



ZYMO RESEARCH

The Beauty of Science is to Make Things Simple

INSTRUCTION MANUAL

Genomic DNA Clean & Concentrator™-25

Catalog Nos. **D4064 & D4065**

Highlights

- Quick (5 minute) spin column recovery of large-sized DNA (e.g., genomic, mitochondrial, plasmid (BAC/PAC), viral, phage, (wga)DNA, etc.) from any enzymatic reaction or impure preparation (e.g., Proteinase K digestion). *No messy precipitations!*
- Unique spin column for low volume ($\geq 35 \mu\text{l}$) elution of ultra-pure, high-yield DNA.
- Eluted DNA is ideal for PCR, restriction endonuclease digestion, Sanger and Next-Gen sequencing, etc.

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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

Genomic DNA Clean & Concentrator™-25 (Kit Size)	D4064 (25 Preps.)	D4065 (100 Preps.)	Storage Temperature
ChIP DNA Binding Buffer	50 ml	2 x 50 ml	Room Temp.
DNA Wash Buffer¹	6 ml	24 ml	Room Temp.
DNA Elution Buffer	4 ml	10 ml	Room Temp.
Zymo-Spin™ IIC-XL Columns	25	100	Room Temp.
Collection Tubes	50	100	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 **DNA Wash Buffer** label.

Specifications

- **DNA Purity** – High-quality ($A_{(260/280)} \geq 1.8$) high molecular weight DNA ideal for ligation, sequencing, labeling, PCR, microarray, transfection, transformation, and restriction digestion procedures.
- **DNA Size Limits** – Capable of purifying small DNA fragments >50 bp and large sized DNAs up to 200 kb.
- **DNA Recovery** – Typically, up to 25 µg total DNA per column can be eluted into ≥35 µl of low salt **DNA Elution Buffer** or water.
- **Sample Sources** – DNA from impure preparations of genomic DNA (e.g., Proteinase K digestions), plasmid DNA (including BAC), viral DNA, and *whole genome amplified* (wga) DNA. Can also be used for the purification of low molecular weight DNA (50 bp to 10 kb) from PCR, endonuclease digestion, post-RT cDNA synthesis, *etc.*
- **Product Detergent Tolerance** – ≤ 5% Triton X-100, ≤ 5% Tween-20, ≤ 5% Sarkosyl, ≤ 1% SDS.

Note: ™ Trademarks of Zymo Research Corporation. This product is for research use only and should only be used by trained professionals. It is not intended 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.

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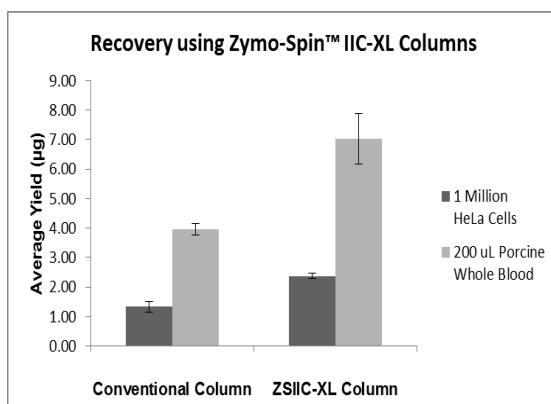
Phone: (949) 679-1190 ▪ Toll Free: (888) 882-9682 ▪ Fax: (949) 266-9452 ▪ info@zymoresearch.com ▪ www.zymoresearch.com

Product Description

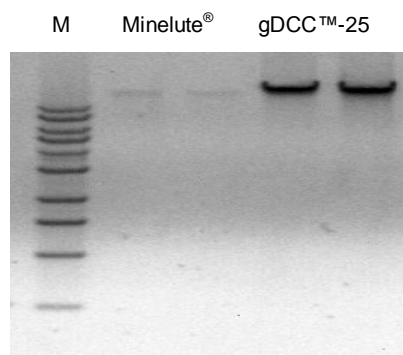
The **Genomic DNA Clean & Concentrator™-25 (DCC™)** is for quick (5 minute) recovery of ultra-pure, large-sized DNA (e.g., genomic, mitochondrial, plasmid (BAC/PAC), viral, phage, (wga)DNA, etc.) from any enzymatic reaction or impure preparation (e.g., Proteinase K digestion). There is no need for organic denaturants, chloroform, or messy precipitations: simply add the specially formulated **ChIP DNA Binding Buffer** to a sample and then transfer the mixture to the supplied **Zymo-Spin™ Column**. Eluted DNA is suitable for sequencing, PCR, endonuclease digestion, and other enzymatic procedures. The product is also compatible with smaller DNAs (50 bp to 10 kb) from PCR, digestions, crude plasmid preparations, cDNA synthesis, etc.



Five minute **Genomic DCC™-25** procedure.






Zymo-Spin™ IIC-XL columns result in superior yields compared to conventional columns. Genomic DNA extracted using the **Zymo-Spin™ IIC-XL Column** results in higher yields from HeLa Cells and Porcine Whole Blood.








High molecular weight DNA is efficiently purified using the Genomic DCC™-25. Lambda (λ) phage DNA (48.5 kb) was purified (in duplicate) from input material using Qiagen's Minelute® and the Genomic DCC™-25 (gDCC™-25). The gDCC™-25 resulted in yields > 40% compared to the Minelute®. Eluted DNAs were analyzed in a 0.8% (w/v) TAE/agarose/EtBr gel (shown above). The size marker "M" is a 1 kb ladder (Zymo Research).

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Available Formats – Genomic DNA Purification

	Genomic DCC™-10	Genomic DCC™-25	ZR-96 Genomic DCC™-5
			
Column	Zymo-Spin™ IC-XL	Zymo-Spin™ IIC-XL	Zymo-Spin™ I-96-XL
Capacity	10 µg/ prep.	25 µg/ prep.	5 µg/ prep.
Elution	≥ 10 µl	≥ 35 µl	≥ 15 µl

Available Formats – DNA Purification (< 23 kb)

	DCC™-5	DCC™-25	DCC™-100	DCC™-500	ZR-96 DCC™-5
					
Column	Zymo-Spin™ I & IC	Zymo-Spin™ II & IIC	Zymo-Spin™ V	Zymo-Spin™ VI	Zymo-Spin™ I-96
Capacity	5 µg/ prep.	25 µg/ prep.	100 µg/ prep.	500 µg/ prep.	5 µg/ prep.
Elution	≥ 6 µl	≥ 25 µl	≥ 150 µl	≥ 2 ml	≥ 10 µl

✓ 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)**.

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 modifying enzymes, RNA polymerases, ligases, kinases, nucleases, phosphatases, endonucleases, <i>etc.</i>
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>i.e.</i> , AMCA, FITC, BIO, DIG, Cy3, Cy5, FAM, <i>etc.</i>) and radiolabeled dNTP derivatives from DNA following <i>in vitro</i> 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 <i>E. coli</i> culture supernatant.

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Buffer Preparation

- ✓ *Before starting:* Add 24 ml 100% ethanol (26 ml 95% ethanol) to the 6 ml **DNA Wash Buffer** concentrate. Add 96 ml 100% ethanol (104 ml 95% ethanol) to the 24 ml **DNA Wash Buffer** concentrate.

Protocol

Note: All centrifugation steps should be performed between 10,000 - 16,000 x g.

- In a 1.5 ml microcentrifuge tube, add 2-5 volumes of **ChIP DNA Binding Buffer** to each volume of DNA sample¹ (see table below). Mix thoroughly.

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

- Transfer mixture to a provided **Zymo-Spin™ IIC-XL Column**² in a **Collection Tube**.
- Centrifuge for 30 seconds. Discard the flow-through.
- Add 400 µl **DNA Wash Buffer** to the column. Centrifuge for 1 minute. Empty the collection tube. Repeat the wash step.
- Transfer the column to a 1.5 ml microcentrifuge tube. Add ≥ 50 µl³ **DNA Elution Buffer**⁴ or water⁵ directly to the column matrix and incubate at room temperature for five minutes and centrifuge at for 30 seconds 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 It may be necessary to add RNase A to cell lysates prior to performing the procedure to ensure RNA-free DNA will be recovered in Step 5.

² The sample capacity of the column is 900 µL. It may be necessary to load and spin a column multiple times if a sample has a volume larger than 900 µL.

³ To increase concentrations of first elution, use ≥ 35 µL **DNA Elution Buffer**. To increase yield, load the eluate directly on the matrix a second time, incubate for 3 minutes at room temperature, and centrifuge again.

⁴ **DNA Elution Buffer:** 10 mM Tris-HCl, pH 8.5, 0.1 mM EDTA

⁵ Elution of DNA from the column is dependent on pH and temperature. If water is used, make sure the pH is >6.0. The total yield may be improved by eluting the DNA with 60-70 °C **DNA Elution Buffer**.

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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 column matrix and not to the walls of the column. Elution buffer requires contact with the matrix for at least 1 minute for large DNA ≥ 10 kb recovery.

- **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

- **Column Tip Contaminated**

When removing the column from the collection tube, be careful that the tip of the column does not come into contact with the flowthrough. Trace amounts of salt from the flowthrough can contaminate a sample resulting in low A_{260}/A_{230} ratios. Ethanol contamination from the flowthrough 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 ($\text{pH} \leq 4$) 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.)
Genomic DNA Clean & Concentrator™-10 (for purification of up to 10 µg genomic DNA per prep.)	D4010 D4011	25 100
Genomic DNA Clean & Concentrator™-25 (for purification of up to 25 µg genomic DNA per prep.)	D4064 D4065	25 100
ZR-96 Genomic DNA Clean & Concentrator™-5 (for 96-well purification of up to 5 µg genomic DNA per well)	D4066 D4067	2 x 96 4 x 96

Related Products

Product Description	Catalog No.	Kit Size (Preps.)
DNA Clean & Concentrator™-5 (for purification of up to 5 µg DNA per prep.) <i>Supplied with capped columns</i>	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.) <i>Supplied with capped columns</i>	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

For Individual Sale	Catalog No.	Size
Chip DNA Binding Buffer	D5201-1-50	50 ml
DNA Wash Buffer (concentrate)	D4003-2-6 D4003-2-24	6 ml 24 ml
DNA Elution Buffer	D3004-4-1 D3004-4-4	1 ml 4 ml
Collection Tubes	C1001-50 C1001-500 C1001-1000	50 tubes 500 tubes 1000 tubes
Zymo-Spin™ IIC-XL Columns	C1102-25	25 Columns

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DNA PURIFICATION



What is Clean-Spin™ Technology?

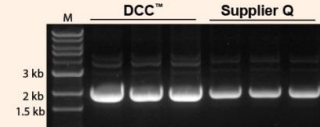
The spin columns from Zymo Research have been designed to ensure complete elution with no binding/wash buffer carryover. The result is ultra-pure inhibitor-free DNA and RNA.

Purify DNA from PCR & other sources

DNA Clean & Concentrator™ (DCC™)

- ✓ Recovery of ultra-pure DNA that is free of salts and contaminants.
- ✓ Small ($\geq 6 \mu\text{l}$) 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)



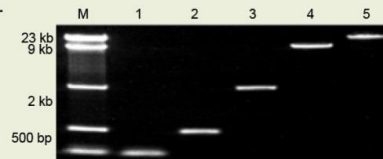
High efficiency DNA recovery with the DCC™-5 compared to Supplier Q.

Boost DNA recoveries from agarose gels to >80%

Zymoclean™ Gel DNA Recovery

- ✓ Rapid (15 min.) recovery of ultra-pure DNA from agarose gels in $\geq 6 \mu\text{l}$.
- ✓ 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



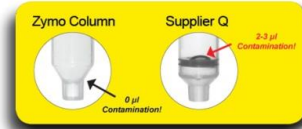
Product	Size (Cat. No.)
Zyppy™ Plasmid Miniprep Kit	50 Preps. (D4036) 100 Preps. (D4019) 400 Preps. (D4020) 800 Preps. (D4037)

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RNA PURIFICATION

Get RNA *directly* from TRIzol® without phase separation



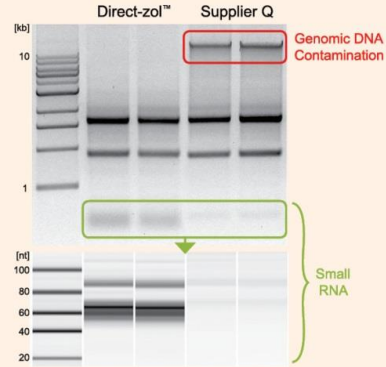
Direct-zol™ RNA

- ✓ 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®



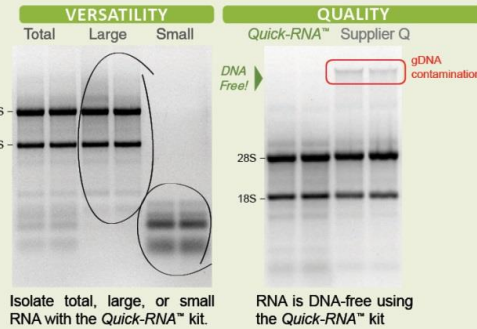
High-quality small and large RNA are effectively recovered with the Direct-zol™ kit. RNA is DNA-free.

Isolate DNA-free RNA from 1 to 10⁷ cells in minutes

Quick-RNA™

- ✓ Isolation of total, large, or small RNA – *You decide!*
- ✓ Ultra clean, high-quality RNA from a single cell to 10⁷ 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)

The following are trademarks of other companies: pGEM®, Promega Corp.; TRIzol® and TRI Reagent®, Molecular Research Center, Inc.; DH5® and DH10B™, Life Technologies, Inc.

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/ μ g plasmid DNA
- ✓ Just Mix & Go! Simply add DNA then spread. Transformation in as little as 20 seconds!

Product	Size (Cat. No.)
Zymo 5 α (Same as DH5 α)	10 x 100 μ l aliquots (T3007) 96 x 50 μ l aliquots (T3009) 96 x 50 μ l aliquots PCR-plate (T3010)
Zymo 10B (Same as DH10B)	10 x 100 μ l aliquots (T3019) 96 x 50 μ l aliquots (T3020)
JM109	10 x 100 μ l aliquots (T3003) 96 x 50 μ l aliquots (T3005)
HB101	10 x 100 μ l aliquots (T3011) 96 x 50 μ l aliquots (T3013)
C600	10 x 100 μ l aliquots (T3015)
TG1	10 x 100 μ l aliquots (T3017)

- ✓ No heat shock
- ✓ No incubations
- ✓ No outgrowth
- ✓ No wait!!!

Mix & Go
Competent cells
E. coli + DNA

Mix & Go!
for 20 second*
transformations!



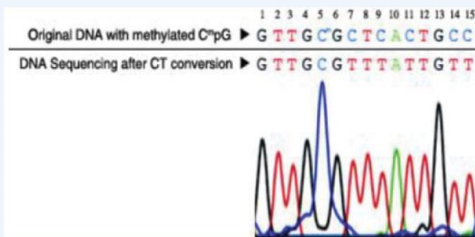
Others
E. coli + DNA
Ice 45 min.
42°C 2 min. (Heatshock)
Place on Ice
Add SOC
Spin to Concentrate Cells
Remove Supernatant

* For Ampicillin selection only.

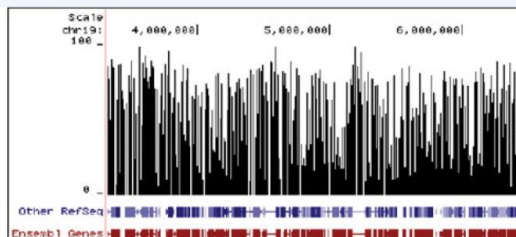
The fastest method for complete bisulfite conversion of DNA

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 2 x 96 rxns. (D5032) Deep-Well 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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