ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZymoPURE™ Plasmid Maxiprep Kit

Catalog Nos. **D4202 & D4203** (Patent Pending)

Highlights

- The fastest spin-column based procedure for purifying up to 1.2 mg of ultra-pure endotoxin-free transfection-grade plasmid DNA.
- Innovative ZymoPURE™ binding technology enables elution of the highest concentration of plasmid DNA directly from a spin-column using a microcentrifuge.
- Routinely recover ≥ 1 µg/µl plasmid DNA that is ideal for transfection and other sensitive downstream applications.

Contents

ZYMO RESEARCH CORP.

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Satisfaction of all Zymo Research products is guaranteed. If you are dissatisfied with this product, please call 1-888-882-9682.

Product Contents:

ZymoPURE™ Plasmid Maxiprep (Kit Size)	D4202 (10 preps.)	D4203 (20 preps.)	Storage Temperature
ZymoPURE™ P1[1] (Red)	150 ml	2x 150 ml	4°C
ZymoPURE™ P2[2] (Green)	150 ml	2x 150 ml	Room Temp.
ZymoPURE™ P3 (Yellow)	150 ml	2x 150 ml	Room Temp.
ZymoPURE™ Binding Buffer	150 ml	2x 150 ml	Room Temp.
ZymoPURE™ Wash 1	55 ml	2x 55 ml	Room Temp.
ZymoPURE™ Wash 2 (Concentrate)	23 ml	2x 23 ml	Room Temp.
ZymoPURE™ Elution Buffer	6 ml	12 ml	Room Temp.
Zymo-Spin™ V-P Column Assemblies[3]	10	20	Room Temp.
ZymoPURE™ Syringe Filters	10	20	Room Temp.
ZymoPURE™ Syringe Plungers	10	20	Room Temp.
Collection Tubes	10	20	Room Temp.
Instruction Manual	1	1	-

Note - Integrity of kit components is guaranteed for up to one year from date of purchase. Reagents are routinely tested on a lot-to-lot basis to ensure they provide maximal performance and reliability.

[1] ZymoPURE™ P1 contains RNase A (200 ng/µl) and is stable at room temperature without loss in RNase activity, however, for long-term storage the product should be stored at 4-8° C.

[2] Caution: ZymoPURE™ P2 Buffer contains NaOH. Please use proper safety precautions.

[3] The Zymo-Spin™ V-P, 15 ml Conical Reservoir and 50 ml Reservoir are pre-assembled as a single unit.

Specifications:

- **DNA Purity:** Eluted DNA is ultrapure and well suited for transfection, transformation, sequencing, restriction endonuclease digestion, *in vitro* transcription, and other sensitive applications.
    - Typical $Abs_{260/280} \geq 1.8$ and $Abs_{260/230} \geq 2.0$
    - Endotoxin levels are typically ≤ 1 EU / µg DNA
- **Plasmid DNA Yield:** Up to 1.2 mg per preparation *(Actual yield is dependent on the plasmid copy number, culture growth conditions, and strain of E. coli utilized)*
- **Plasmid DNA Size:** Up to 25 kb
- **Recovery Volume:** ≥ 200 µl of ZymoPURE™ Elution Buffer or DNase free water
- **Required Equipment:** Microcentrifuge and vacuum/vacuum manifold (recommended) or swinging bucket centrifuge.
- **Processing Time:** 18 min

Notes:

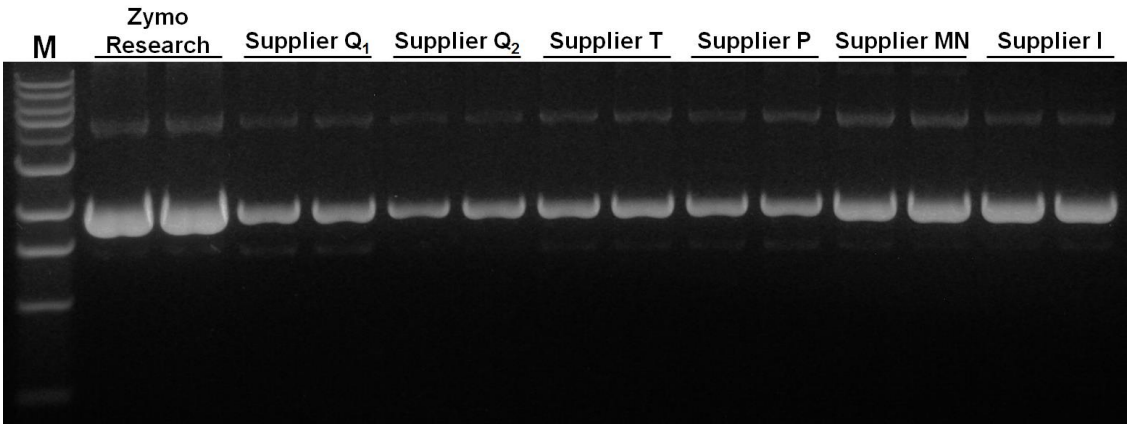
This product is for research use only and should only be used by trained professionals. It is not for use in diagnostic procedures. Some reagents included with this kit are irritants. Wear protective gloves and eye protection. Follow the safety guidelines and rules enacted by your research institution or facility.

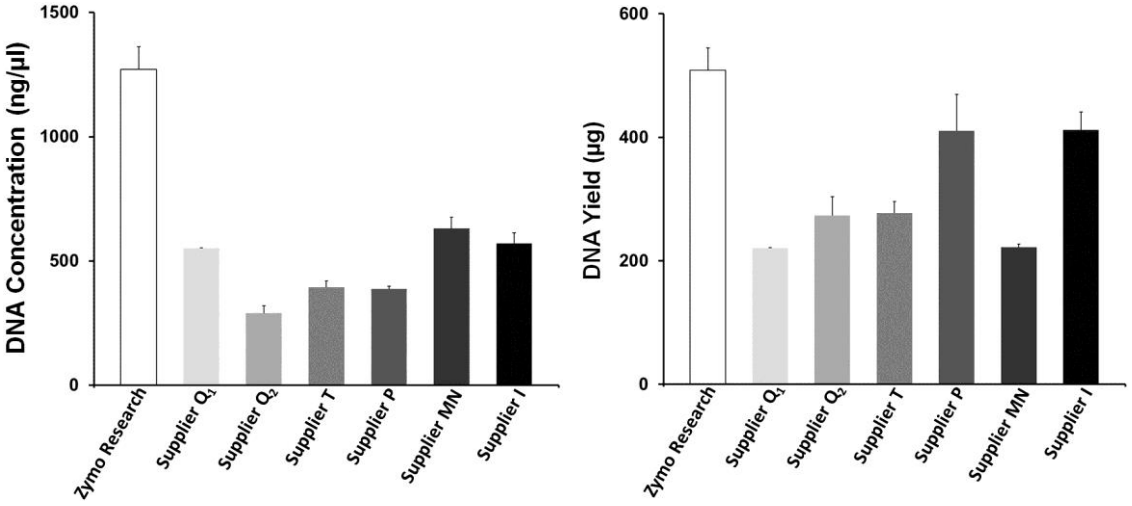
Product Description

The **ZymoPURE™ Plasmid Maxiprep** kit features a spin column-based method for the purification of up to 1.2 mg of high-quality plasmid DNA in less than 20 minutes. The eluted plasmid DNA is ready for immediate use, avoiding the need for subsequent precipitation steps.

ZymoPURE™ technology uses a modified alkaline lysis method and features novel binding chemistry that yields highly concentrated plasmid DNA (up to 2.5 µg/µl). In addition, the wash regimen has been optimized to ensure the plasmid DNA is free of endotoxins, salt, protein, and RNA. The result is plasmid DNA suitable for transfection, restriction endonuclease digestion, bacterial transformation, PCR amplification, DNA sequencing, and other sensitive downstream applications.

As an added convenience, the **ZymoPURE™ Plasmid Maxiprep** contains colored buffers that permit error-free visualization and identification of complete bacterial cell lysis and neutralization.

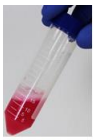




Plasmid DNA yield and concentration from the ZymoPure™ Maxiprep kit compared to other major suppliers. Plasmid DNA (pGEM®) was isolated from 150 ml of JM109 *E. coli* culture grown overnight following the manufacturer's suggested protocol (in duplicate). One (1) µl of eluted plasmid DNA was visualized post agarose gel electrophoresis. M, ZR 1 kb DNA Marker (Zymo Research).

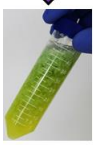
For **Technical Assistance**, please contact **Zymo** at 1-888-882-9682 or E-mail tech@zymoresearch.com.

Procedure Overview:



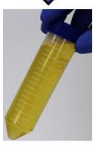
Bacterial cells are resuspended in **ZymoPURE™ P1** (red).

The solution will turn dark purple and viscous following the addition of **ZymoPURE™ P2** (green) indicating bacterial lysis is complete.



The solution will turn yellow and a precipitate will form after adding **ZymoPURE™ P3** (yellow) indicating neutralization is complete.

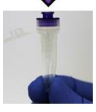
The neutralized lysate is loaded into the **ZymoPURE™ Syringe Filter** and clarified into a new 50 ml conical tube.



ZymoPURE™ Binding Buffer is added to the cleared lysate and mixed thoroughly.

The mixture is loaded into the **Zymo-Spin™ V-P Column** using a vacuum manifold.

The **50 ml Reservoir** is removed and the **Zymo-Spin™ V-P Column** is washed using a vacuum manifold.



Ultra-pure plasmid DNA is eluted from the **Zymo-Spin™ V-P Column** using a microcentrifuge.

Buffer Preparation:

- ✓ Add 88 ml of 95% ethanol to the 23 ml **ZymoPURE™ Wash 2 (Concentrate)** (D4202 & D4203) before use.
- ✓ The **ZymoPURE™ P2** and **ZymoPURE™ Binding Buffer** may have precipitated. If this occurs, dissolve the precipitate by incubating the bottles at 30-37 ºC for 10-20 minutes and mix by inversion. Do not microwave!

Protocol:

The following procedure should be performed at room temperature (15-30°C).

1. Centrifuge up to 150 ml of bacterial culture at ≥ 3,400 x *g* for 10 minutes to pellet the cells[1]. Discard supernatant.
2. Add 14 ml of **ZymoPURE™ P1 (Red)** to the bacterial cell pellet and resuspend completely by vortexing or pipetting.
3. Add 14 ml of **ZymoPURE™ P2 (Green)** and immediately mix by gently inverting the tube 6 times. Do not vortex! Let sit at room temperature for 2-3 minutes[2]. *Cells are completely lysed when the solution appears clear, purple, and viscous.*
4. Add 14 ml of **ZymoPURE™ P3 (Yellow)** and mix gently but thoroughly by inversion. Do not vortex! *The sample will turn yellow when the neutralization is complete and a yellowish precipitate will form.*
5. Ensure the plug is attached to the Luer Lock at the bottom of the **ZymoPURE™ Syringe Filter.** Place the syringe filter upright in a tube rack and load the lysate into the ZymoPURE™ Syringe Filter[3] and wait 8 minutes for the precipitate to float to the top.
6. Remove the Luer Lock plug from the bottom of the syringe and place it into a clean 50 ml conical tube. Place the plunger in the syringe and push the solution through the ZymoPURE™ Syringe Filter to clear the debris[4]. Save the cleared lysate!
7. Add 14 ml **ZymoPURE™ Binding Buffer** to the cleared lysate from step 6 and mix thoroughly by inverting the capped tube 10 times.

   To continue processing the lysate using the recommended vacuum protocol, proceed to the next page. If a vacuum is not available, proceed to page 6 for an alternative centrifugation method.

Notes:

[1] A vessel with a minimum volume of 50 ml is required to prepare the bacterial lysate.

[2] Do not allow the lysis reaction to proceed for more than 3 minutes. Excessive lysis can result in denatured plasmid DNA.

[3] If the precipitate has formed a homogenous layer at the surface of the neutralized lysate then invert the tube 3-4 times prior to loading the lysate into the **Zymo PURE™ Syringe Filter**.

[4] Approximately 33-35 ml of cleared lysate will be recovered from the **ZymoPURE™ Syringe Filter**.

Notes:

[1] To achieve optimal performance, the vacuum pump should be able to apply at least 400 mm Hg pressure. If less pressure is applied, centrifuge the column prior to washing to remove any residual lysate remaining in the matrix.

[2] The **ZymoPURE™ Elution Buffer** contains 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If required, pure water can also be used to elute the DNA.

[3] The DNA yield can be increased by pre-warming the **ZymoPURE™ Elution Buffer** to 50 ºC and/or increasing the incubation period up to 5 minutes prior to centrifugation.

[4] For low copy number plasmids or if higher concentration is desired, the plasmid DNA can be eluted in as little as 200 µl.

Vacuum Protocol: *(Recommended)*

This product is compatible with any conventional vacuum-based manifold. The vacuum pump should be a single or double-staged unit capable of producing up to 400 mm Hg pressure at the vacuum manifold[1].

8. Ensure the connections of the **Zymo-Spin™ V-P Column Assembly** are finger-tight and place onto a vacuum manifold. (If vacuum is not available, see page 6 for the centrifugation protocol.)

9. Add the entire mixture from step 7 into the Zymo-Spin™ V-P Column Assembly, and then turn on the vacuum[1] until all of the liquid has passed completely through the column.

10. Remove and discard the **50 ml Reservoir** from the top of the Zymo-Spin™ V-P Column Assembly.

11. With the vacuum off, add 5 ml of **ZymoPURE™ Wash 1** to the **15 ml Conical Reservoir.** Turn on the vacuum until all of the liquid has passed completely through the column.

12. With the vacuum off, add 5 ml of **ZymoPURE™ Wash 2** to the 15 ml Conical Reservoir. Turn on the vacuum until all of the liquid has passed completely through the column. Repeat this wash step.

13. Remove and discard the 15 ml Conical Reservoir and place the **Zymo-Spin™ V-P Column** in a **Collection Tube**. Centrifuge at ≥ 10,000 x *g* for 1 minute, in a microcentrifuge, to remove any residual wash buffer.

14. Transfer the column into a clean 1.5 ml tube and add 400 µl of **ZymoPURE™ Elution Buffer**[2,3,4] directly to the column matrix. Incubate at room temperature for 2 minutes, and then centrifuge at ≥ 10,000 x *g* for 1 minute in a microcentrifuge. Store the eluted plasmid DNA at ≤ -20°C.

Centrifugation Protocol: *(Alternative)*

Perform steps 1-7 as indicated in the general protocol, see page 4.

8. Remove the **50 ml Reservoir** from the top of the **Zymo-Spin™ V-P Column Assembly**. Ensure the connection between the **15 ml Conical Reservoir** and **Zymo- Spin™ V-P column** is finger-tight and place the assembly into a 50 ml conical tube.

9. Add 14 ml of the mixture from step 7 into the **15 ml Conical Reservoir/Zymo-Spin™ V-P column assembly,** and then centrifuge the column at 500 x *g* for 2 minutes. Empty the 50 ml conical tube and repeat this step until the entire sample has passed through the column.

10. Add 5 ml of **ZymoPURE™ Wash 1** to the Zymo-Spin™ V-P column assembly and centrifuge the column at 500 x *g* for 2 minutes.

11. Add 5 ml of **ZymoPURE™ Wash 2** to the Zymo-Spin™ V-P column assembly and centrifuge the column for 2 minutes at 500 x *g*. Repeat the wash step.

12. Remove and discard the 15 ml Conical Reservoir and place the Zymo-Spin™ V-P Column into a **Collection Tube**. Centrifuge the column at ≥ 10,000 x *g* for 1 minute, in a microcentrifuge, to remove any residual wash buffer.

13. Transfer the column into a clean 1.5 ml tube and add 400 µl of **ZymoPURE™ Elution Buffer**[1,2,3] directly to the column matrix. Incubate at room temperature for 2 minutes, and then centrifuge at ≥ 10,000 x *g* for 1 minute in a microcentrifuge. Store the eluted plasmid DNA at ≤ -20°C.

Notes:

[1] The **Zymo PURE™ Elution Buffer** contains 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA. If required, pure water can also be used to elute the DNA.

[2] The DNA yield can be increased by pre-warming the **Zymo PURE™ Elution Buffer** to 50 ºC and/or increasing the incubation period up to 10 minutes prior to centrifugation.

[3] For low copy number plasmids or if higher concentration is desired, the plasmid DNA can be eluted in as little as 200 µl.

Troubleshooting Guide:

Problem	Possible Causes and Suggested Solutions
Low DNA Yield	
Culture growth conditions	• **Poor aeration of culture.** The optimal culture volume to air volume ratio is 1:5 or less. For best aeration, use baffled culture flasks, or a vented or gas-permeable seal on the culture vessel. • **The culture was overgrown, undergrown or contaminated.** Use a fresh culture for optimal performance. (An A_{600} of 0.2-0.35 is the optimal optical density of a tenfold dilution of the culture.) • **Antibiotics were omitted from the growth medium.**
Cell density is too high	• **Too much culture used.** Lysis and neutralization will be incomplete and the Zymo PURE™ Syringe Filter may clog during filtration. More culture does not always equal more plasmid. Incomplete lysis and neutralization are two of the most common causes of failed plasmid preps and both are caused by too much culture being used. • **Incomplete lysis**: After addition of ZymoPURE™ P2, the solution should change from opaque pink to a clear viscous purple, indicating complete lysis. Different *E. coli* strains often require different growth conditions and may vary in their susceptibility to alkaline lysis. • **Incomplete neutralization:** The solution should not be viscous following neutralization and the yellowish precipitate should appear fluffy and readily float to the surface. Make sure the neutralization is complete prior to filtration. Invert the tube an additional 2-3 times after the sample turns yellow following the addition of ZymoPURE™ P3.
ZymoPURE P2 and ZymoPURE Binding Buffer precipitated	• **Both buffers may have precipitated during shipping.** To completely resuspend the buffers, incubate the bottles at 30-37 ºC for 10 minutes and mix by inversion. DO NOT MICROWAVE.
Wash buffer	• **Ensure that ethanol has been added** to the ZymoPURE™ Wash 2. • **Ensure that the bottle cap is screwed on tightly** after each use to prevent evaporation of the ethanol.
DNA elution	• **Incomplete elution:** For large size plasmids (> 10 kb), add ZymoPURE™ Elution Buffer and incubate the column for 5-10 minutes before centrifugation. Also, pre-warm the ZymoPURE™ Elution Buffer to 50 ºC prior to elution.
Low DNA Quality	
DNA does not perform well	• **Incomplete neutralization:** Incomplete neutralization generates poor quality supernatant. Ensure that neutralization is complete by inverting the sample an additional 2-3 times after the addition of ZymoPURE™ P3 and extending the incubation. • **Ethanol contamination in eluate.** Centrifuge the Zymo-Spin™ V-P column matrix to dryness as indicated in the protocol prior to adding the ZymoPURE™ Elution Buffer.
RNA in eluate	• Ensure that ZymoPURE™ P1 has been stored at 4°C. RNase A can be purchased separately if necessary.
Genomic DNA in eluate	• **Improper handling** (Sample was vortexed or handled too roughly). Genomic DNA contamination is usually caused by excessive mechanical shearing during the lysis and neutralization steps. Also, prolonged lysis or incomplete mixing of lysis or neutralization buffers may contribute to genomic DNA contamination in your sample. • **Overgrown culture.** Overgrown or old cultures may contain more genomic DNA contamination than fresh cultures.

Ordering Information

Product Description	Kit Size	Catalog No.
ZymoPURE™ Plasmid Maxiprep Kit	10 preps.	D4202
ZymoPURE™ Plasmid Maxiprep Kit	20 preps.	D4203

For Individual Sale	Amount	Catalog No.
ZymoPURE™ P1 (Red)	150 ml 210 ml 410 ml	D4200-1-150 D4200-1-210 D4200-1-410
ZymoPURE™ P2 (Green)	150 ml 210 ml 410 ml	D4200-2-150 D4200-2-210 D4200-2-410
ZymoPURE™ P3 (Yellow)	150 ml 210 ml 410 ml	D4200-3-150 D4200-3-210 D4200-3-410
ZymoPURE™ Binding Buffer	150 ml 210 ml 410 ml	D4200-4-150 D4200-4-210 D4200-4-410
ZymoPURE™ Wash 1	20 ml 55 ml 410 ml	D4200-5-20 D4200-5-55 D4200-5-410
ZymoPURE™ Wash 2 (Concentrate)	10 ml 23 ml	D4200-6-10 D4200-6-23
ZymoPURE™ Elution Buffer	6 ml 12 ml 30 ml	D4200-7-6 D4200-7-12 D4200-7-30
Zymo-Spin™ V-P Column Assemby w/ 15 ml Conical and 50 ml Reservoir	5	C1042-5
15 ml Conical Reservoir	25	C1031-25
50 ml Reservoir	25	C1032-25
ZymoPURE™ Syringe Filter	5	C1036-5
ZymoPURE™ Syringe Plunger	5	C1037-5
Collection Tubes	50 500 1000	C1001-50 C1001-500 C1001-1000

DNA PURIFICATION

What is Clean-Spin™ Technology?

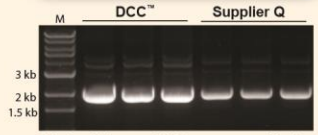
The spin columns from Zymo Research have been designed to ensure complete elution with no binding/wash buffer carryover. The result is ultra-pure inhibitor-free DNA and RNA.

Purify DNA from PCR & other sources

DNA Clean & Concentrator™ (DCC™)

- ✓ Recovery of ultra-pure DNA that is free of salts and contaminants.
- ✓ Small (≥6 µl) elution volume.
- ✓ DNA is ideal for ligation, PCR, Next-Gen sequencing, etc.

Product	Size (Cat. No.)
DNA Clean & Concentrator™-5	50 Preps. (D4013) 200 Preps. (D4014)
ZR-96 DNA Clean & Concentrator™-5	2 x 96 Preps. (D4023) 4 x 96 Preps. (D4024)
Genomic DNA Clean & Concentrator™	25 Preps. (D4010) 100 Preps. (D4011)



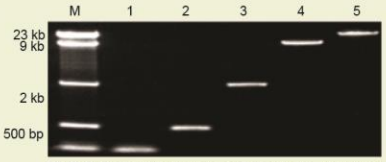
High efficiency DNA recovery with the DCC™-5 compared to Supplier Q .

Boost DNA recoveries from agarose gels to >80%

Zymoclean™ Gel DNA Recovery

- ✓ Rapid (15 min.) recovery of ultra-pure DNA from agarose gels in ≥6 µl.
- ✓ Ultra-pure DNA ideal for DNA ligation, sequencing, etc.
- ✓ Format also available for large DNA >20 kb.

Product	Size (Cat. No.)
Zymoclean™ Gel DNA Recovery Kit	50 Preps. (D4001) 200 Preps. (D4002)
Zymoclean™ Large Fragment DNA Recovery Kit	25 Preps. (D4045) 100 Preps. (D4046)



DNA fragments recovered from an agarose gel using the Zymoclean™ Gel DNA Recovery Kit. Lanes: M: DNA Ladder; 1-5: individual ladder DNA fragments.

Recover transfection-quality plasmid DNA directly from culture

Zyppy™ Plasmid Prep Kits

- ✓ The fastest, simplest method available for purifying high quality plasmid DNA from *E. coli.*
- ✓ Pellet-Free™ procedure omits conventional cell-pelleting and resuspension steps.
- ✓ Transfection quality plasmid DNA directly from culture in under 15 minutes.

Easy, Pellet-free Procedure: Add Lysis Buffer ***Directly*** to Bacterial Culture

Product	Size (Cat. No.)
Zyppy™ Plasmid Miniprep Kit	50 Preps. (D4036) 100 Preps. (D4019) 400 Preps. (D4020) 800 Preps. (D4037)

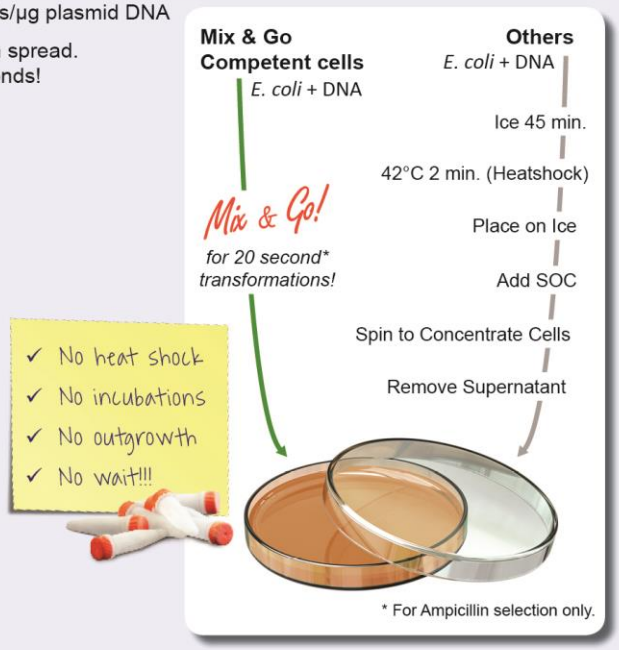
OTHER INNOVATIVE PRODUCTS FROM ZYMO RESEARCH...

Competent cells for transformations *without* heat shock!

Mix & Go! Pre-made Competent *E. Coli*

- ✓ High efficiency: 10^8-10^9 transformants/µg plasmid DNA
- ✓ Just *Mix & Go!* Simply add DNA then spread. Transformation in as little as 20 seconds!

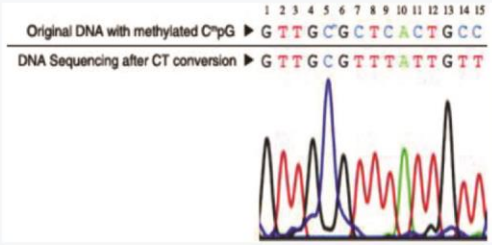
Product	Size (Cat. No.)
Zymo 5α (Same as DH5α)	10 x 100 µl aliquots (T3007) 96 x 50 µl aliquots (T3009) 96 x 50 µl aliquots PCR-plate (T3010)
Zymo 10B (Same as DH10B)	10 x 100 µl aliquots (T3019) 96 x 50 µl aliquots (T3020)
JM109	10 x 100 µl aliquots (T3003) 96 x 50 µl aliquots (T3005)
HB101	10 x 100 µl aliquots (T3011) 96 x 50 µl aliquots (T3013)
C600	10 x 100 µl aliquots (T3015)
TG1	10 x 100 µl aliquots (T3017)



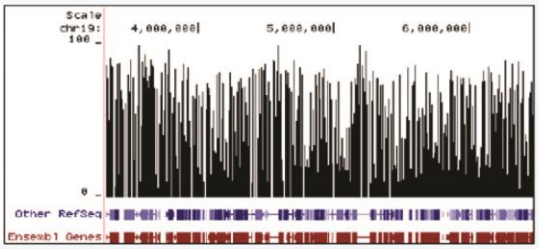
The fastest method for complete bisulfite conversion of DNA

EZ DNA Methylation-Lightning™ Kits

- ✓ The next generation of bisulfite conversion technology by the most cited provider in the industry
- ✓ Guarantees high conversion efficiencies of cytosine (>99.5%)
- ✓ Maintains the highest template integrity following bisulfite conversion
- ✓ Recovered DNA is ideal for PCR, MSP, array, bisulfite, and next-generation sequencing.



DNA Sequencing Results Following Bisulfite Treatment



Methylation Plot From Reduced Representation Bisulfite Sequencing (RRBS)

Product		Size (Cat. No.)
EZ DNA Methylation-Lightning™ Kit		50 rxns. (D5030) 200 rxns. (D5031)
EZ-96 DNA Methylation-Lightning™ Kit	Shallow-Well Deep-Well	2 x 96 rxns. (D5032) 2 x 96 rxns. (D5033)
EZ-96 DNA Methylation-Lightning™ MagPrep		4 x 96 rxns. (D5046) 8 x 96 rxns. (D5047)

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