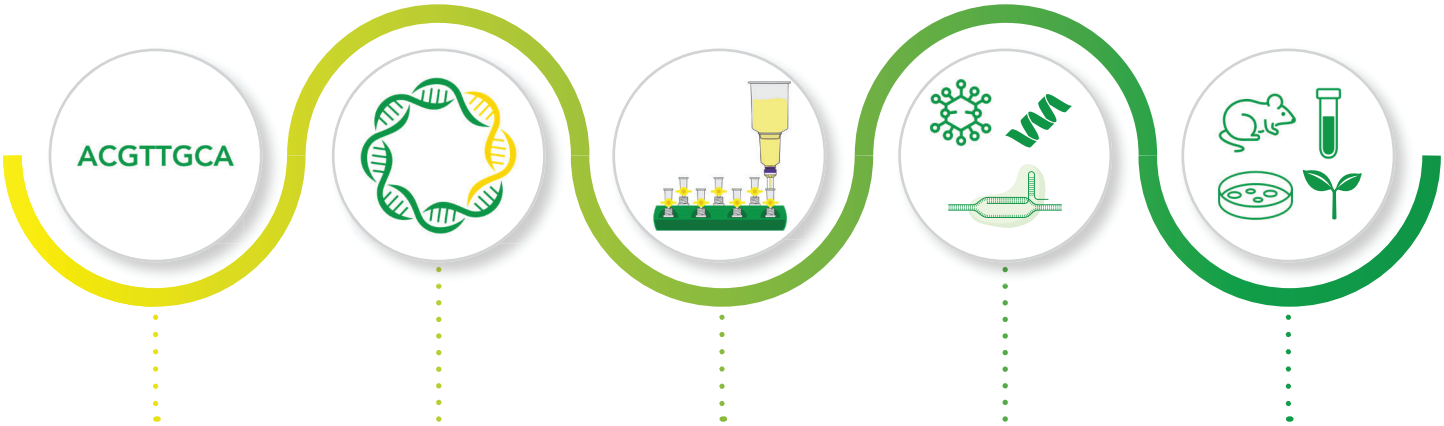
ZYMO RESEARCH

The Beauty of Science is to Make Things Simple®

Cloning & Synthetic Biology

Cloning & Synthetic Biology

Cloning is one of the most widely used techniques in molecular biology because it enables the creation of recombinant DNA and the expression of foreign DNA within a host organism. Cloning is instrumental for the growing field of synthetic biology, in which scientists aim to address various challenges related to healthcare, agriculture, the environment, and manufacturing. Synthetic biologists are capable of engineering innovative biofuels, pharmaceuticals, gene therapies, biosensors, diagnostic tools, and more. Plasmid DNA has become a key element in synthetic biology research due to its ability to transfer and store genetic information, ease of DNA manipulation and propagation, and natural capacity to be taken up by a variety of cells. Plasmids can act as a direct vector for gene therapies or be used *in vitro* to create recombinant viral vectors, mRNA, and protein products. However, traditional cloning and plasmid purification workflows involve long and complicated techniques that are prone to inconsistency, making the development and testing of new potential therapeutics, biomaterials, genetically modified organisms, and biosensors a long and arduous process. Zymo Research strives to give researchers the best foundation possible for synthetic biology discovery through its collection of innovative products that provide fast, simple, and efficient solutions for cloning and plasmid purification. Our DNA clean-up and gel extraction kits utilize Zymo-Spin™ technology, which has revolutionized the microcentrifuge column to ensure DNA purified from PCR and restriction digestions is ultra-pure and highly concentrated, making it ideal for cloning techniques such as Gibson and Golden Gate assembly. Zymo Research's patented ZymoPURE™ technology reduces the time it takes to purify endotoxin-free plasmid DNA by 9-fold compared to traditional plasmid prep methods and enables up to 3 mg of plasmid DNA to be eluted directly from a microcentrifuge column, providing a fast and simple solution for preparing plasmids suitable for mammalian cell transfection. Our unique *Mix & Go!*™ competent *E. coli* cells streamline the plasmid transformation process by eliminating the traditional heat shock and long outgrowth steps without sacrificing transformation efficiency, enabling a more efficient and high-throughput approach to cloning. Additionally, our innovative solutions for yeast cell lysis and transformation make it possible to easily transfer plasmids between *E. coli* and yeast for strain engineering. With our diverse selection of innovative cloning technologies, Zymo Research has everything you need to elevate your synthetic biology research.



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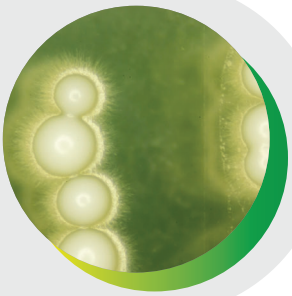
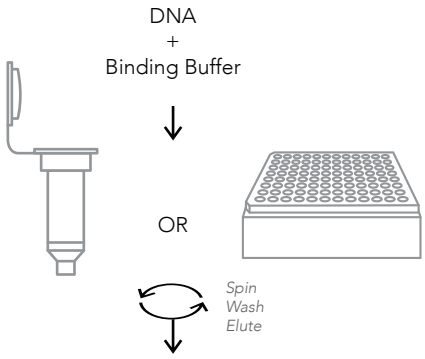


DNA Clean-Up

DNA Clean & Concentrator® Kits

- **Fast & Simple:** Clean and concentrate DNA in 2 minutes.
- **Highly Concentrated:** Elute in as little as 6 µl.
- **Ultra-Pure:** Ready for Next-Gen Sequencing, PCR, ligations, Gibson Assembly, etc.

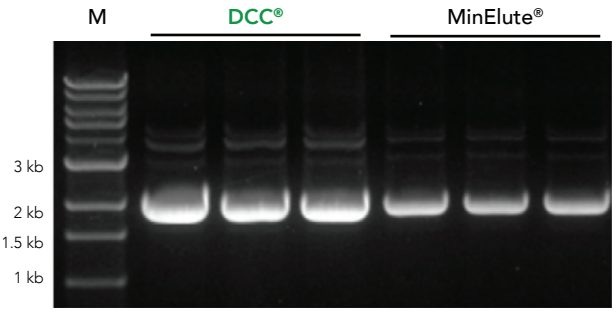
Simple, Innovative Workflow



Ultra-pure DNA for...

- ✓ Sequencing
- ✓ DNA Ligation
- ✓ Endonuclease Digestion, etc.

Recover Ultra-Pure, High-Quality DNA



High-efficiency DNA recovery using the DNA Clean & Concentrator®-5 (DCC®-5). Equivalent amounts of DNA were purified using the DCC®-5 or MinElute® Kit (Supplier Q) and analyzed by electrophoresis in a 0.8% (w/v) agarose/TAE/EtBr gel.

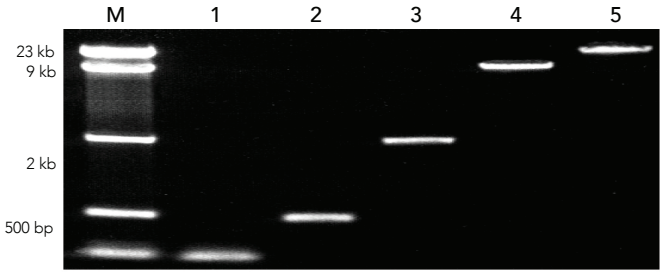
Product	Cat. No.	Size
DNA Clean & Concentrator®-5 (uncapped columns)	D4003T D4003 D4004	10 preps 50 preps 200 preps
DNA Clean & Concentrator®-5 (capped columns)	D4013 D4014	50 preps 200 preps
DNA Clean & Concentrator®-25 (uncapped columns)	D4005 D4006	50 preps 200 preps
DNA Clean & Concentrator®-25 (capped columns)	D4033 D4034	50 preps 200 preps
ZR-96 DNA Clean-up Kit™	D4017 D4018	2 x 96 preps 4 x 96 preps
ZR-96 DNA Clean & Concentrator®-5	D4023 D4024	2 x 96 preps 4 x 96 preps
DNA Clean & Concentrator® MagBead Kit	D4012	100 preps
Genomic DNA Clean & Concentrator®-10	D4010 D4011	25 preps 100 preps
Genomic DNA Clean & Concentrator®-25	D4064 D4065	25 preps 100 preps
ZR-96 Genomic DNA Clean & Concentrator®-5	D4066 D4067	2 x 96 preps 4 x 96 preps

DNA Clean-Up

Zymoclean™ Gel DNA Recovery Kits

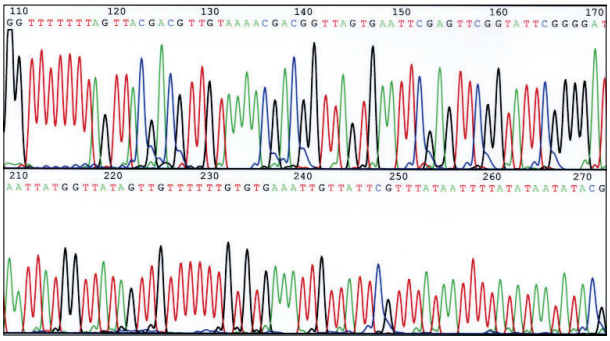
- **Superior Yields:** Recover 80% of DNA from agarose gels.
- **Highly Concentrated:** Elute in as little as 6 µl.
- **Ultra-Pure:** Ready for Next-Gen Sequencing, PCR, ligations, Gibson Assembly, etc.

Boost DNA Yield from Agarose Gels Regardless of Size



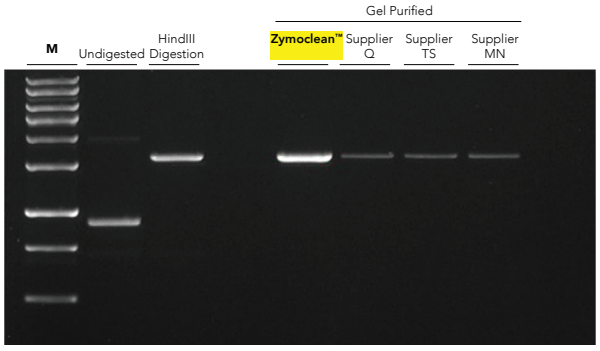
DNA fragments recovered from an agarose gel using the Zymoclean™ Gel DNA Recovery Kit. Lanes: M: DNA Ladder; 1-5: individual ladder DNA fragments.

Recover Sequencing-Ready DNA



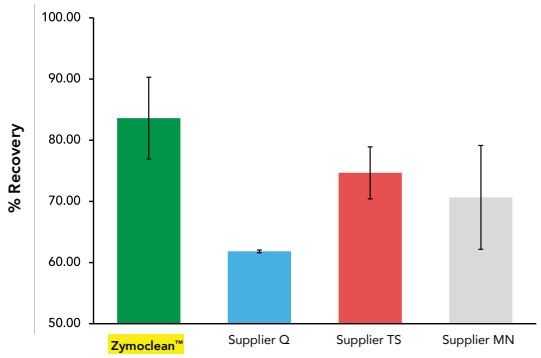
DNA sequencing chromatogram of a PCR product recovered using the Zymoclean™ Gel DNA Recovery Kit. DNA was recovered from a 2% (w/v) agarose gel and used directly for Sanger sequencing.

Lowest Elution Volume, Highest Concentration



Concentration of DNA recovered from an agarose gel using the Zymoclean™ Gel DNA Recovery Kit compared to products from other suppliers. 1 µg of plasmid DNA (pGEM®) was digested with HindIII and visualized on a 0.8% (w/v) agarose gel. Linearized plasmid bands were excised from the gel and the DNA was purified using either the Zymoclean™ Gel DNA Recovery Kit or equivalent kits from Suppliers Q, TS, or MN and eluted using the manufacturer's lowest recommended elution volume. 1 µl of each eluate was loaded on a gel. M: 1 kb DNA Ladder (Zymo Research).

Achieve Recoveries > 80%



Percentage of DNA recovered from an agarose gel using the Zymoclean™ Gel DNA Recovery Kit compared to products from other suppliers. 1 µg of plasmid DNA (pGEM®) was digested with HindIII and visualized on a 0.8% (w/v) agarose gel (in duplicate). Linearized plasmid bands were excised from the gel and the DNA was purified using either the Zymoclean™ Gel DNA Recovery Kit or equivalent kits from Suppliers Q, TS, or MN. DNA concentration of the resulting eluted DNA was measured using Nanodrop (Thermo) and % recovery was calculated for each kit.

Product	Cat. No.	Size
Zymoclean™ Gel DNA Recovery Kit (uncapped columns)	D4001T D4001 D4002	10 preps 50 preps 200 preps
Zymoclean™ Gel DNA Recovery Kit (capped columns)	D4007 D4008	50 preps 200 preps
ZR-96 Zymoclean™ Gel DNA Recovery Kit	D4021 D4022	2 x 96 preps 4 x 96 preps
Zymoclean™ Large Fragment DNA Recovery Kit	D4045 D4046	25 preps 100 preps

Bringing the Power of Chromosome Synthesis to Every Lab

A Novel Approach to Chromosome Synthesis Using Natural Components in Yeast

Artificial chromosome synthesis is a powerful technique that enables scientists to elucidate the mechanisms underlying cellular life and its diversity. From mapping the genetic basis of trait differences to discerning the structural requirements of genomes, synthesizing chromosomes makes it possible to explore fundamental biological questions that have yet to be answered.[1] However, *de novo* chromosome synthesis is expensive and time-consuming, which greatly limits its use.

To overcome these challenges and break down the barrier of high costs, molecular biologists at the University of Southern California created a new method for building synthetic chromosomes from natural components in the budding yeast *Saccharomyces cerevisiae*.[2] In this study published in *Nature*, the researchers presented a detailed protocol for their method and demonstrated the viability of this procedure. Their technique, named CReATiNG (Cloning, Reprogramming, and Assembling Tiled Natural Genomic DNA), consists of cloning natural chromosome segments flanked by unique adapter sequences. The adapter sequences specify how the segments will recombine during assembly. The cloned segments are then transformed into yeast cells and assembled *in vivo* via homologous recombination.

To accomplish this, target-specific cloning vectors were produced and harvested from *E. coli* using the ZymoPURE™ II Plasmid Midiprep Kit. Next, *S. cerevisiae* cells constitutively expressing Cas9 were transformed with a linearized version of the cloning vector, a repair template containing a selectable marker, and multiple guide RNAs used to direct Cas9 to target segments. The cloned chromosome segments were extracted from the yeast cells using the ZymoPURE™ II Plasmid Midiprep Kit and lyticase and the isolated plasmids were amplified in *E. coli*. After another round of plasmid purification, the cloned chromosome segments were excised from the cloning vector using restriction enzyme digestion. The digested chromosome segments were then analyzed using agarose gel electrophoresis and recovered from the gel using the Zymoclean™ Large Fragment DNA Recovery Kit. Once recovered, the chromosome segments were co-transformed into a yeast cell with a centromere cassette and a centromere-free version of the cloning vector, enabling *in vivo* assembly via homologous recombination. The native chromosome was then removed through centromere destabilization and counterselection.

The authors then demonstrated potential ways this method can be used to analyze phenotypic effects tied to specific chromosome segments. First, the researchers recombined chromosomes between different yeast strains and species and successfully produced a viable strain with a hybrid phenotype. They also utilized CReATiNG to restructure chromosome 1 in *S. cerevisiae* in non-natural configurations, which significantly impeded growth. Extracting RNA from the modified yeast using Zymo Research's YeaStar™ RNA Kit for RT-qPCR analysis revealed that the chromosome restructuring resulted in the overexpression of non-essential genes that are known to cause growth defects. Lastly, the authors successfully used their technique to create a viable *S. cerevisiae* strain that was missing about 40% of chromosome 1.

With the help of Zymo Research's innovative nucleic acid purification technologies, the researchers successfully developed a precise and cost-effective chromosome synthesis method that leverages natural components within yeast cells. Using CReATiNG, scientists can efficiently recombine, modify, or remove specific segments of natural chromosomes. This flexibility enables precise modifications, such as introducing beneficial traits or removing undesirable elements. In addition, since this method relies on the recombination between the synthetic chromosome and its native complement, defects in synthetic chromosome design can be overcome. CReATiNG opens doors to innovative biotechnological applications such as:

- Metabolic Engineering: Custom chromosomes can optimize metabolic pathways for more efficient production of valuable compounds (e.g., biofuels, pharmaceuticals).
- Gene Therapy: Synthetic chromosomes may be used to deliver therapeutic genes to treat genetic disorders.
- Synthetic Organisms: By assembling entire synthetic genomes, scientists can create novel organisms with beneficial properties.

REFERENCES

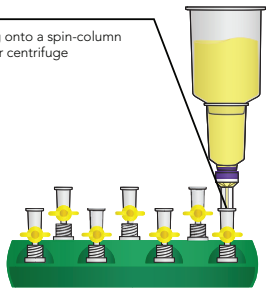
1. Hutchison, C. A. 3rd, Chuang, R. Y., Noskov, V. N., Assad-Garcia, N., Deerinck, T. J., Ellisman, M. H., Gill, J., Kannan, K., Karas, B. J., Ma, L., Pelletier, J. F., Qi, Z. Q., Richter, R. A., Strychalski, E. A., Sun, L., Suzuki, Y., Tsvetanova, B., Wise, K. S., Smith, H. O., Glass, J. I., Merryman, C., Gibson, D. G., & Venter, J. C. (2016). Design and synthesis of a minimal bacterial genome. *Science* 351(6280), aad6253.
2. Coradini, A. L. V., Ne Ville, C., Krieger, Z. A., Roemer, J., Hull, C., Yang, S., Lusk, D. T., & Ehrenreich, I. M. (2023). Building synthetic chromosomes from natural DNA. *Nat. Commun.* 14(1), 833.

ZymoPURE™ II Plasmid Purification Kits

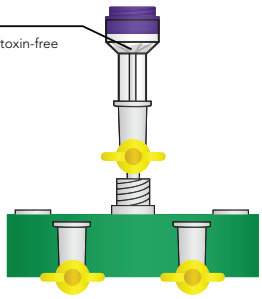
- **Fastest:** Simple 16-minute midi/maxi preps.
- **Highest Yield:** Up to 3 mg from a spin column.
- **Ultra-Pure:** Endotoxin-free and transfection-ready.

Perform Transfection-Ready Midi & Maxi Preps in Only 16 Minutes

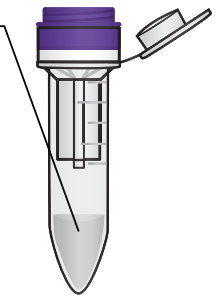
bind
rapid loading onto a spin-column via vacuum or centrifuge

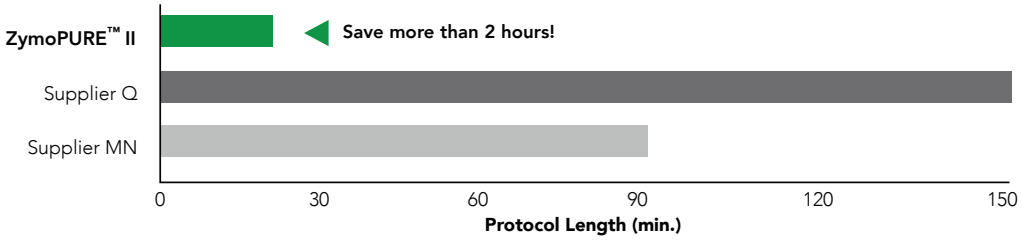


wash
for ultra-pure endotoxin-free plasmid DNA



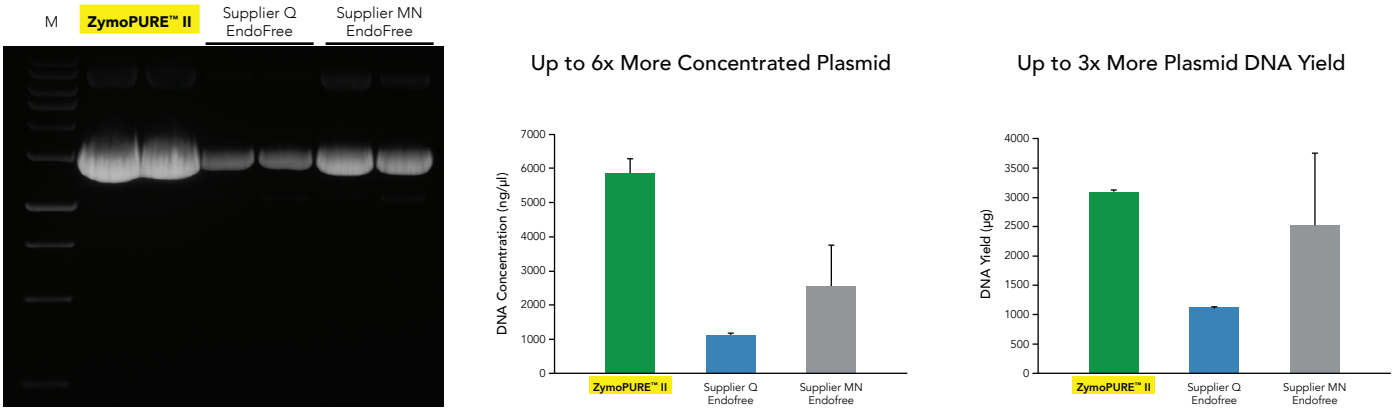
elute
transfection-ready from a spin-column





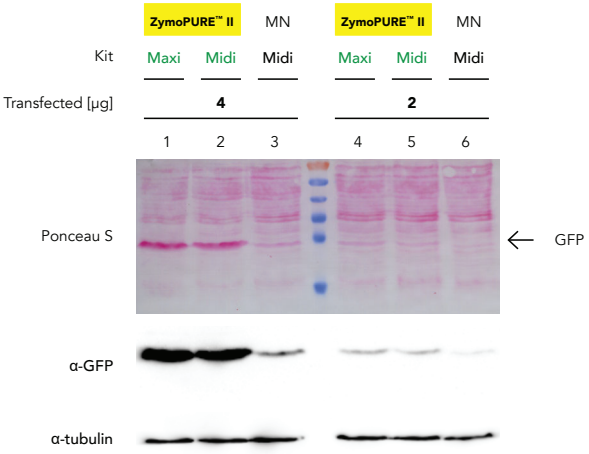
Our patented ZymoPURE™ plasmid purification system features a novel binding chemistry and EZ-Flow™ spin column design that enables simple purification of transfection-ready plasmid DNA up to 9x faster using a vacuum manifold or centrifuge. No gravity flow columns or tedious alcohol precipitation steps are required.

Consistent Highly Concentrated Transfection-Grade Plasmid



Yield and concentration for plasmid DNA isolated using the ZymoPURE™ II Maxiprep Kit compared to two endotoxin-free kits from Supplier Q and Supplier MN. Plasmid DNA (pGL3®) 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).

Dependable Purification of Transfection-Grade Plasmid



Plasmid DNA prepared with ZymoPURE Kits exhibit superior transfection efficiency. HeLa cells seeded in a 6-well plate were transfected with either 2 or 4 µg of pCI-neo®+GFP plasmid isolated from 100 ml of bacteria culture using the ZymoPURE™ II Midiprep Kit, ZymoPURE™ II Maxiprep Kit, or MN Midiprep Kit. GFP expression was assessed 48 hours later in cell lysates using western blot and Ponceau S staining. The blot was also probed with an antibody against α-tubulin in order to verify equal loading of samples. **Data generated by V.B. at University of Cologne.**

Product	Cat. No.	Size	Processing Time	Culture Volume	Minimum Elution Volume	Maximum Plasmid Yield	Endotoxins
ZymoPURE™ II Plasmid Midiprep Kit	D4200 D4201	25 preps 50 preps	≤ 18 minutes	≤ 50 ml	150 µl	1.2 mg	≤ 0.025 EU/µg
ZymoPURE™ II Plasmid Maxiprep Kit	D4202 D4203	10 preps 20 preps	≤ 18 minutes	≤ 150 ml	300 µl	3.0 mg	≤ 0.025 EU/µg
ZymoPURE™ II Plasmid Gigaprep Kit	D4204	5 preps	≤ 45 minutes	≤ 2.5 L	3 ml	25 mg	≤ 0.025 EU/µg

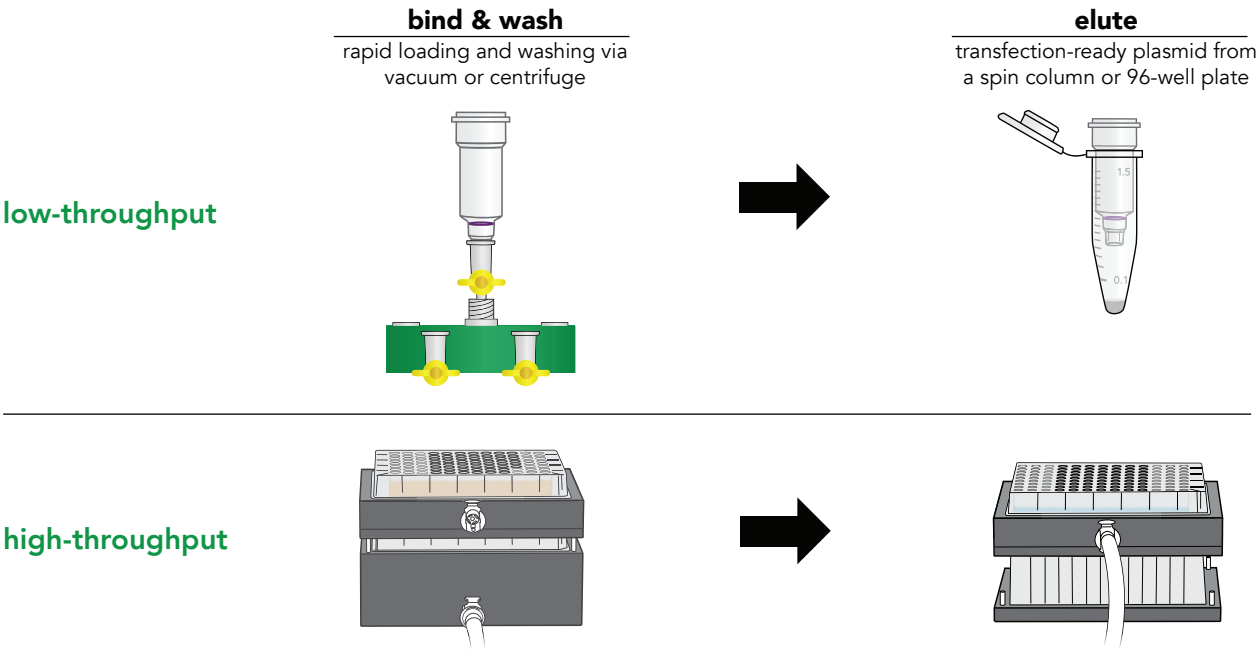
Plasmid Purification

ZymoPURE™ Plasmid Miniprep Kits

- **Convenient Formats:** Available in spin column or 96-well plate.
- **Highest Yield:** Up to 100 µg of highly concentrated plasmid DNA.
- **Transfection-Ready:** Low endotoxins and ultra-pure.

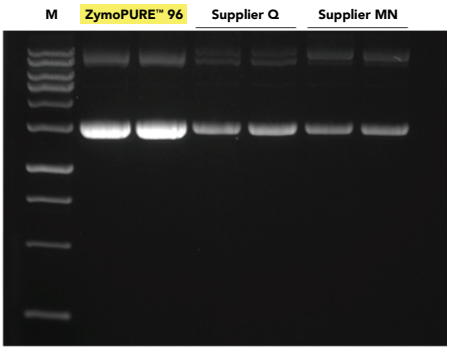
Get Your Transfection-Grade Plasmid from a Miniprep!

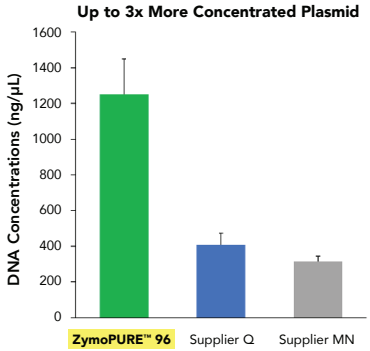
Conveniently Available in Spin Column and 96-Well Plate Formats

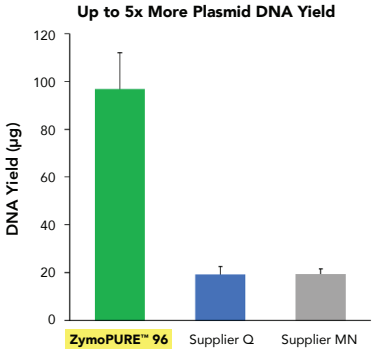


The ZymoPURE™ Miniprep is conveniently available in a spin column or 96-well plate format that allows for the purification of transfection-ready plasmid DNA using a vacuum manifold or centrifuge.

Achieve the Highest Yield of Plasmid DNA from a Miniprep



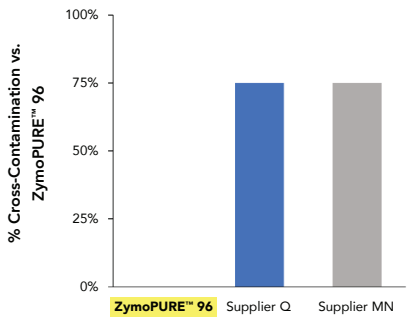




Concentration and yield for plasmid DNA using the ZymoPURE™ 96 Plasmid Miniprep Kit compared to two popular 96 vacuum plate kits from Supplier Q and Supplier MN. Plasmid DNA (pGL3®) was isolated from 5 ml of JM109 *E. coli* culture grown overnight following the manufacturer's suggested protocol (in duplicate). One (1) µl of eluted plasmid DNA was visualized post agarose gel electrophoresis. M, ZR 1 kb DNA Marker (Zymo Research).

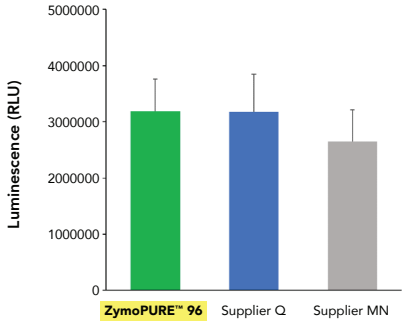
Robust Purification of High-Quality Plasmid DNA

Novel 96-Well Wash Plate Significantly Reduces Cross-Contamination



Percent cross-contamination of two popular 96 vacuum plate kits from Supplier Q and Supplier MN compared to ZymoPURE™ 96 Plasmid Miniprep Kit. Plasmid DNA (pGL3®) was isolated from 1 ml of JM109 *E. coli* culture grown overnight following the manufacturer's suggested protocol (in duplicate). Unused wells adjacent to sample-processed wells were eluted and quantified for the presence of pGL3® plasmid DNA through qPCR. The number of cross-contaminated unused wells was compared between kits.

Eluted Plasmid DNA is Transfection-Ready



Transfection efficiency of plasmid prepared using ZymoPURE chemistry compared to two popular endotoxin-free kits from Supplier Q and Supplier MN. HeLa cells seeded in a 96-well plate were transfected with 0.2 µg of pGL3® plasmid isolated from an overnight *E. coli* culture. Luciferase activity was measured after 48 hours.

Product	Cat. No.	Size	Processing Time	Culture Volume	Minimum Elution Volume	Maximum Plasmid Yield	Endotoxins
ZymoPURE™ Plasmid Miniprep Kit	D4208T D4209 D4210 D4211 D4212	10 preps 50 preps 100 preps 400 preps 800 preps	≤ 15 minutes	≤ 5 ml	25 µl	100 µg	≤ 1.0 EU/µg
ZymoPURE™ 96 Plasmid Miniprep Kit	D4214 D4215	2 x 96 preps 4 x 96 preps	≤ 60 minutes	≤ 5 ml	125 µl	100 µg	≤ 1.0 EU/µg

Advancing Cancer Immunotherapy with Exosomes

Genetically Engineered Exosomes Elicit Targeted Anti-Cancer Immunity

Cancer immunotherapy is a type of cancer treatment that utilizes the patient's own immune system to fight cancer cells, either by stimulating the immune system's ability to target and attack cancer cells or by using synthetic substances that behave like naturally occurring immune system components.[1] Traditional cancer treatments, like chemotherapy, are only effective when the drugs are present in the body and impact healthy tissues as well as cancer cells, which results in extreme side effects such as hair loss, anemia, nausea, and increased likelihood of infection. The objective of cancer immunotherapy is to provide a long-term cancer-fighting response that overcomes these adverse side effects by using the immune system to directly target any type of cancer cell.

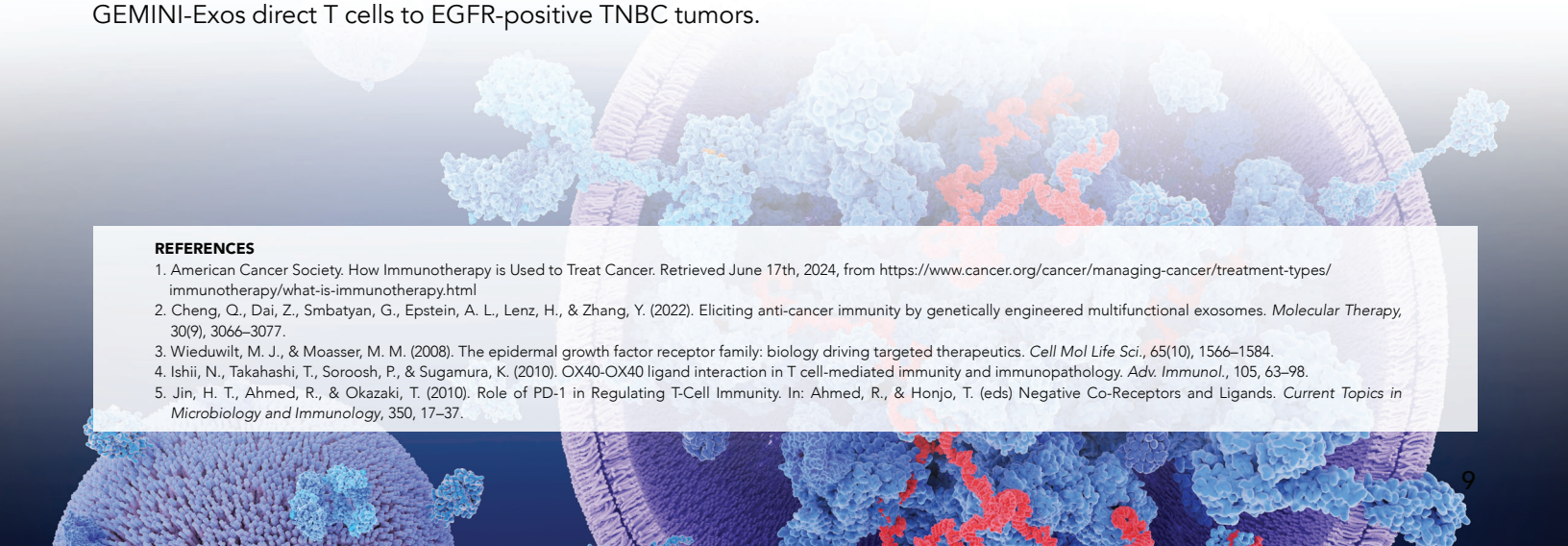
Exosomes are naturally occurring nanovesicles with therapeutic capabilities. Recently, there has been significant interest in using exosomes for cancer immunotherapy. In a study published in *Molecular Therapy*, cancer researchers with the University of Southern California explored the potential of expressing both antibody targeting groups and immunomodulatory proteins on the surface of exosomes for cancer immunotherapy.[2] The authors reasoned that because these multifunctional exosomes could both guide and stimulate T cells toward killing cancer cells, they would elicit a better immune response than current immunotherapy techniques. They tested their hypothesis by designing a genetically engineered multifunctional immune-modulating exosome (GEMINI-Exos) with antibodies specific for human T cells and cancer cells as well as immune checkpoint modulators fused to exosomal membrane proteins. The effectiveness of GEMINI-Exos was then evaluated in cellular and animal models of triple-negative breast cancer (TNBC).

Four proteins were selected for expression to generate anti-cancer immunity: αCD3, αEGFR, OX40 ligand (OX40L), and programmed death 1 (PD-1). CD3 is a T-cell co-receptor and EGFR is a growth factor receptor commonly overexpressed in cancer cells.[3] The surface-displayed monoclonal antibodies (αCD3, αEGFR) expressed by GEMINI-Exos direct T cells to EGFR-positive TNBC tumors. OX40L binds to the T-cell receptor OX40 to help stimulate an immune response.[4] Conversely, when the T-cell receptor PD-1 is bound by its ligands (PD-L1/L2), it produces an inhibitory signal.[5] Cancer cells will often express PD-L1/L2 to inactivate T cells. GEMINI-Exos-expressed PD-1 binds PD-L1/L2, effectively blocking cancer cells from inactivating T cells.

Fusion gene fragments for the proteins were created by overlap extension PCR and integrated into a plasmid mammalian expression vector using restriction enzyme cloning. Plasmid vectors were purified using ZymoPURE™ II Plasmid Kits to produce transfection-grade plasmid DNA. GEMINI-Exos were generated by transiently transfecting the purified fusion gene constructs into Expi293F cells. Centrifugation was used to isolate the genetically modified exosomes from cell culture supernatant for downstream analysis.

Immunoblot data revealed expression of the fusion proteins in the engineered exosomes and ELISA and flow cytometry results confirmed strong binding affinity of the exosomes to their protein targets and cells. Furthermore, mice treated with GEMINI-Exos exhibited the greatest tumor growth inhibition compared to combined or separate treatment with exosomes displaying only αCD3 and αEGFR or OX40L and PD-1. Tumors from GEMINI-Exos-treated mice also contained the highest percentage of CD8+ T cells and ratio of CD8+ T cells to regulatory T cells. This suggests that co-expression of both surface-displayed antibody targeting groups and immunomodulatory proteins on the same exosome vesicle is a more effective treatment than expressing them on separate exosomes. In addition, no overt toxicity or significant weight loss was observed in the treated mice.

These promising results and the potential adaptability of this technique to other kinds of cancers demonstrate the potential of genetically modified multifunctional exosomes as a potent, safe, and versatile method for cancer immunotherapy.



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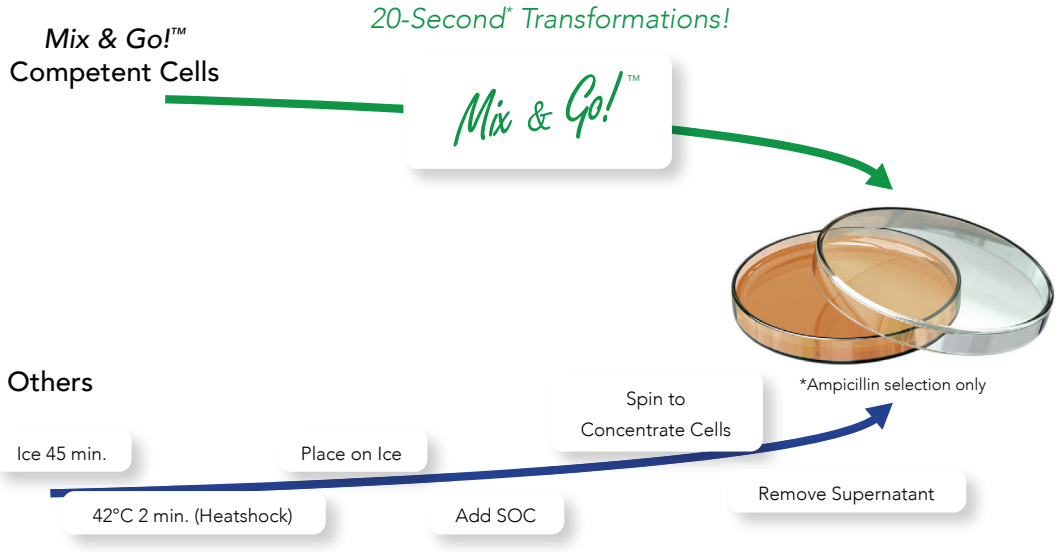
Mix & Go!™ Competent Cells

Premade strains available or kits to prepare your own

- **Simple 20-Second Transformation:** No heat shock! Just add DNA and spread on a plate.
- **High Transformation Efficiencies:** Achieve 10^8 – 10^9 transformants per µg of plasmid DNA.
- **Versatile:** Excellent for general cloning, blue-white screening, and plasmid isolation.
- **Prepare Your Own:** Easy 3-step protocol to produce reliable chemically competent *E. coli* in ≤ 45 minutes.

Simple 20-Second Transformation



Product	Cat. No.	Size	Uses
Mix & Go!™ E. coli Transformation Kit	T3001	up to 20 ml	Preparation of competent *E. coli*
Mix & Go!™ E. coli Transformation Buffer Set	T3002	up to 60 ml	Preparation of competent *E. coli*

Strain	Cat. No.	Size
JM109	T3003 T3005	10 x 100 µl aliquots (10 tubes) 96 x 50 µl aliquots (12 x 8-tube strips)
DH5 Alpha	T3007 T3009 T3010	10 x 100 µl aliquots (10 tubes) 96 x 50 µl aliquots (12 x 8-tube strips) 96 x 50 µl aliquots (PCR plate)
HB101	T3011 T3013	10 x 100 µl aliquots (10 tubes) 96 x 50 µl aliquots (12 x 8-tube strips)
TG1	T3017	10 x 100 µl aliquots (10 tubes)
Zymo 10B	T3019 T3020	10 x 100 µl aliquots (10 tubes) 96 x 50 µl aliquots (12 x 8-tube strips)

Product Guide: *Mix & Go!™* Competent *E. coli*

	JM109	DH5 Alpha	HB101	TG1	Zymo 10B
Specifications					
Strain Background	K-12	K-12	K-12	K-12	K-12
General Cloning	✓	✓	✓	✓	✓
Plasmid Isolation	✓	✓	✓	✓	✓
Recombinant Protein Expression	✓				
Production of ssDNA (F'episome)	✓			✓	
Suppression of Amber Mutations (glnV44 or supE44)	✓	✓	✓	✓	
Blue-White Selection (lacZΔM15)	✓	✓		✓	✓
High-quality and Yield of Plasmid DNA (endA1)	✓	✓			✓
Reduced Recombination & Insert Stability (recA1 or recA13)	✓	✓	✓		✓
Plasmid Size	Up to 10-15 kb		Up to 10-15 kb	Up to 10-15 kb	
Transformation of Large Plasmids (deoR)		Up to 20-32 kb			Up to 20-32 kb
Ampicillin Resistant (bla or ampR)					
Chloramphenicol Resistant (cat or cmR or camR)					
Tetracycline Resistant (Tn10 or tetR)					
Kanamycin Resistant (kanR)					
Nalidixic Acid Resistant (gyrA96 or nalR)	✓	✓			
Streptomycin Resistant (strR)			✓		✓
Genotype	F`[traD36 proA+B+ lacIq Δ(lacZ)M15] Δ(lac-proAB) glnV44 (supE44) e14- (mcrA-) thi gyrA96 (nalR) endA1 hsdR17(rk-mk+) relA1 recA1	F- φ80lacZΔM15 Δ(lacZYA-argF)U169 deoR nupG recA1 endA1 hsdR17(rK-mK+) phoA glnV44 (supE44) thi-1 gyrA96 relA1, λ-	F- Δ(gpt-proA)62 leuB6 glnV44 (supE44) ara-14 galK2 lacY1 Δ(mcrC-mrr) xyl-5 mtl-1 recA13 thi-1 rpsL20 (smR)	F'[traD36 lacIq Δ(lacZ) M15 proA+B+] glnV (supE) thi-1 Δ(mcrB-hsdSM)5 (rK- mK-mcrB-) thi Δ(lac-proAB)	F- mcrA Δ(mrr-hsdRMS-mcrBC) Φ80lacZΔM15 ΔlacX74 recA1 endA1 araD139 Δ(ara leu) 7697 galU galK rpsL nupG λ-
Catalog Number	T3003	T3007	T3011	T3017	T3019

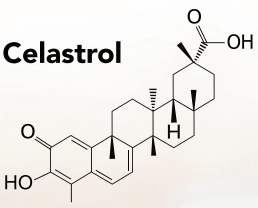
A Sweet Solution to Obesity

Synthesizing the Anti-Obesity Agent Celastrol from Table Sugar

Obesity is a global health risk characterized by excessive fat deposits that affects 1 in 8 people around the world.[1] The health consequences of obesity are severe, with obese individuals facing an increased risk of heart disease, type 2 diabetes, stroke, cancer, breathing difficulties, joint problems, gallbladder disease, and mental illnesses such as anxiety and depression.[2] Given these pressing issues, it is crucial to develop effective strategies and treatment options to manage obesity. While lifestyle changes such as dieting and increased physical activity are always recommended, certain cases require greater intervention using pharmacological medications or even bariatric surgery.[3]

Celastrol, a pentacyclic triterpenoid found in the roots of *Tripterygium wilfordii*, has long been used to treat inflammatory disorders. Recently, it was discovered that celastrol could potentially be used to treat obesity, as it triggered substantial weight loss in obese mice.[4] However, harvesting enough celastrol from plant tissue to meet global demand is not feasible and its biosynthesis is not well documented. Recognizing these challenges, researchers with the University of Copenhagen conducted a study to characterize the celastrol synthesis pathway and develop a protocol for its scalable production.[5]

Three cytochrome P450 enzymes, CYP712K1, CYP712K2 and CYP712K3, were known to catalyze oxidation reactions that generate key intermediate products in the celastrol synthesis pathway. The scientists began their study by identifying transcripts corresponding to homologous cytochrome P450 enzymes that were overexpressed in root tissue of *Tripterygium wilfordii*.



Chemical structure of celastrol

RNA transcripts were isolated, converted to cDNA, amplified by PCR, and then cloned for further characterization. Mach1 *Escherichia coli* cells were made highly competent in less than 45 minutes with Zymo Research's *Mix & Go!™ E. coli* Transformation Kit and Buffer Set and then used for cloning. Plasmid constructs were purified from the cells, transformed into *Agrobacterium tumefaciens*, and then infiltrated into tobacco leaves for metabolite analysis.

Two cytochrome P450 enzymes exhibiting activity linked to the celastrol biosynthesis pathway were identified, enabling the researchers to rebuild the pathway in yeast cells and systematically define each intermediate compound. Digestion with the Not1 enzyme was used to release genes of interest from plasmids prior to transformation. *Saccharomyces cerevisiae* cells were then transformed using a lithium acetate protocol. YPD plates containing 5-Fluoroorotic Acid (5-FOA), a genetic counter-selection agent provided by Zymo Research, were used to easily screen for the correct yeast strains. The researchers discovered the enzymes that oxidize friedelin, a precursor of celastrol, to celastrogenic acid, which then undergoes non-enzymatic conversion to celastrol.

Having confirmed the chemical pathway for celastrol synthesis, the researchers then focused their efforts on developing a scalable celastrol production system in yeast strains. They succeeded in developing an efficient synthesis method whereby table sugar was first converted to the key intermediate celastrogenic acid in an 8-step enzymatic pathway, and finally to celastrol with seven spontaneous steps split into two modules in engineered yeast.

This breakthrough method constitutes an easier, more scalable method for obtaining celastrol that previously could only be extracted from plant roots. Though the efficiency of this method must be improved for commercial deployment, the synthetic production of celastrol starting from sugar in engineered yeast shows great promise. Given celastrol's potent anti-obesity effects, the scalable production of this compound could beget new medications with the potential to dramatically reduce the health burden of obesity.

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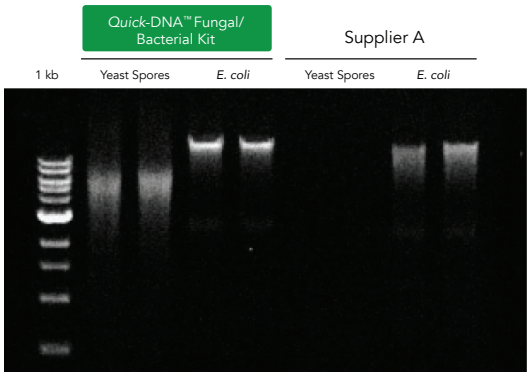
Quick-DNA™ & *Quick*-RNA™ Fungal/Bacterial Kits

- **Boost Detection:** Included BashingBeads™ ensure complete lysis of tough-to-lyse samples.
- **Ultra-Pure:** Isolated DNA or RNA is ready for PCR, Next-Gen Sequencing, arrays, etc.
- **Simple & Quick Workflow:** Extract microbial DNA or RNA in less than 20 minutes.

Description:

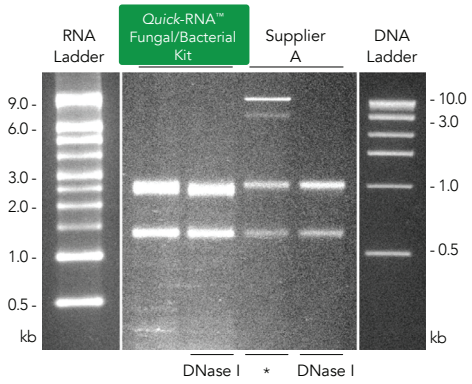
The *Quick*-DNA™ and *Quick*-RNA™ Fungal/Bacterial Kits enable rapid isolation of nucleic acid from tough-to-lyse bacteria (e.g., Gram-positive), yeast, and/or fungi. These kits use ultra-high-density BashingBeads™ for sample homogenization and a robust buffer system for nucleic acid purification. Zymo-Spin™ column technology allows eluted DNA or RNA volumes in as little as 6 µl (Microprep). Highly pure DNA or RNA is suitable for sensitive downstream applications such as qPCR, RT-PCR, and Next-Gen Sequencing.

Highest DNA Yields



DNA isolated from *Saccharomyces cerevisiae* (spores) and *E. coli* using the *Quick*-DNA™ Fungal/Bacterial Kit was high-quality and structurally intact. Equivalent amounts of yeast and bacteria were processed using the *Quick*-DNA™ Fungal/Bacterial Kit or the Supplier A kit. Equal volumes of eluted DNA were analyzed on a 0.8% (w/v) agarose gel stained with EtBr.

Recover High-Quality RNA



Total RNA was isolated from equal amounts of *E. coli* cells containing plasmid DNA (pGEM®) using the *Quick*-RNA™ Fungal/Bacterial Microprep Kit or the Supplier A kit. The samples were resolved in a 2% (w/v) agarose gel. RNA Millenium™ Markers (Ambion) and ZR 1 kb DNA Marker (Zymo Research) were used.

* = genomic (> 10 kb) and plasmid (> 3 kb) DNA contamination
DNase I = samples treated with DNase I.

Product	Cat. No.	Size	Specifications	Uses
Quick-DNA™ Fungal/Bacterial Microprep Kit	D6007	50 preps	Format: Spin Column Binding Capacity: 5 µg Elution Volume: ≥ 6 µl Processing Time: 15 minutes	Total DNA isolation from: Gram (+) and (-) bacteria; Yeast; Filamentous fungi; Unicellular algae; Filamentous algae; Protists; Either fungi or bacteria grown in media
Quick-DNA™ Fungal/Bacterial Miniprep Kit	D6005	50 preps	Format: Spin Column Binding Capacity: 25 µg Elution Volume: ≥ 35 µl Processing Time: 15 minutes	
Quick-DNA™ Fungal/Bacterial Midiprep Kit	D6105	25 preps	Format: Spin Column Binding Capacity: 125 µg Elution Volume: ≥ 150 µl Processing Time: 20 minutes	
Quick-DNA™ Fungal/Bacterial 96 Kit	D6006	2 x 96 preps	Format: 96-Well Binding Capacity: 5 µg Elution Volume: ≥ 25 µl Processing Time: 40 minutes	
Quick-RNA™ Fungal/Bacterial Microprep Kit	R2010	50 preps	Format: Spin Column Binding Capacity: 10 µg Elution Volume: ≥ 6 µl Processing Time: 10 minutes	Total RNA isolation from: Gram (+) and (-) bacteria; Yeast; Filamentous fungi; Unicellular algae; Filamentous algae; Protists; Soft tissue (limited); Food
Quick-RNA™ Fungal/Bacterial Miniprep Kit	R2014	50 preps	Format: Spin Column Binding Capacity: 50 µg Elution Volume: ≥ 25 µl Processing Time: 10 minutes	

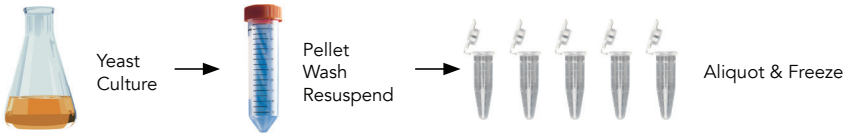
Frozen-EZ Yeast Transformation II™ Kit

- **Fast:** Yeast cells with high transformation efficiencies can be prepared in under 10 minutes.
- **Simple:** Easy method to transform yeast with single or multiple plasmids in ≤ 1 hour without carrier DNA.
- **Versatile:** Can be used with *S. cerevisiae*, as well as other fungi, including *C. albicans*, *S. pombe*, and *P. pastoris*. Compatible with both circular and linear DNA.

Description:

The Frozen-EZ Yeast Transformation II™ Kit is designed to make yeast transformations and library screening easier and more efficient than currently available methods. The yeast cells can be transformed immediately or can be stored at ≤ -70°C for later use.

Simple 10-Minute Protocol



Product	Cat. No.	Size	Transformation Efficiency	Transformation DNA Input	Competent Cell Stability	Uses
Frozen-EZ Yeast Transformation II™ Kit	T2001	120 rxns	10^5 - 10^6 cfu/µg	0.2 - 1.0 µg	≥ 1 year at -70°C	Competent yeast cell preparation

Zymoprep™ Yeast Plasmid Miniprep Kits

- **Simple:** Quickly and easily rescue plasmid from yeast.
- **Efficient Isolation:** Works well with low-copy and hard-to-isolate plasmids.
- **High-Quality:** Isolated DNA is ideal for molecular biology techniques, such as PCR, transformation, hybridization, etc.

Description:

The Zymoprep™ Yeast Plasmid Miniprep provides all the necessary reagents for plasmid isolation from *S. cerevisiae*, *C. albicans*, *S. pombe*, and any fungi whose cell walls are susceptible to yeast lytic enzyme lysis. The procedure is simple and efficient, with no need for glass beads or phenol. Reliably recover plasmid DNA from yeast colonies, patches on plates, or liquid cultures.

Yeast Plasmid Purification

Product	Cat. No.	Size	Specifications	Uses
Zymoprep™ Yeast Plasmid Miniprep I	D2001	100 preps	• Format: Isopropanol Precipitation • Elution Volume: ≥ 35 µl • Processing Time: 35 - 90 minutes • DNA Size Limits: ≤ 23 kb	Plasmid recovery from yeast
Zymoprep™ Yeast Plasmid Miniprep II	D2004	50 preps	• Format: Spin Column • Elution Volume: ≥ 10 µl • Processing Time: 35 - 90 minutes • Binding Capacity: 5 µg • DNA Size Limits: ≤ 23 kb	
Zymoprep™-96 Yeast Plasmid Miniprep	D2005 D2006 D2007	2 x 96 preps 4 x 96 preps 8 x 96 preps	• Format: 96-Well • Elution Volume: ≥ 10 µl • Processing Time: 60 - 90 minutes • Binding Capacity: 5 µg • DNA Size Limits: ≤ 25 kb	

Zymolyase - Yeast Lytic Enzyme

- **100T Equivalent:** Prepared from *Arthrobacter luteus*. Essential enzyme activities are β-1,3-glucanase and β-1,3-glucan laminaripentao-hydrolase.
- **Convenient:** Provided lyophilized along with a storage buffer for reconstitution.
- **Efficient Cell Wall Digestion:** Supplied storage buffer has been optimized to confer maximum levels of enzymatic activity.

Description:

Zymolyase is composed of essential enzymatic activities targeting yeast and fungi cell wall components. It is supplied with a storage buffer that has been optimized to confer maximum enzymatic activity.

Susceptible fungal genera: *Ashbya, Candida, Debaryomyces, Eremothecium, Endomyces, Hansenula, Hanseniaspora, Kloeckera, Kluyveromyces, Lipomyces, Metschnikowia, Pichia, Pullularia, Saccharomyces, Saccharomycodes, Saccharomycopsis, Schizosaccharomyces, Torulopsis.*

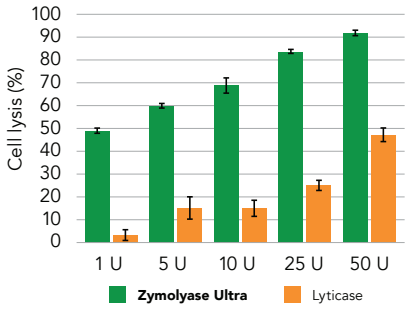
Zymolyase Ultra

- **Ultra-Efficient:** > 50 times more efficient than lyticase, works even at 4°C.
- **Ultra-Low Bioburden:** Up to 7000% less DNA contamination compared to other suppliers.
- **Exceptional Solubility & Stability:** Dissolves in seconds and stable for multiple freeze-thaw cycles.

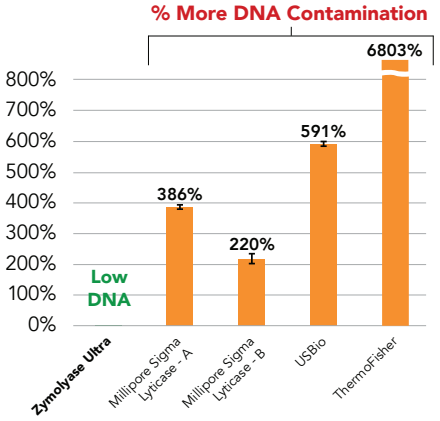
Description:

Compared to Zymolyase, Zymolyase Ultra has additional lytic activities, further enhancing its efficiency in cell wall digestion. It is extensively purified using an innovative DNA/RNA removal technology that results in extremely low nucleic acid contamination, a critical feature for various downstream applications including PCR and NGS.

Over 50x More Efficient Than Lyticase



1-50 U Zymolyase Ultra or Lyticase were used to test lysing 8 x 10^7 *C. albicans* cells at 37°C for 30 min.



Up to 7000% less DNA contamination

Bacterial and fungal DNA contamination was measured using qPCR and these measurements were converted to genomic DNA copies/Unit enzyme.

Product	Cat. No.	Size	Specifications	Uses
Zymolyase	E1004 E1005	1,000 U 2,000 U	**Concentration:** 5 U/µl after resuspension **Storage:** -20°C **Unit Definition:** One lytic unit is defined as catalyzing a 10% decrease in absorbance at 800 nm at 30°C in 30 minutes.	Spheroplast/Protoplast formation; Yeast cell fusion; Yeast transformation; Plasmid, DNA, and RNA purification; Protein purification; Yeast/fungi detection
R-Zymolyase (with RNase A)	E1006	1,000 U		
Zymolyase Ultra	E1007T E1007-2 E1007-10	100 U 2,000 U 10,000 U		

Cutting-Edge Mosquito Management

Engineering Yeast Strains for Eco-Friendly Insecticide Production

Despite advancements in modern medicine and pest control, mosquitoes remain the deadliest animal in the world, causing more than 700,000 human deaths each year.[1] There were 249 million cases of malaria alone in 2022, illustrating the importance of developing effective malaria prevention strategies and treatments.[2] Malaria together with other common mosquito-borne diseases such as Zika virus, West Nile virus, Chikungunya virus, Dengue, and Yellow Fever cause infections in over 700 million people annually, with most cases concentrated in tropical and subtropical regions where medical resources are limited.[3]

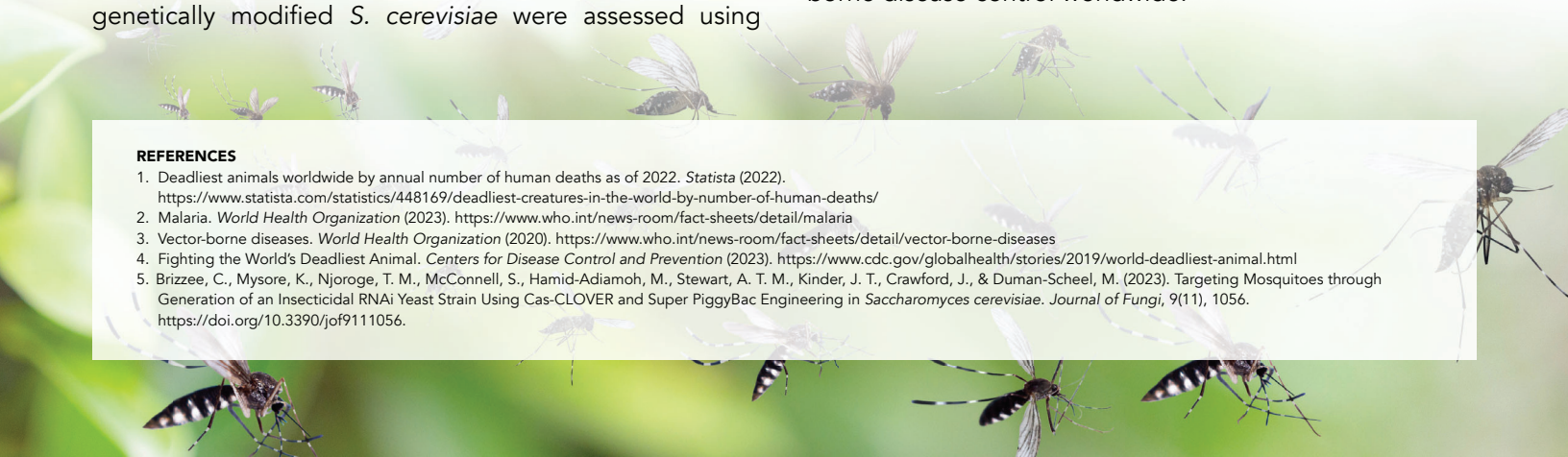
Taking appropriate measures to prevent mosquito bites, particularly in areas where mosquito-borne diseases are prevalent, is critical to controlling these illnesses and reducing their health burden. However, the global deployment of affordable vector-borne disease control programs remains challenging and growing insecticide resistance hinders progress.[4] Thus, there is an acute need for species-specific, environmentally friendly insecticides that can protect vulnerable populations against mosquito-borne illnesses.

In a 2023 study, scientists with Demeetra Ag Bio, Indiana University School of Medicine, and the University of Notre Dame applied genome editing technology in the budding yeast *Saccharomyces cerevisiae* to develop a new class of eco-friendly insecticides based on elevated shRNA expression.[5] Cas-CLOVER, an RNA-guided system similar to the original CRISPR/Cas9 technique, was used to generate auxotrophic yeast strains with *URA3* and *LEU2* gene deletions. These engineered strains were used in combination with sPB transposase/transposon technology, which was employed to prepare plasmids containing copies of the *Sh.463* short hairpin RNA (shRNA) insecticide expression cassette. The mutant *S. cerevisiae* strains were transformed with the transposon "cargo" plasmid using Zymo Research's Frozen-EZ Yeast Transformation II™ Kit for simple and efficient transformation in less than one hour.

Sh.463 expression levels in the selected clones of genetically modified *S. cerevisiae* were assessed using RT-qPCR. Yeast cells were pelleted and then resuspended in Zymo Research's YR Digestion Buffer and Zymolyase, a yeast lytic enzyme optimized for maximal digestion of the fungal cell wall. After forming spheroplasts, highly pure total RNA was extracted using the YeaStar™ RNA Kit from Zymo Research. *Sh.463* expression levels were quantified using RT-qPCR and strains with the highest expression were further characterized. Whole genome sequencing was used to verify that select *S. cerevisiae* strains retained multiple integrated copies of the *Sh.463* insecticide expression cassette in the genome.

The second-generation *S. cerevisiae* strains were then used in laboratory and field trials to assess their insecticidal activity against *A. gambiae, A. aegypti, A. albopictus, C. quinquesfasciatus,* and *C. pipiens* mosquito species. Laboratory trials demonstrated that the *S. cerevisiae* strains effectively killed mosquito larvae in the third or fourth instar of larval development prior to adult emergence. The same genetically modified yeast strains were also evaluated for insecticidal efficacy in adult mosquitoes. Strains with the highest *Sh.463* expression levels were suspended in a sucrose solution that was used as bait and fed to adult female mosquitoes. The sugar bait solution exhibited a median lethal dose of just 0.0192 mg/µL in adult mosquitoes, which is tenfold less than other insecticides prepared using similar methods. This suggests that the genome editing technology developed in this study can be used as a cost-effective alternative for controlling mosquitoes.

Pilot fermentations were then initiated and confirmed that the modified *S. cerevisiae* strains could be cultured with high growth levels and robust expression of insecticidal *Sh.463* shRNA. This suggests that genetically engineered insecticidal yeast strains may be feasible to deploy in larger scale fermentations such as malaria prevention strategies. While additional field studies and regulatory approval is still required, this research represents a significant step toward eco-friendly and targeted mosquito management, with potential applications in pesticide distribution and vector-borne disease control worldwide.



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- **ZymoScript™ One-Step RT-qPCR Kit:** Rapid cDNA synthesis and efficient qPCR in a single reaction

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