ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

ZR-96 DNA Clean & Concentrator™-5

Catalog Nos. **D4023 & D4024**

Highlights

- Quick, high throughput recovery of ultra-pure DNA from PCR, enzymatic reactions, and other sources.
- DNA can be eluted in as little as 10 µl and is ideal for DNA ligation, sequencing, labeling, PCR, microarray, transfection, transformation, restriction digestion, etc.

Contents

Product Contents……. 1
Specifications……..…1
Product Description……...2-3
Buffer Preparation…….....4
Protocol…… 4
Appendices
ssDNA Purification…….…..... 5
Troubleshooting……6
Ordering Information……7
List of Related Products……..…...8-10

ZYMO RESEARCH CORP.

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

Product Contents

ZR-96 DNA Clean & Concentrator™-5 (Kit Size)	D4023 (2 x 96 Preps.)	D4024 (4 x 96 Preps.)	Storage Temperature
DNA Binding Buffer	100 ml	2 x100 ml	Room Temp.
DNA Wash Buffer*	24 ml	48 ml	Room Temp.
DNA Elution Buffer	10 ml	16 ml	Room Temp.
Zymo-Spin™ I-96 Plate	2	4	Room Temp.
Collection Plate	2	4	Room Temp.
Elution Plate	2	4	Room Temp.
Instruction Manual	1	1	-

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 the highest performance and reliability.

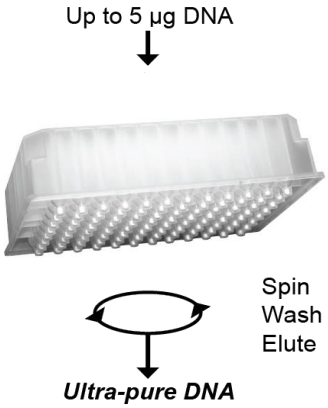
[1] Ethanol must be added prior to use as indicated on the **DNA Wash Buffer** label.

Specifications

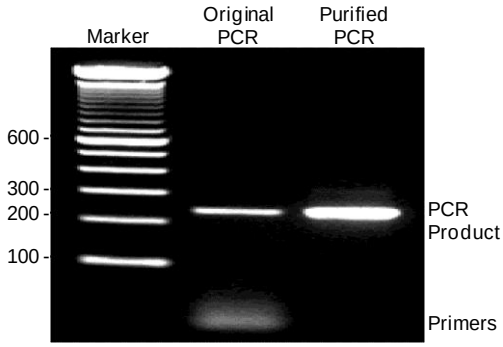
- **DNA Purity** – High-quality DNA (A_{260}/A_{280} >1.8) ideal for ligation, sequencing, labeling, PCR, microarray, transfection, transformation, and restriction digestion procedures.
- **DNA Size Limits** – From ~50 bp to 23 kb.
- **DNA Recovery** – Typically, up to 5 µg total DNA per well can be eluted into as little as 10 µl of low salt **DNA Elution Buffer** or water. For DNA 50 bp to 10 kb, the recovery is 70-90%. For DNA 11 kb to 23 kb, the recovery is 50-70%.
- **Sample Sources** – DNA from enzymatic reactions (e.g., PCR, restriction endonuclease digestions), plasmid preparations, and impure preparations.
- **Product Detergent Tolerance** – ≤ 5% Triton X-100, ≤ 5% Tween-20, ≤ 5% Sarkosyl, ≤ 0.1% SDS.

Product Description

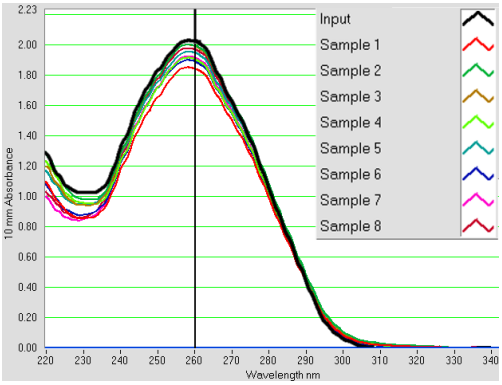
The **ZR-96 DNA Clean & Concentrator™-5** (**ZR-96 DCC™-5**) provides a hassle-free method for the rapid, high throughput purification and concentration of high-quality DNA from PCR, endonuclease digestions, cell lysates and other impure DNA preparations. It can also be used for post-RT cDNA clean-up and purification of sequencing-ready DNA from M13 phage. Simply add the specially formulated **DNA Binding Buffer** to your sample and transfer to the wells of the supplied **Zymo-Spin™ I-96 Plate**. There is no need for organic denaturants or chloroform. Instead, the product features *Fast-Spin* technology to yield DNA that is free of salts and contaminants in just minutes. DNA purified using the **ZR-96 DCC™-5** is suitable for nucleotide sequencing, microarray analysis, PCR, and restriction endonuclease digestion procedures.



ZR-96 DCC™-5 procedure.



DNA samples, such as the PCR products shown here, can be efficiently purified and concentrated using the **DNA Clean & Concentrator™-5**.



Pure and Reliable Recovery with the ZR-96 DCC™-5. Shown here is the recovery of 1 µg of 100 bp marker DNA eluted into 10 µl of water analyzed using a NanoDrop® spectrophotometer. The DNA Clean & Concentrator™-5 consistently recovers > 90% of input DNA.

Available Formats

	DCC™-5	DCC™-25	DCC™-100	DCC™-500	Genomic DCC™	ZR-96 DCC™-5
						
Name	Zymo-Spin™ I & IC	Zymo-Spin™ II & IIC	Zymo-Spin™ V	Zymo-Spin™ VI	Zymo-Spin ™ IC-XL	Zymo-Spin™ I-96
Capacity	5 µg/ prep.	25 µg/ prep.	100 µg/ prep.	500 µg/ prep.	10 µg/ prep.	5 µg/ well.
Elution Vol.	≥ 6 µl	≥ 25 µl	≥ 150 µl	≥ 2 ml	≥ 10 µl	≥ 10 µl
Cat. Nos.	D4003, D4013	D4005, D4033	D4029, D4030	D4031, D4032	D4010, D4011	D4023, D4024

Typical DCC™ Applications

Post-PCR DNA Clean-up	Efficient desalting of DNA with the removal of DNA polymerases, primers and free dNTPs.
DNA Clean-up From Enzymatic Reactions	Efficient desalting of DNA with the removal of DNA polymerases, modifying enzymes, RNA polymerases, ligases, kinases, nucleases, phosphatases, endonucleases, *etc.*
Post-Reverse Transcription (RT) & cDNA Clean-up	Efficiently purifies DNA following RT, either as a DNA/RNA complex or as single stranded cDNA following chemical hydrolysis of the RNA template.
Plasmid DNA Clean-up	Efficiently purifies plasmid DNA from "home-made" preparations of cell free lysates or from commercial kits. Plasmid DNA purified and concentrated using the **DCC™** has proven an excellent substrate for high quality DNA sequencing.
Isotope and Dye Removal	Efficiently removes unincorporated fluorescent (*i.e.*, AMCA, FITC, BIO, DIG, Cy3, Cy5, FAM, *etc.*) and radiolabeled dNTP derivatives from DNA following *in vitro* labeling reactions.
Purification of M13 ssDNA	The **DCC™** can be used for the rapid isolation of single stranded M13 phage DNA directly from phage-infected *E. coli* culture supernatant.

- ✓ For purification of short DNA or RNA oligonucleotides ≥16 nt, use the **Oligo Clean & Concentrator (D4060, D4061)**.
- ✓ For ChIP (Chromatin Immunoprecipitation) sample cleanup, use **the ChIP DNA Clean & Concentrator (D5201, D5205)** for high quality DNA from any step in a standard ChIP protocol.
- ✓ For post-cycle sequencing samples, use the **ZR Sequencing DNA Clean-up Kit (D4050, D4051)** for dye blob elimination.
- ✓ For samples containing PCR inhibitors, use the ***OneStep*™ PCR Inhibitor Removal Kit (D6030, D6035)**.

Selected Citations

Li, N. (2010). Whole genome DNA methylation analysis based on high throughput sequencing technology. *Methods, 52* (3), 221-232.

Lee, EJ. (2011). Targeted bisulfite sequencing by solution selection and massively parallel sequencing. *Nucleic Acids Research, 39*(19), e127, doi:10.1093/nar/gkr598

Papageorgiou, EA. (2009). Sites of differential DNA methylation between placenta and peripheral blood. *Am J Pathol, 174* (5), 1609-1618.

Ferguson, A.A. et al. (2009). Retrofitting ampicillin resistant vectors by recombination for use in generating *C. elegans* transgenic animals by bombardment. *Plasmid, 62*, 140-145.

Buffer Preparation

✓ *Before starting:* Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA Wash Buffer** concentrate. Add 192 ml 100% ethanol (208 ml 95% ethanol) to the 48 ml **DNA Wash Buffer** concentrate.

Protocol

Note: All centrifugation steps should be performed between 3,000 - 5,000 x g.

1. Add 2-7 volumes of **DNA Binding Buffer** to each volume of DNA sample (see table below). Mix briefly by vortexing.

Application	DNA Binding Buffer : Sample	Example
Plasmid, genomic DNA (>2 kb)	2 : 1	200 µl : 100 µl
PCR product, DNA fragment	5 : 1	500 µl : 100 µl
ssDNA (*e.g.* cDNA, M13 phage[1])	7 : 1	700 µl : 100 µl

For efficient recovery of high molecular weight DNA (> 20kb to > 200kb), use the **Genomic DNA Clean & Concentrator™ (Cat. Nos. D4010, D4011)**.

2. Transfer sample mixtures to the wells of a **Zymo-Spin™ I-96 Plate**[2] mounted on a **Collection Plate**.

3. Centrifuge for 5 minutes until sample mixtures have been completely filtered. Discard the flow-through.

4. Add 300 µl **DNA Wash Buffer** to each well of the **Zymo-Spin™ I-96 Plate**. Centrifuge for 5 minutes. Repeat wash step, but centrifuge for 15 minutes. (Alternatively, one wash can be performed using 600 µl **Wash Buffer**).

5. Add ≥ 10 µl **DNA Elution Buffer**[3] or water[4] directly to the column matrix in each well. Transfer the **Zymo-Spin™ I-96 Plate** onto an **Elution Plate** and centrifuge for 3 minutes to elute the DNA.

   Ultra-pure DNA is now ready for use.

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

Notes:

[1] Centrifuge phage-infected bacterial culture at 8,000 x *g* for 1 minute prior to mixing an aliquot of the phage-containing supernatant with the **DNA Binding Buffer**.

[2] The capacity of each well of the **Zymo-Spin™ I-96 Plate** is approximately 1.1 ml. The capacity of each well of the **Collection Plate** is approximately 800 µl. Therefore, it may be necessary to load and spin the plate multiple times if a sample has a volume larger than 800 µl.

[3] **DNA Elution Buffer**: 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA.

[4] Elution of DNA from the column is dependent on pH and temperature. If water is used, make sure the pH is >6.0. Waiting 1 minute prior to elution may improve the yield of larger (> 6 kb) DNA. For even larger DNA (> 10 kb), the total yield may be improved by eluting the DNA with 60-70 °C **DNA Elution Buffer**.

For the clean-up of short cDNAs or ESTs (≥16 nt), we recommend the **Oligo Clean & Concentrator ™ (Cat. Nos. D4060, D4061)**.

Appendix A: ssDNA Purification

cDNA clean-up

The DCC™ kit can be used to effectively clean and concentrate cDNA (>500 nt) following reverse transcription (RT) in the presence/absence of fluorescent dyes. Unincorporated free nucleotides and fluorescent derivatives are efficiently removed using the DCC™, and the recovered cDNA may be used directly for microarray analysis, second-strand cDNA synthesis, or indirect labeling with a fluorescent dye such as NHS ester Cy3 or Cy5.

Hydrolysis

1. Add 10 µl 0.5 M EDTA and 10 µl 1 N NaOH to 50 µl of RT reaction.

   The volumes of EDTA and NaOH should be scaled proportionally depending on the Starting volume of the RT reaction.

2. Incubate at 65°C for 15 minutes.

Clean-up

1. Add 490 µl (7 volumes) of **DNA Binding Buffer** to the hydrolysis reaction above. Mix well.

   Neutralization (pH) following RNA hydrolysis is not necessary as the **DNA Binding Buffer** will effectively neutralize the NaOH added to the reaction.

2. Continue with *Step 2* of the Protocol on page 4.

M13 phage ssDNA purification

1. Centrifuge phage-infected bacterial culture at 8,000 x *g* for 1 minute
2. Transfer 100 µl of phage-containing supernatant to a 1.5 ml microcentrifuge tube and add 700 µl (7 volumes) of **DNA Binding Buffer**. Mix briefly by vortexing.

   Increased supernatant volumes may be processed by proportionally increasing the amount of **DNA Binding Buffer** added to the sample.

3. Continue with *Step 2* of the Protocol on page 4.

Appendix B: Troubleshooting

Low Recovery

- **Improperly Prepared/Stored DNA Wash Buffer**
  Make sure ethanol has been added to the **DNA Wash Buffer** concentrate. Cap the bottle tightly to prevent evaporation over time.
- **Addition of DNA Elution Buffer**
  Add elution buffer directly to the well matrix and not to the walls of the well. Elution buffer requires contact with the matrix for at least 1 minute for large DNA ≥ 10 kb.
- **Incomplete Elution**
  1. DNA elution is dependent on pH, temperature, and time. For large genomic DNA (≥ 50 kb), apply heated elution buffer (60-70 °C) and incubate for several minutes prior to elution.
  2. Sequential elutions may be performed for quantitatively higher recovery but lower final DNA concentration. This is recommended for DNA ≥ 10 kb.

Low A_{260}/A_{230} Ratios

- **96-Well Tip Contaminated**
  When removing the Zymo-Spin I-96 plate from the Collection Plate, be careful that the tips of the 96-well plate does not come into contact with the flow-through. Trace amounts of salt from the flow-through can contaminate a sample resulting in low A_{260}/A_{230} ratios. Ethanol contamination from the flow-through can also interfere with DNA elution. Zymo-Spin™ columns are designed for complete elution with no buffer retention or carryover.

Following Clean-up with the DCC™, Multiple Bands Appear in an Agarose Gel

- **Acidification of DNA Loading Dye**
  Most loading dyes do not contain EDTA and will acidify (pH ≤ 4) over time due to some microbial growth. This low pH is enough to cause DNA degradation. Therefore, if water is used to elute the DNA, 6X Loading Dye containing 1 mM EDTA is recommended.

Ordering Information

Product Description	Catalog No.	Kit Size (Preps.)
DNA Clean & Concentrator™-5 (for purification of up to 5 µg DNA per prep.) *Supplied with **uncapped columns***	D4003 D4004	50 200
DNA Clean & Concentrator™-5 (for purification of up to 5 µg DNA per prep.) *Supplied with **capped columns***	D4013 D4014	50 200
ZR-96 DNA Clean & Concentrator™-5 (for 96-well purification of up to 5 µg DNA per well)	D4023 D4024	2 x 96 4 x 96
DNA Clean & Concentrator™-25 (for purification of up to 25 µg DNA per prep.) *Supplied with **uncapped columns***	D4005 D4006	50 200
DNA Clean & Concentrator™-25 (for purification of up to 25 µg DNA per prep.) *Supplied with **capped columns***	D4033 D4034	50 200
DNA Clean & Concentrator™-100 (for purification of up to 100 µg DNA per prep.)	D4029 D4030	25 50
DNA Clean & Concentrator™-500 (for purification of up to 500 µg DNA per prep.)	D4031 D4032	10 20
Oligo Clean & Concentrator™ (for purification of up to 5 µg of oligonucleotides per prep.)	D4060 D4061	50 200
Genomic DNA Clean & Concentrator™ (for purification of up to 10 µg genomic DNA per prep.)	D4010 D4011	25 100

Refer to Page 3 for column design specifics in each kit.

For Individual Sale	Catalog No.	Size
DNA Binding Buffer	D4003-1-L D4004-1-L	50 ml 100 ml
DNA Wash Buffer (concentrate)	D4003-2-24 D4003-2-48	24 ml 48 ml
DNA Elution Buffer	D3004-4-10 D3004-4-16	10 ml 16 ml
Zymo-Spin™ I-96 Plate	C2004-2	2 plates
Collection Plate	C2002	2 plates
Elution Plate	C2003	2 plates

DNA PURIFICATION

What is Clean-Spin™ Technology?

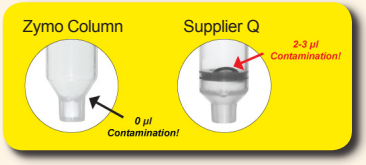
The spin columns from Zymo Research have been designed to ensure complete elution with no binding/wash buffer carryover. The result is ultra-pure inhibitor-free DNA and RNA.

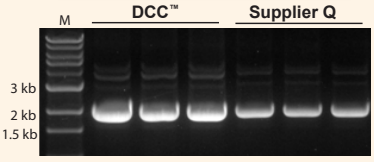
Purify DNA from PCR & other sources

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)





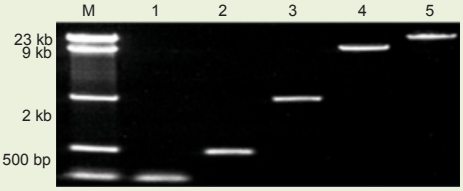
High efficiency DNA recovery with the DCC™-5 compared to Supplier Q .

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)

RNA 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.

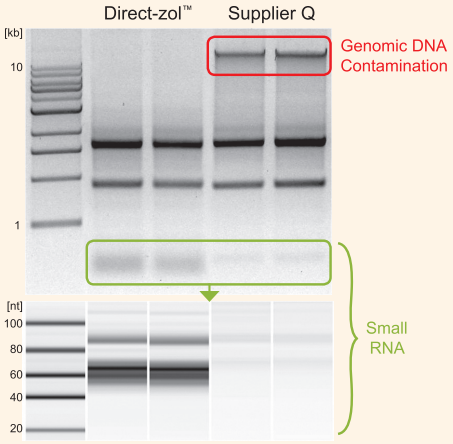
Get RNA *directly* from TRIzol® without phase separation

Direct-zol™ RNA

- ✓ For purification of high-quality small and large RNA *directly* from TRIzol®, TRI Reagent®, or similar.
- ✓ Bypasses phase separation and precipitation procedures allowing for unbiased recovery of miRNA

Product	Size (Cat. No.)
Direct-zol™ RNA MiniPrep	50 Preps. (R2050) 50 Preps. (R2051)* 200 Preps. (R2052) 200 Preps. (R2053)*
96-well and MagBead formats also available!	

DNase I included in all kits.
* Supplied with *TRI-Reagent®*



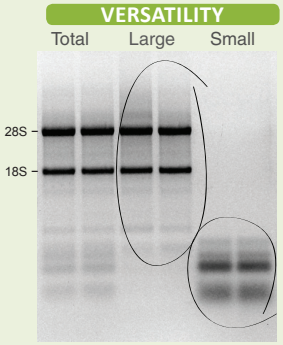
High-quality small and large RNA are effectively recovered with the Direct-zol™ kit. RNA is ***DNA-free***.

Isolate DNA-free RNA from 1 to 10^7 cells in minutes

Quick-RNA™

- ✓ Isolation of total, large, or small RNA – *You decide!*
- ✓ Ultra clean, high-quality RNA from a single cell to 10^7 cells.
- ✓ DNA-free RNA ideal for any downstream application – *DNase I included.*

Product	Size (Cat. No.)
Quick-RNA™ MicroPrep	50 Preps. (R1050) 200 Preps. (R1051)
Quick-RNA™ MiniPrep	50 Preps. (R1054) 200 Preps. (R1055)
ZR-96 *Quick-RNA*™	2 x 96 Preps. (R1052) 4 x 96 Preps. (R1053)



Isolate total, large, or small RNA with the *Quick-RNA*™ kit.

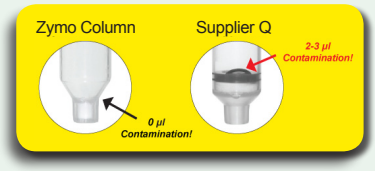
RNA is DNA-free using the *Quick-RNA*™ kit

Purify RNA from enzymatic and labeling reactions in 5 minutes

RNA Clean & Concentrator™

- ✓ Recover ultra-pure RNA in small (≥6 µl) elution volumes.
- ✓ Compatible with TRIzol®, phenol, choloform, and RNase inhibitors (RNAlater®).
- ✓ RNA is ideal for RT-PCR, q-PCR, hybridization, arrays, RNA interference, etc.

Product	Size (Cat. No.)
RNA Clean & Concentrator™-5	50 Preps. (R1015) 200 Preps. (R1016)
RNA Clean & Concentrator™-25	50 Preps. (R1017) 100 Preps. (R1018)
ZR-96 RNA Clean & Concentrator™	2x96 well plates (R1080)
DNA-Free RNA Kit™	50 Preps. (R1013) 200 Preps. (R1014)



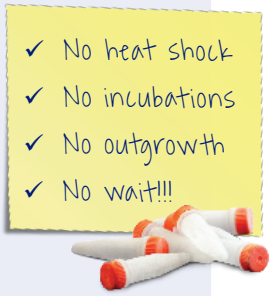
OTHER INNOVATIVE PRODUCTS FROM ZYMO RESEARCH...

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!

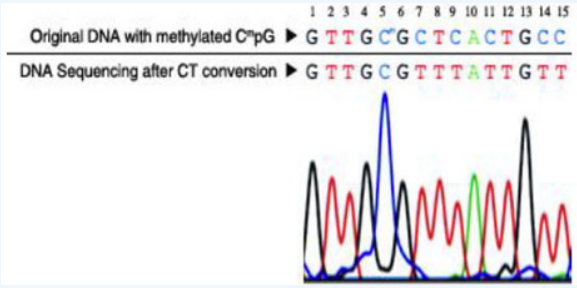
Product	Size (Cat. No.)
Zymo 5α (Same as DH5α)	10 x 100 µl aliquots (T3007) 96 x 50 µl aliquots (T3009) 96 x 50 µl aliquots PCR-plate (T3010)
Zymo 10B (Same as DH10B)	10 x 100 µl aliquots (T3019) 96 x 50 µl aliquots (T3020)
JM109	10 x 100 µl aliquots (T3003) 96 x 50 µl aliquots (T3005)
HB101	10 x 100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



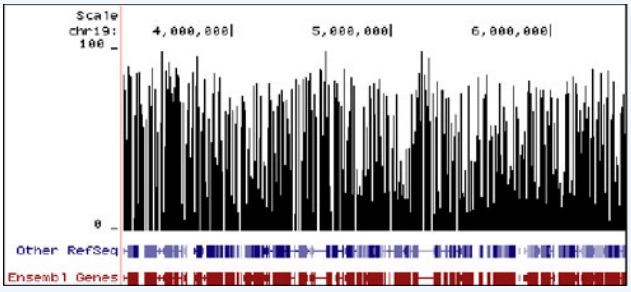
The fastest method for complete bisulfite conversion of DNA

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.

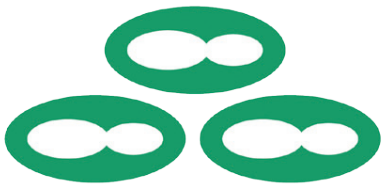


DNA Sequencing Results Following Bisulfite Treatment



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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The Beauty of Science is to Make Things Simple