process of Hoffman-La Roche. Use of the PCR process requires a license.

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