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DECODING THE GENE

Sometimes our greatest advancements come from the most unlikely of places. Such is the case with Genetics, which finds its roots in the pea plants of the Austrian Scientist Gregor Mendel. Mendel's work on the inheritance patterns observed in *Pisum sativum* went largely unnoticed in his own time but would ultimately serve as the basis for the modern field of genetics.

In the century and a half since Mendel published his work, we have seen our knowledge grow by leaps and bounds; highlighted by Watson and Crick's landmark discovery of the double helix structure of DNA and the completion of the Human Genome. Today we find ourselves at the dawn of a new era. One in which we have the capacity to sequence a genome in hours and the potential to engineer that sequence to our liking. The potential applications of our Decoding the Genome is revolutionizing all aspects of biological research and medicine. To think, it all started with the humble pea.

CONTENTS

Rapid Detection of Gene-Editing Events and Genotyping Using DNA Isolated with QuickExtract™ DNA Extraction Solution

By Ken Doyle

Gene-editing techniques have attracted considerable attention in recent years due to their therapeutic potential and application in diverse areas of research, such as disease modeling, drug discovery, and agrigenomics. A fundamental requirement common to all gene-editing methods is validation of the results. The QuickExtract DNA Extraction Solution has emerged as a popular reagent to extract genomic DNA for PCR-based validation, due to its speed, convenience, and ease of automation. It has also been used in PCR-based genotyping with a broad range of sample types.

INTRODUCTION

When isolating nucleic acids for downstream applications, researchers generally choose between two types of methods: purification, which is a multi-step, longer procedure that provides high-quality DNA or RNA, and extraction, which is a rapid procedure designed specifically for subsequent PCR or RT-PCR. The QuickExtract family of products provides a simple, rapid method for the extraction of PCR-ready DNA from a variety of sources. For most sample types, the procedure can be completed in as little as 8 minutes. The QuickExtract products are easy to use, with a single-tube protocol that does not require the use of spin columns, which can cause considerable sample loss.

The QuickExtract workflow avoids the use of toxic chemicals and is easily adapted for multiwell plates with robotic automation systems. Because of the speed of the QuickExtract method, it is ideal for applications where the results from PCR amplification are required to proceed with further experiments, such as screening and genotyping assays.

QuickExtract DNA Extraction Solution is suitable for a broad range of sample types, from hair follicles to cultured cells. Due to the more challenging nature of some sample types, additional QuickExtract kits were developed to handle these difficult samples. The QuickExtract FFPE DNA Kit eliminates the need for tedious extraction procedures that use organic solvents for formalin-fixed, paraffin-embedded (FFPE) samples. The QuickExtract Plant DNA Extraction Solution enables rapid extraction of plant genomic DNA from leaf tissues for both endpoint and real-time PCR. This white paper provides a brief overview of published applications that represent the diverse uses for the QuickExtract DNA Extraction Solution.

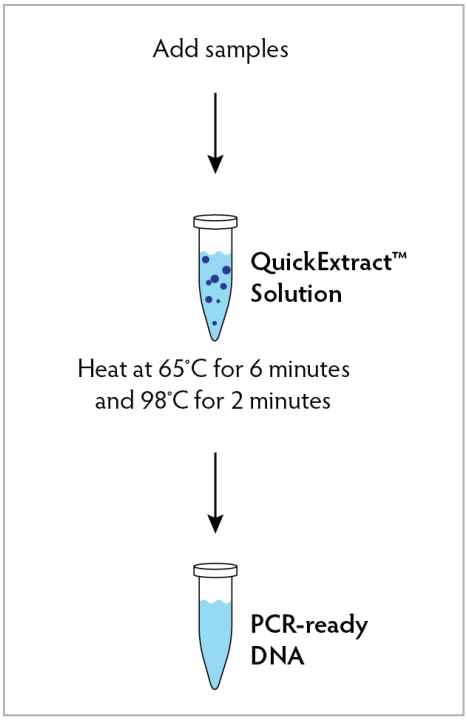

Fig. 1. The QuickExtract DNA Extraction Solution isolates PCR-ready DNA in 8 minutes or less.

CRISPR-CAS GENE EDITING

The ability to make precise, targeted changes to genes within living organisms has undergone a revolution in recent years, largely thanks to the rapid development of CRISPR-Cas technology. This technology has its origins in a bacterial immune defense mechanism that uses clustered regularly interspaced short palindromic repeats (CRISPR) together with CRISPR-associated (Cas) nucleases to recognize and destroy foreign DNA from invading viruses. Several types of CRISPR-Cas systems have been identified, of which the CRISPR-Cas9 system is the most commonly used.

Typically, foreign DNA is fragmented within bacteria and incorporated into CRISPR sequences. When the CRISPR locus is transcribed, the transcripts are processed into smaller CRISPR RNAs (crRNAs). In conjunction with bacterial trans-activating CRISPR RNAs (tracrRNA), these crRNAs can target and cleave complementary sequences in bacteriophage DNA during future infections.

Genome-editing techniques take advantage of the relatively small number of components in the CRISPR-Cas9 system.

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The sequences of the crRNAand tracrRNA are often combined into a single guide RNA (sgRNA). Sequences coding for sgRNA and Cas9 can be incorporated into a single vector that is used to transfect or transduce the host cell. Alternatively, purified Cas9 and transcribed guide RNA can be complexed and delivered to the host cell as a ribonucleoprotein (RNP). Once inside the cell, the guide will target Cas9 activity to a specific locus. Cas9 activity results in a double-stranded break (DSB), which will be repaired by the error-prone nonhomologous end-joining (NHEJ) pathway. This type of repair often produces insertion and/or deletion (indel) mutations at the target site. If the sgRNA and Cas9 sequences are delivered to the cell with a "repair template," then the cell uses an alternative repair mechanism called homology-directed repair (HDR). A repair template contains a DNA sequence to be inserted so that a locus can be modified in a specific manner. The modified target sequences are then validated, typically by PCR-based screening or next-generation sequencing (NGS).

Screening for Mutations

An important part of any gene-editing method is the screening of mutated and wild-type cells to confirm the success and efficiency of the procedure. Several assays have been developed for this purpose. Of these, enzyme mismatch cleavage (EMC) assays have been widely used in CRISPR-Cas- based methods. The first EMC assays used the bacteriophage resolvase enzymes T4 endonuclease VII[1] and T7 endonuclease I[2]. Subsequently, plant-based S1 nucleases were introduced, and the technology is currently commercialized in the line of Surveyor mutation-detection assays from Integrated DNA Technologies (IDT).

In a typical EMC assay, genomic DNA is isolated from a population of cells, containing wild-type and mutant alleles. The targeted region is amplified by PCR and then denatured and reannealed. This process results in mismatched duplexes at the targeted sites, due to cross-annealing of the wild-type and mutated sequences. Digestion of the amplicons with T7 endonuclease I, which selectively cleaves distorted duplex DNA, is followed by analysis of the DNA fragments by polyacrylamide gel electrophoresis.

The use of QuickExtract DNA Extraction Solution to prepare genomic DNA for an EMC assay was originally reported to screen for mutations introduced by zinc-finger nucleases (ZFNs)[3]. The assay is sensitive down to ~1% gene modification in a population, and the authors cite its speed, low cost, and convenience as advantages over other mutation-detection methods for ZFN-based gene editing.

Subsequently, the laboratory of Dr. Feng Zhang at the Massachusetts Institute of Technology developed a standard EMC protocol for mutation detection following CRISPR-Cas9 gene editing, using genomic DNA isolated with QuickExtract DNA Extraction Solution[4]. Extract workflow contribute to this specific, scalable, and cost-efficient assay for genome editing in mammalian cells.

Genome-Scale Knockout

Joung et al.[5] described a system for performing pooled genome-scale knockout or transcriptional activation screening using CRISPR-Cas9 in a variety of human and mouse cell lines. They constructed plasmids containing variable sgRNAs that, when pooled, make up an sgRNA library for genome-scale targeting. The system format can either encode the sgRNA, Cas9, and other necessary components on the same plasmid or on separate plasmids. The sgRNA libraries were packaged into lentivirus and transduced into the cell lines being studied. After applying selective pressure to the cells, the resulting population was screened by NGS for changes in sgRNA distribution and, from the results, a number of candidate target genes were selected for validation. Following cloning and lentiviral packaging of validation sgRNAs targeting the candidate genes of interest, these select sgRNAs were transduced into cells. Once the cells reached confluency, genomic DNA was isolated using QuickExtract DNA Extraction Solution in 96-well plates, and the regions containing the sgRNA sequences were amplified by a two-step PCR for initial validation by targeted NGS on an Illumina® platform. The researchers report that although their method is designed for loss-of-function and gain-of-function screening, it can be used for other types of screening as well.

Improved Gene-Editing Efficiency

Research using human pluripotent stem cells (hPSCs) has enabled an array of novel technologies in the study of human development, as well as disease progression and therapeutic intervention. The ability of CRISPR-Cas9 technology to introduce specific, targeted mutations in hPSCs has added a new dimension to this rapidly expanding field of research. Xie et al.[6]

describe a novel, episomal vector-based CRISPR-Cas9 system (epiCRISPR) for the generation of knockout mutations in hPSCs. The vector includes components from Epstein-Barr virus that allow it to replicate once per cell division in eukaryotes, allowing persistent expression of Cas9 and sgRNAs. Further, the transfected cells can be enriched by puromycin selection. The epiCRISPR system was tested in separate experiments for gene knockout and genomic deletions, using custom-designed sgRNA panels. After transfection of hPSCs and puromycin selection, genomic DNA was isolated using the QuickExtract DNA Extraction Solution. The target region was amplified by PCR, purified, and analyzed by restriction fragment length polymorphism (RFLP). Off-target effects were also examined using NGS. The authors reported high efficiency of gene knockouts and up to 100% efficiency for indel generation with the epiCRIPSR system, with no off-target effects.

Allele Exchange

While the majority of CRISPR-Cas9 methods are used for gene knock-out or knock-in experiments, the exchange of an entire allele is an attractive prospect for potential therapeutic applications. Kelton et al.[7] provide a proof-of-concept method to reprogram the polymorphic major histocompatibility complex (MHC) locus in murine-derived antigen- presenting cell lines, using a CRISPR-Cas9 system. The authors used Cas9 with sgRNAs that introduced DSBs flanking the MHC-I allele (~3.4kb), enabling its replacement with a MHC donor cassette, which was introduced as a double-stranded DNA template. To confirm insertion of MHC donor DNA, candidate cells were treated with QuickExtract DNA Extraction Solution, and the genomic DNA was amplified using primers covering the putative cleavage sites at the MHC locus for analysis. The authors conclude that their approach demonstrates the feasibility of replacing large MHC alleles at the native locus, suggesting its future utility for correcting MHC mismatches in allogenic cellular transplantations.

TALENS

Concern about the off-target effects of CRISPR-derived gene-editing methods has spurred renewed interest in TALENs, which typically offer high targeting specificity. Wang et al.[8] optimized a TALEN design and tested their method in human cultured cell lines, as well as human embryonic stem cells (hESCs). The method combined an optimized TALEN with a single-strand oligodeoxynucleotide (ssODN) for high-efficiency gene editing. The cutting efficiency of the optimized TALEN was validated in HEK293T cells after transfection with TALEN plasmid by treating the cells with QuickExtract DNA Extraction Solution and analyzing the percentage of gene modification using a Surveyor nuclease assay. Subsequent experiments established human tumor cell lines and hESCs with homozygous deletions of two microRNA genes, miR-21 and miR-9-2. The authors suggest that the versatility of the TALEN-ssODN method could contribute to its utility in therapeutic applications.

GENOTYPING

The speed of the QuickExtract DNA workflow has made it popular for PCR-based genotyping in a variety of organisms, from bivalves to mice.

Mussels

Ferguson et al.[9] characterized population structure, dispersal potential, and reproductive strategies in the freshwater mussel *Lampsilis cardium* from three watershed sites in Ohio, USA. Genomic DNA was extracted from adult mussels using the PureGene kit (Gentra) and from ethanol-preserved glochidia (mussel larvae) using QuickExtract DNA Extraction Solution. The QuickExtract procedure was modified to extend the time for both heating steps: 30 minutes at 65°C followed by 7 minutes at 98°C. The extracted DNA was used in PCR with primers for 12 microsatellite loci to assess local population structure relative to within-population patterns of relatedness and parentage. The study concluded that long-distance fertilization observed in this mussel species may have implications for improved recovery of imperiled freshwater mussel populations.

Mice

The G protein-coupled trace amine-associated receptor 1 (TAAR1) is stimulated by neurotransmitters and metabolites, and the receptor has multiple physiological roles, including predisposition to drug abuse and related effects. Shi et al.[10] studied the functional effects of single-nucleotide polymorphisms (SNPs) in the TAAR1 gene in mice. Genomic DNA from several mouse strains was extracted from ear or tail tissue using QuickExtract DNA Extraction Solution and amplified by PCR with primers flanking the SNP-containing regions. The amplicons were purified and sequenced. The results showed that TAAR1 mutants resulted in expression of functional, subfunctional, and nonfunctional receptors, with widely differing responses to methamphetamine. The authors suggest that SNPs in TAAR1 could provide a useful screening tool for determining predisposition to a variety of human diseases, as well as for individualizing treatments using TAAR1-specific therapies.

Transgenic Mice

Tumor-specific antigens (TSAs) constitute attractive therapeutic targets in a wide range of cancers. Unlike TSAs, tumor-associated antigens (TAAs) are expressed at varying levels in normal cells as well as tumors, making therapeutic strategies more challenging. Yong et al.[11] studied the TAA human epidermal growth

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factor 2 (Her2), which is expressed in normal breast tissue but upregulated in 15%-30% of breast cancers. They used a transgenic mouse model to examine therapies that specifically targeted Her2 antigen in tumor cells. While heterozygous $Her2^{+/-}$ mice appear to develop in a similar manner to wild-type mice ($Her2^{-/-}$), it has proven difficult to generate homozygous $Her2^{+/+}$ mice, potentially due to embryonic lethality. Genomic DNA was extracted from tail snips of $Her2^{+/+}$, $Her2^{+/-}$ and $Her2^{-/-}$ mice using QuickExtract DNA Extraction Solution, followed by PCR to examine the integration site of the Her2 transgene.

Subsequent whole-genome sequencing confirmed that the integration site of the Her2 transgene was indeed responsible for embryonic lethality, due to its insertion into the gene encoding precocious dissociation of sisters (Pds5b), which is implicated in embryonic development.

CONCLUSION

The speed and convenience of the QuickExtract methods make them ideal for PCR validation during intermediate steps in gene-editing and genotyping workflows. The QuickExtract procedure allows for a large number of samples to be screened in a rapid, automation-friendly process. The utility of the QuickExtract DNA Extraction Solution in applications requiring PCR-based screening has been recognized by its incorporation into standard CRISPR-Cas9 gene-editing protocols provided by IDT (Alt-R™ Genome Editing Detection Kit, Protocol CRS-10056-PR) and New England Biolabs (EnGen® Mutation Detection Kit, Technical Manual E3321).

REFERENCES

For a complete list of references, visit ***vwr.com/vwrbiomarke.***

Description	Cat. No.	Unit
QuickExtract™ DNA Extraction Solution	76081-768	5 mL
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No ROX	20 mL	**76204-724**	**Each**
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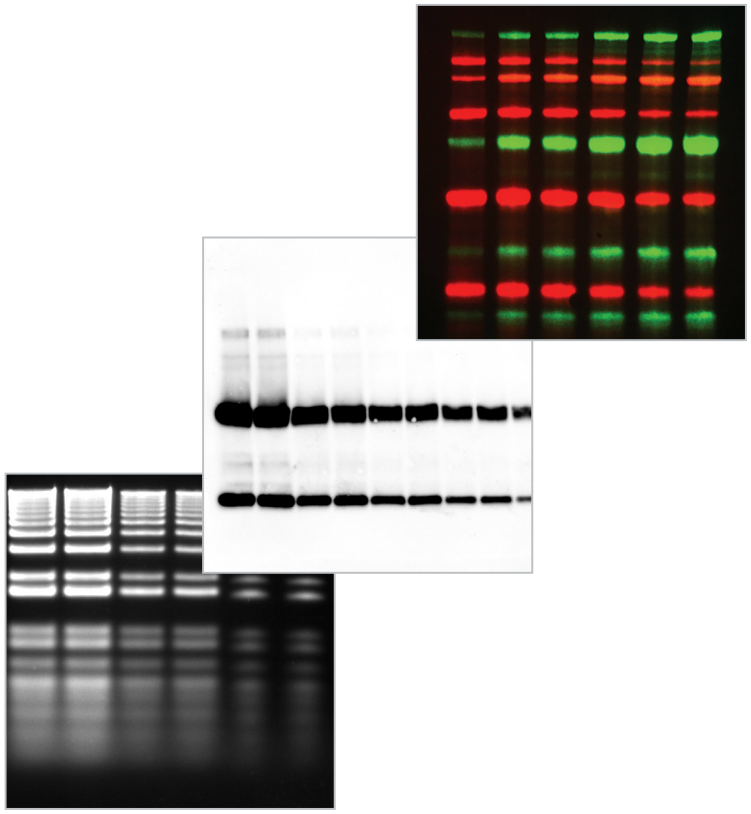

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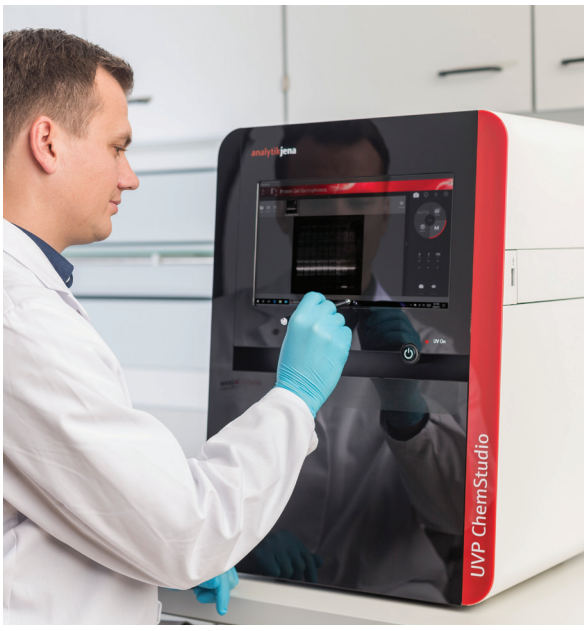

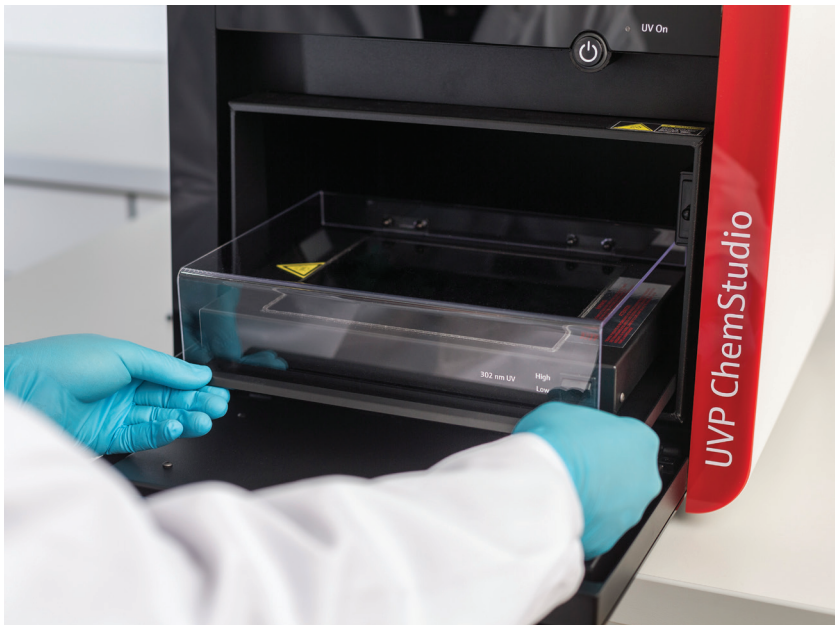

Demonstration of FlashGel™ Recovery with Sheared DNA

By Christopher Veilleux and Hugh White, Lonza Rockland, Inc.

Introduction

More and more molecular biologists are working with sheared DNA. Sheared DNA is critical for constructing genomic libraries, and is utilized in DNA reassociation and hybridization analysis. Published methods for DNA fragmentation can be classified into four categories: sonication; enzymatic digestion; hydrodynamic shearing; and nebulization. Regardless of the fractionation method, gel electrophoresis is one of the best ways to estimate mean size and distribution of the DNA material.[1]

The FlashGel™ system for recovery is a fast and simple tool for separating and recovering DNA, including fragmented DNA.[2] With the recent addition of a double-tier 2.2% concentration cassette format, it is possible to separate and recover a narrow window within a sheared DNA sample. DNA may be recovered in as little as 5 minutes, without the need for UV light or downstream purification.

Capabilities of the FlashGel™ System for Recovery of Sheared DNA

The sensitivity of the FlashGel™ system allows for a wide range of starting material concentrations. As with most gel recovery systems, the higher the concentration of starting sample, the higher the recovery yield. Intensity of recovered DNA from FlashGel™ cassettes scales well with load levels of starting DNA, (Figures 1A and 1B). The FlashGel™

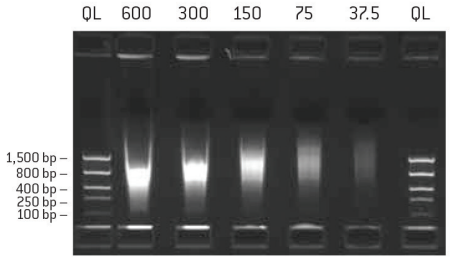

Figure 1A: Sheared DNA on the FlashGel™ system
A dilution series of sheared DNA 600–37.5ng in amount and approximately 200–4,000bp in size were run on a double-tier 1.2% FlashGel™ recovery cassette at 275V for 4 minutes. Lanes QL contain FlashGel™ quantladder.

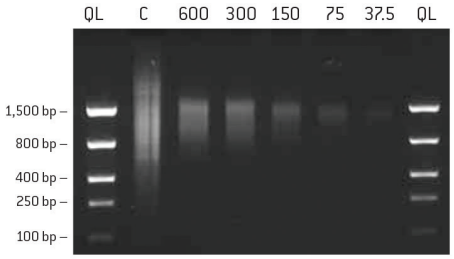

Figure 1B: Recovered samples from sheared DNA
4µL aliquots of Figure 1A samples post recovery were run on a single-tier 1.2% FlashGel™ cassette at 275V for 5 minutes. Lanes QL contain the FlashGel™ quantladder. Lane C contains 50ng of the original sheared DNA sample as a reference. Numeric lane labels indicate amount of starting material correlated from Figure 1A.

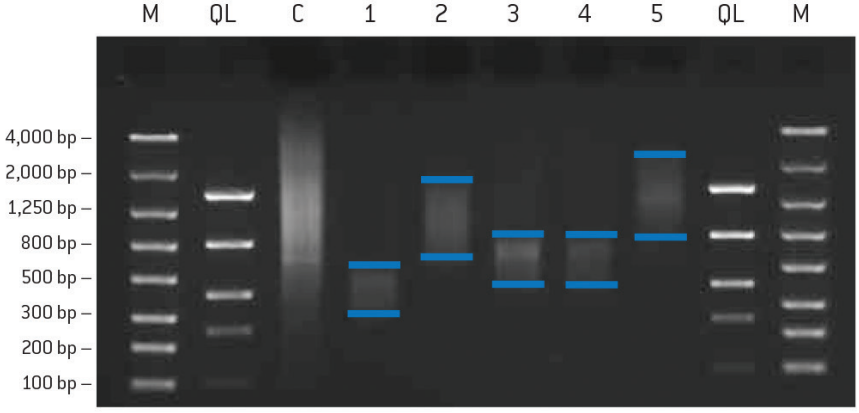

Figure 2: Recovered samples of various size selections
Recovered samples of various size selections from fragmented genomic lambda DNA. Lanes M contain the FlashGel™ 100–4,000bp marker, lanes QL contain the FlashGel™ quantladder, lane C contains 50ng of sheared DNA, lanes 1–5 contain DNA extractions from several experiments taken from 1.2% FlashGel™ recovery cassettes. Samples were run on a single-tier 1.2% FlashGel™ cassette at 275V for 7 minutes. Lane 1: 280–550bp; lane 2: 650–1,190bp; lane 3: 425–800bp; lane 4: 425–800bp (smaller load volume of Lane 3); lane 5: 800–3,000bp M QL C 1 2 3 4.

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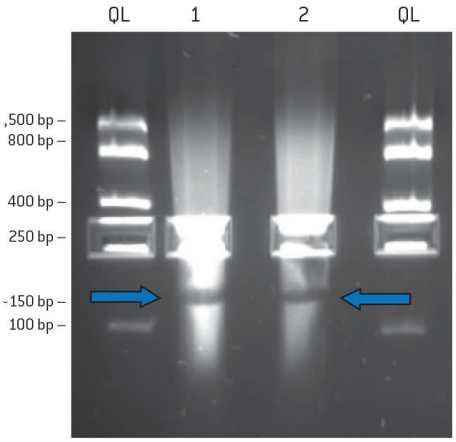

Figure 3: Sheared DNA samples following size selection recovery
Lower MW fragments from two sheared DNA samples were recovered from a double-tier 2.2% FlashGel™ recovery cassette. Several seconds of 275V current were applied after the selected areas were removed from the samples. The images show the area where the recovered samples were removed. Samples of higher molecular weight are ready to be recovered. Lanes QL contain FlashGel™ quantladder.

recovery system is capable of recovering various size ranges and various size range windows within the sheared DNA (Figure 2).

Recovery steps may be performed in parallel lanes to recover samples from multiple lanes, and multiple samples may be recovered from a single lane. Simply run the smallest of the desired size range in to the recovery well, stop the voltage, add FlashGel™ recovery buffer, and collect the sample. Then continue running the unwanted DNA through the recovery well, until the next desired size range reaches the well. Then stop the voltage, add more recovery buffer, and collect the next sample. As long as current is applied, DNA that is not recovered will migrate through the well and leave no trace contamination (Figure 3). Only when the current is stopped, and the FlashGel™ recovery buffer is added, will DNA remain in the recovery well. Recovering DNA using the FlashGel™ system is highly efficient. Figure 3 shows clear voids of samples removed during recovery.

Capabilities of 1.2% and 2.2% FlashGel™ Cassettes

Extraction range windows are predefined by selecting and using the 1.2% or the 2.2% recovery cassettes. Optimal separation range for the 1.2% FlashGel™ recovery cassettes is 50–4,000bp, and optimal separation range for the 2.2% recovery cassettes is 10–1,000bp. The 2.2% cassettes provide a larger spread of bands in the 10–400bp range, while the 1.2% cassettes show a wider spread in the larger size range (Figure 4). The 2.2% cassettes will provide a tighter and more defined range than the 1.2% cassettes within the same size range (Figure 5). The 1.2% cassettes may be better suited for a wider size selection range in a single extraction cycle.

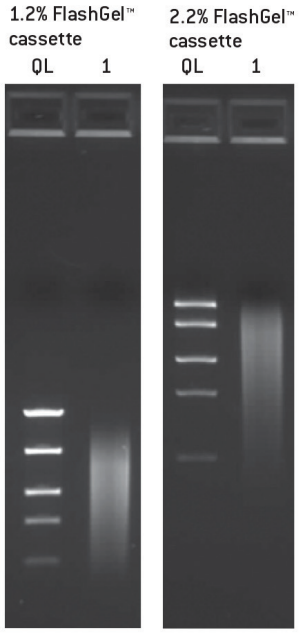

Figure 4: Demonstration of size separation Sheared DNA consisting of fragments
50–3,000bp run on 1.2% and 2.2% FlashGel™ cassettes. Lane QL is FlashGel™ quantladder, lane 1 is the DNA.

Summary

The FlashGel™ system is an efficient tool for fragmented DNA size selection. The two recovery cassette concentrations (1.2% and 2.2%) are optimized to provide narrow separation over a wide size range. With the FlashGel™ system, DNA fragments may be separated, recovered and photographed in as little as 5 minutes, without the use of UV light. Recovered samples are immediately ready for downstream applications — eliminating agarose gel preparation, band excision and purification.

REFERENCES

For a complete list of references, visit ***vwr.com/vwrbiomarke.***

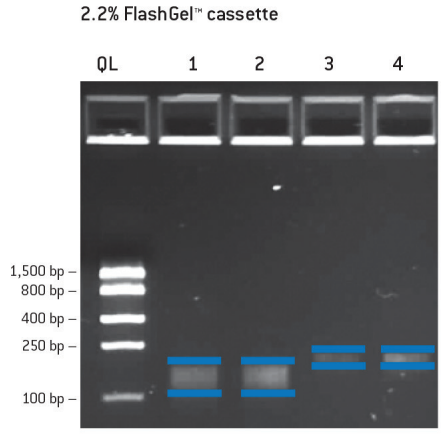

Figure 5: Demonstration of size recovery
Lanes labeled 1 and 3 are extractions of samples through recovery well window from 1.2% and 2.2% cassettes respectively. Lanes 2 and 4 are slightly higher load volumes of lanes 1 and 3 respectively. Lane QL contains the FlashGel™ quantladder.

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Instant Sticky-end Ligase Master Mix	250 Reactions	102715-944	Each

Agilent CRISPRa/i Libraries

Agilent Technologies

Designed with Content from UCSF

- Synthesized with industry's lowest error rates
- Full control of targets and guide sequence
- Available as Ready-to-Amplify and Ready-to-Clone libraries in catalog or custom options

Agilent synthesizes the highest fidelity, longest oligos in the industry, and we are now applying this technology to make CRISPR gRNA libraries. CRISPRa/i libraries with gene targets from UCSF are available in genome-wide and prevalidated subsets relevant to disease-related research.

US ONLY

Description	Cat. No.	Unit
Genome Wide Libraries		
SureGuide Human CRISPRa - Genome Wide	**76204-942**	**Each**
SureGuide Human CRISPRi - Genome Wide	**76204-940**	**Each**
SureGuide Mouse CRISPRa - Genome Wide	**76204-946**	**Each**
SureGuide Mouse CRISPRi - Genome Wide	**76204-944**	**Each**
Genome Wide Libraries		
SureGuide Human CRISPRa - Cancer/Apoptosis	**76204-950**	**Each**
SureGuide Human CRISPRa - Drug/Kinase/Phosphatase	**76204-954**	**Each**
SureGuide Human CRISPRa - Stress/Proteostasis	**76204-958**	**Each**
SureGuide Human CRISPRi - Gene Expression	**76204-966**	**Each**
SureGuide Human CRISPRi - Membrane Proteins	**76204-970**	**Each**
SureGuide Human CRISPRi - Mito/Traffic/Motility	**76204-960**	**Each**

The above six sublibraries are all available for Human and Mouse, Activation and Interference.
For Research Use Only. Not for use in diagnostic procedures.

These products will be available soon in Canada. Please contact your VWR Sales Rep to learn about similar options available in your region in the meantime.

Take the 20 Minute Route to Mycoplasma Detection

Lonza

Get Reliable Results within 20 Minutes Using the Lonza MycoAlert™ PLUS Assay

- Bioluminescence-based technology with no DNA extraction necessary
- Simply add two reagents to the culture supernatant and perform two luminescence readings
- Detects all common mollicute contaminations, except Ureaplasma (Mycoplasma, Acholeplasma, Entomoplasma, and Spiroplasma)

The MycoAlert PLUS Assay is a selective biochemical test that exploits the activity of mycoplasmal enzymes, which are not present in eukaryotic cells. If viable mycoplasma are present, the enzymes react with the MycoAlert PLUS Substrate and generate ATP. The ATP is then transferred into a light signal via the luciferase enzyme in the MycoAlert PLUS Reagent to show that mycoplasma is present. The MycoAlert Assay Control Set (does NOT contain mycoplasma) is sold separately.

Visit vwr.com/lonzamycoalert for more information!

Description	Size	Cat. No.	Unit
MycoAlert™ Plus Mycoplasma Detection Kit	10 Tests	**75860-358**	**Each**
MycoAlert™ Plus Mycoplasma Detection Kit	30 Tests	**75860-360**	**Each**
MycoAlert™ Plus Mycoplasma Detection Kit	50 Tests	**75860-362**	**Each**
MycoAlert™ Plus Mycoplasma Detection Kit	100 Tests	**75866-212**	**Each**
MycoAlert™ Assay Control Set	10 Tests	**75870-450**	**Each**

Capture Your Genetic Snapshot

By Shaun Veran, Sr. Research Associate, Zymo Research

The Importance of Sample Collection & Preservation

Sample collection is the first step in the purification of all workflows that involve nucleic acids. In this critical step, it is imperative that steps are taken to ensure proper storage and preservation of the samples as these methodologies can dramatically affect downstream analyses. We have all heard the saying "garbage in, garbage out." Compositional changes and bias can occur due to nucleic acid degradation, cellular growth/decay, and the logistics of collection.

Current preservation methods use cold-chain logistics to prevent or slow these changes to a sample. However, these systems are very costly and without proper storage conditions, can result in a misrepresentation of an analyte's abundance, bias results, reduce assay sensitivity, and decrease reproducibility.

These steps are most critical for RNA which is especially vulnerable to degradation from the prevalence of RNases and an unstable phosphodiester bond of the backbone. However, DNA can also be prone to rapid degradation and complete signal loss. Because of this, it is necessary to reliably preserve these nucleic acids.

Stabilization of Nucleic Acids

Things change, make sure your results do not! Standardized sample collection in the research and clinical setting is becoming more important. But rather than using costly cold-chain logistics, Zymo Research has reimagined the sample collection process with a single preservation reagent, DNA/RNA Shield™.

DNA/RNA Shield™ stabilizes both DNA and RNA without the need for cold storage (-80°C), protecting samples even at ambient temperatures for up to 30 days. This eliminates the need for costly storage conditions and specialized sample collection procedures while maintaining a preserved snapshot of the DNA/RNA profile, even through multiple freeze-thaw cycles.

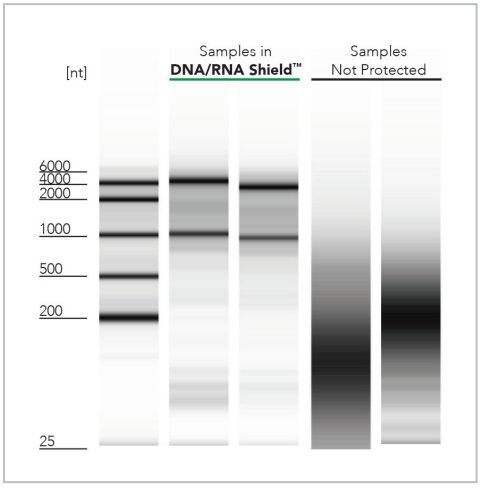


Figure 1. Whole blood samples +/- DNA/RNA Shield™ were subjected to > 2 freeze thaw cycles. Total RNA was subsequently purified using the Quick-RNA™ Whole Blood kit.

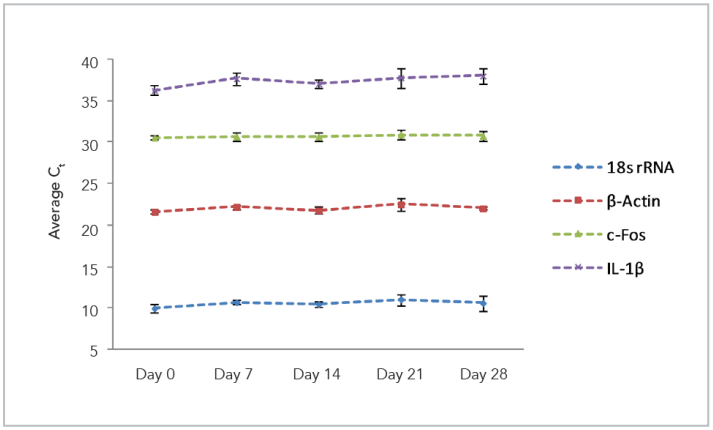


Figure 2. RNA from tissue stored in DNA/RNA Shield™ (included with the Quick-RNA™ Miniprep Plus Kit) is preserved at ambient temperature. RNA from muscle tissue (mouse) was purified using the Quick-RNA™ Miniprep Plus Kit and analyzed by RT-PCR.

RNA samples are very susceptible to degradation. Time, temperature, and freeze-thaw cycling all contribute to issues with sample stabilization. DNA/RNA Shield™ provides worry-free protection against these changes. As seen in Figure 1, the DNA/RNA Shield™ effectively prevents degradation after whole blood samples underwent multiple freeze-thaw cycles before purification.

DNA/RNA Shield™ protects samples kept at ambient temperatures over time. Analysis of RNA purified from tissue stored in DNA/RNA Shield™ shows there is no change in the average C_t values of muscle tissue stored in DNA/RNA Shield™ for up to 30 days at ambient temperatures (Figure 2).

Maintain Nucleic Acid Profiles

Shifting nucleic acid profiles can also be observed for unprotected samples. This is most exemplified in the microbial composition of a stool sample (Figure 3). Without proper stabilization, high levels of variability and bias are introduced. The compositional proportion of DNA for specific species changes drastically over time in unprotected samples. Conversely, DNA/RNA Shield™-protected samples change little compared to the original collected sample.

ZYMO RESEARCH
The Beauty of Science is to Make Things Simple

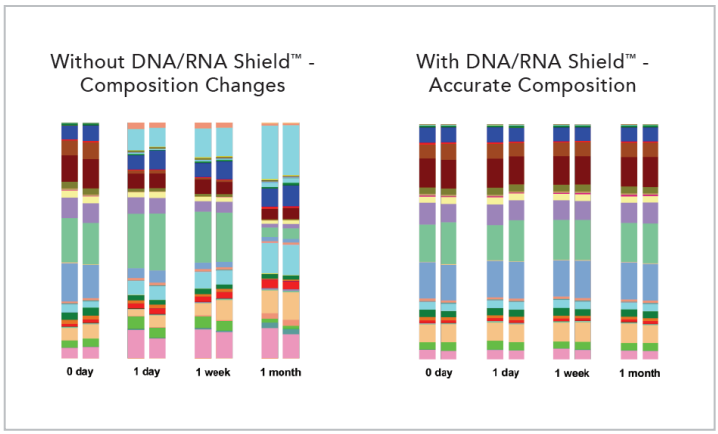
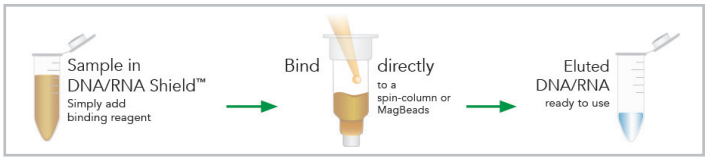


Figure 3. Microbial composition (indicated by different colors for different species) from a stool sample is unchanged after one month at ambient temperature with DNA/RNA Shield™. Stool samples suspended in DNA/RNA Shield™ and stored at room temperature were compared to stool without preservative for one month. Samples were collected at the indicated time points and DNA was subsequently extracted with the ZymoBIOMICS® DNA Miniprep Kit. The DNA was then subjected to microbial composition profiling via 16S rRNA gene targeted sequencing. Samples stored with DNA/RNA Shield™ had a constant microbial composition while the samples stored without shifted dramatically.

It provides an easy and effective method to stabilize both DNA and RNA over time at various temperatures, despite freeze-thaw cycles.

Simplified Purification

DNA/RNA Shield™ drastically streamlines the process of sample collection and nucleic acid purification. It is compatible with all Zymo Research purification kits, and unlike other preservatives such as RNAlater®, it does not need to be removed prior to sample processing. Simply add binding reagent to a sample protected in DNA/RNA Shield™ and purify.



To help determine which kit would work best for your needs, please refer to the Sample Collection Product Guide listed at the end of this article. DNA/RNA Shield™ is offered in a variety of collection vessels to best suit the needs of your workflow: Blood Collection Tube, Fecal Collection Tube, Lysis Tube, or Swab & Collection Tube. Once a vessel is chosen, the appropriate extraction kit can be determined from the starting sample type.

SAMPLE COLLECTION PRODUCT GUIDE

1. Choose a Collection Device:

Swabs

8mL tube with breakable sterile swab

- Mouth
- Nose
- Throat
- Fluid

Stool

15mL tube with screwcap scoop

- Virus
- Microbe
- Host

Blood

10mL blood tube

- Fresh
- EDTA
- Citrate
- Heparin

Swabs

2mL tube with high density beads

- Animal
- Plant
- Insect
- Microbe

2. Choose a Purification Kit:

SAMPLE TYPE	DNA	RNA	BOTH
Blood Collection Tube			
Entire Blood Tube	Quick-DNA/RNA™ Blood Tube Kit		
200µL Whole Blood Input	Quick-DNA™ Plus Kits	Quick-RNA™ Whole Blood Kit	Quick-DNA/RNA™ Kit
Fecal or Soil Samples	Quick-DNA™ Plus Kits	Quick-RNA™ Plus Kits	—
Cell or Soft Tissue	Quick-DNA™ Plus Kits	Quick-RNA™ Plus Kits	Quick-DNA/RNA™ Kit
Microbiomic Samples	ZymoBIOMICS® DNA Kits	ZymoBIOMICS® RNA Kits	ZymoBIOMICS® DNA/RNA Miniprep Kits
Viral Samples	Quick-DNA™ Viral Kits	Quick-RNA™ Plus Kits	Quick-DNA/RNA™ Viral Kit

US ONLY

Description	Size	Cat. No.	Unit
DNA/RNA Shield Blood Collection Tube	50 Tubes	**76020-434**	**Each**
DNA/RNA Shield Fecal Collection Tube	10 Tubes	**76020-422**	**Each**
DNA/RNA Shield Lysis Tube (Microbe)	50 Tubes	**76020-426**	**Each**
DNA/RNA Shield Lysis Tube (Tissue)	50 Tubes	**76020-098**	**Each**
DNA/RNA Shield Collection Tube with Swab (1 mL fill)	10 Tubes	**76020-100**	**Each**
DNA/RNA Shield Collection Tube with Swab (2 mL fill)	10 Tubes	**76020-430**	**Each**
DNA/RNA Shield Reagent	50 mL	**76020-420**	**Each**
DNA/RNA Shield Reagent (2X Concentrate)	25 mL	**76020-444**	**Each**

These specific products are not available in Canada. Please contact your VWR Sales Rep to learn about similar options available in your region.

Monarch® Kits for DNA and RNA Purification

NEW ENGLAND BioLabs Inc.

Sustainably-Designed Purification Kits for Total RNA Extraction, Isolation of Plasmids, and Cleanup of DNA Fragments

- **Excellent performance —** Consistently high nucleic acid yields and purity
- **Ease of use —** Enjoy fast, simple protocols
- **Flexibility and convenience —** In addition to convenient kit formats, buffers and columns are available separately

Monarch kits enable quick and easy purification of high-quality DNA and RNA, suitable for direct use in a variety of downstream applications.
Recover pure, intact DNA and RNA in minutes with fast, user-friendly protocols and optimized buffer systems, and focus your time on the experiments that will drive your research forward. Request a free sample from your VWR representative today.

Description	Size	Cat. No.	Unit
Monarch Total RNA Miniprep Kit	50 Preps	103529-148	Each
Monarch Plasmid Miniprep Kit	50 Preps	102971-698	Each
Monarch Plasmid Miniprep Kit	250 Preps	102971-696	Each
Monarch DNA Gel Extraction Kit	50 Preps	102971-670	Each
Monarch DNA Gel Extraction Kit	250 Preps	102971-668	Each
Monarch PCR and DNA Cleanup Kit	50 Preps	102971-674	Each
Monarch PCR and DNA Cleanup Kit	250 Preps	102971-672	Each
Monarch DNA Cleanup Columns (5 µg)	100 Columns	102971-676	Each

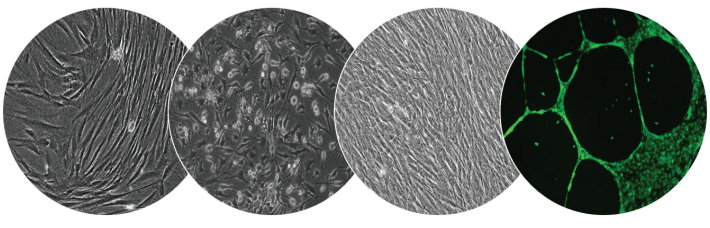
CELLvo™ Cells

StemBioSys®

The Cell of Choice for Discerning Scientists. Never Exposed to Artificial Substrates.

- More proliferative
- More natural response to experimental variables
- Less phenotypic drift

CELLvo™



CELLvo Cells are primary cells isolated and grown on CELLvo Matrix, a cell-derived substrate that mimics the natural extracellular microenvironment containing over 150 proteins. CELLvo Cells are never exposed to artificial substrates and when grown on CELLvo Matrix express a more natural gene profile compared to cells grown on standard tissue culture plastic.

Description	Size	Cat. No.	Unit
CELLvo Human Bone-Marrow Mesenchymal Stem Cells	>1,000,000	76203-012	Each
CELLvo Human Cord Blood Endothelial Progenitor Cells from a Single Donor Cryopreserved at P4	>500,000	76169-452	Each
CELLvo Human Mesenchymal Stem Cell – Wharton's Jelly Cryopreserved at P2	>500,000	76200-216	Each
CELLvo Primary H-Chondrocytes (HC-a) P0	>1,000,000	76203-918	Each
CELLvo H-Chondrocytes (HC-a) P1	>1,000,000	76203-926	Each

Mirus Bio TransIT® Lentivirus System

An Optimized Transfection Reagent and Lentivirus Packaging Mix for High, Functional Titers with a Streamlined Workflow

US ONLY

- Produce 2–3X higher functional titers than Lipofecatmine® 2000 and 3000
- Achieve higher titers without the need to concentrate
- No media change required, single harvest

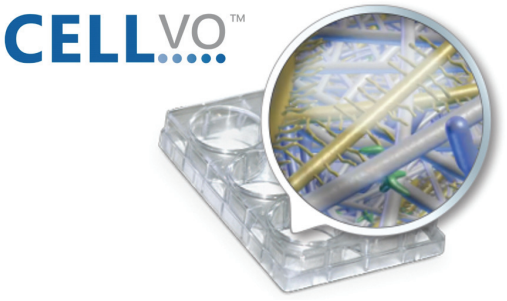
Combined with an efficient host-genome integration mechanism capable of infecting both dividing and non-dividing cells, recombinant lentivirus has become a central gene delivery tool for robust and stable transgene expression in target cells. The lentivirus platform is often limited by insufficient viral titers requiring concentration before use. To address this limitation, Mirus Bio developed the TransIT® Lentivirus System for enhanced delivery and expression of the essential lentivirus genes required for higher-titer lentivirus production in adherent or suspension HEK 293 cell types. The TransIT® Lentivirus System is composed of the TransIT®-Lenti Transfection Reagent, Lentivirus Packaging Mix Powered by MISSION® Genomics, and TransduceIT™ Reagent.

Description	Cat. No.	Unit
TransIT® Lentivirus System RXN5	**76210-950**	**Each**
TransIT® Lentivirus System RXN34	**76210-952**	**Each**
TransIT®-Lenti Transfection Reagent 0.3mL	**75814-982**	**Each**
TransIT®-Lenti Transfection Reagent 1.5mL	**75814-980**	**Each**
Lentivirus Mix Powered by MISSION® RXN5	**76218-672**	**Each**
Lentivirus Mix Powered by MISSION® RXN34	**76211-034**	**Each**
TransIT®-Lenti Transfection Reagent 5 x 1.5 mL (7.5mL)	**75814-986**	**Each**
TransIT®-Lenti Transfection Reagent 10 x 1.5 mL (15 mL)	**75814-994**	**Each**

These specific products are not available in Canada. Please contact your VWR Sales Rep to learn about similar options available in your region.

CELLvo™ Matrix — The Next Evolution in Cell Culture™

Use in Mammalian, Adherent Cell Culture to Avoid Unnatural Cell-Matrix Interactions



- Cells grown on CELLvo Matrix express a more natural gene profile and reduced donor-donor variability
- Cells grown on CELLvo Matrix exhibit more natural responses to experimental variables
- CELLvo Matrix enhances isolation of primary cells

CELLvo Matrix is an extracellular matrix of proteins synthesized *in vitro* by bone marrow stromal cells and contains more than 150 proteins that were secreted and assembled by the cells during production. The final product is cell free with only the ECM attached to the surface of the dish providing a native three-dimensional microenvironment for rapid expansion of hiqh quality adherent cells...There is no place like home!

Description	Growth Area	Cat. No.	Unit
CELLvo Matrix			
6-Well Flat Bottom Plates	9.5 cm^2	**75853-938**	**Pack of 5**
T75 Vented Flasks	75 cm^2	**75853-856**	**Pack of 5**
T150 Vented Flasks	150 cm^2	**75853-858**	**Pack of 5**
CELLvo XF Matrix			
XF-Matrix 6-Well Flat Bottom Plates	9.5 cm^2	**76183-420**	**Pack of 5**
XF-Matrix T75 Vented Flasks	75 cm^2	**76183-422**	**Pack of 5**
XF-Matrix T150 Vented Flasks	150 cm^2	**76183-424**	**Pack of 5**

VWR Seradigm LIFE SCIENCE

designed for discovery

VWR Life Science Seradigm FB Essence

Proven Performance and Consistency

- Nutritionally rich, cost-effective alternative to Fetal Bovine Serum (FBS)
- Proven effective across a broad range of cell types and origins
- Long term stability of supply and pricing
- 100% US Origin

FB Essence is a nutritionally rich, cost-effective alternative to Fetal Bovine Serum (FBS) containing FBS, Bovine Calf Serum, Equine Serum, and a proprietary blend of supplements, vitamins, minerals, and growth factors.

FB Essence offers proven performance and consistency, low endotoxin and hemoglobin levels, and can produce equivalent results to FBS in many applications. FB Essence is 100% US origin.

Description	Size	Cat. No.	Unit
FB Essence	50 mL	10805-184	Each
FB Essence	500 mL	10803-034	Each
FB Essence, Heat Inactivated	50 mL	10799-384	Each
FB Essence, Heat Inactivated	500 mL	10799-390	Each

Gamma irradiation available upon request.

Reagents that Advance Scientific Discovery

PEPROTECH
OUR SUPPORT, YOUR DISCOVERY

PeproTech Cytokines and Growth Factors

- RUO and animal-free grade offered in human, murine, and rat
- Flexible sizes
- Lot-to-lot consistency

PeproTech offers a comprehensive line of full length and fully biologically active recombinant proteins that are developed in-house by PeproTech's experienced protein scientists.

Description	Size	Cat. No.	Unit
Human FGF-basic (146aa)	10 µg	10771-944	Each
Human FGF-basic (154aa)	10 µg	10778-898	Each
Human GM-CSF	20 µg	10780-398	Each
Human IL-2	50 µg	10779-566	Each
Human IL-4	20 µg	10779-606	Each
Human M-CSF	10 µg	10773-706	Each
Human SCF	10 µg	10780-450	Each
Human TPO	10 µg	10773-600	Each

How Can a Chromatography Bead Free Up Your Time?

Distributor
GE Healthcare

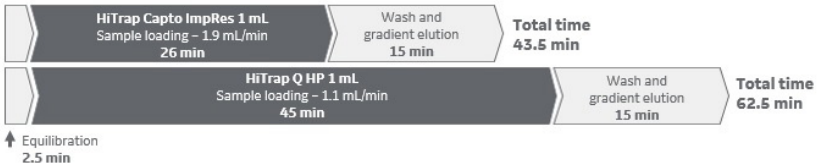
By Emma Lind, Product Manager for Ion Exchange Chromatography at GE Healthcare

Have you ever considered how a resin bead in your chromatography column can affect your ion exchange (IEX chromatography) run time?

Today's protein researcher is pressed to deliver results faster than ever before. The less time spent on purification, the more time you will have to focus on studying your protein.

Ion exchange chromatography can be used in the first steps of protein purification for both native proteins and untagged recombinant proteins. While protein purification has become almost routine, new technology and materials always provide an opportunity to look for improvements. That is the case when choosing a Capto™ ImpRes resin for use with viscous samples.

Example with **50 mL** *E. coli* lysate **run in cold room**



Speeding Up Run Time for Viscous Samples

Viscous samples, such as *E. coli* extract, can increase back pressure on the column and force you to reduce the flow rate, resulting in longer load times. Utilizing a robust, modern, and rigid resin — such as Capto Q ImpRes — reduces sample run time significantly by allowing high flow rate with low column backpressure, as seen in the example above.

HiTrap Capto ImpRes contains Capto ImpRes high-flow agarose resin, which has much better pressure/flow properties than Sepharose™ High Performance resin. (Capto ImpRes resin has maximum flow rate of 300cm/h vs 150cm/h for Sepharose High Performance.) This allows more flexibility — even in the HiTrap format — for viscous samples such as *E. coli* lysate, and allows them to be loaded at higher flow rates in a HiTrap Capto ImpRes than a HiTrap HP column. In this example, the IEX total run time is 30% shorter with HiTrap Capto Q ImpRes, than a HiTrap Q HP, with an otherwise similar performing column.

HiTrap Capto ImpRes: a Versatile Tool

HiTrap Capto ImpRes columns can be used for viscous samples as well as for other high-resolution IEX chromatography procedures with a broad range of samples. Since the resolution is similar to Sepharose High Performance, Capto ImpRes can be used in these applications as well, and there is no need to have both columns on hand.

Capto ImpRes resin has been used in diverse applications, including:

- Analyzing the influence of a mitochondrial protein in mammalian cytosolic Fe-S repair
- Researching the mode of action of antimalarial drug primaquine

HiTrap™ Capto™ ImpRes can serve as a universal column for routine work in a research lab. Use HiTrap Capto Q ImpRes for anion exchange chromatography, and HiTrap Capto SP ImpRes for cation exchange chromatography.

REFERENCES

For a complete list of references, visit ***vwr.com/vwrbiomarke.***

Product Description	Pack Size	Cat. No.	Unit
HiTrap™ Capto™ SP ImpRes Prepack Columns	5 x 1 mL	**97067-776**	**Each**
HiTrap Capto SP ImpRes Prepack Columns	5 x 5 mL	**97067-778**	**Each**
HiTrap Capto SP ImpRes Resin	25 mL	**97067-934**	**Each**
HiTrap Capto SP ImpRes Resin	100 mL	**97067-932**	**Each**
HiTrap Capto Q ImpRes Prepack Columns	5 x 1 mL	**97067-780**	**Each**
HiTrap Capto Q ImpRes Prepack Columns	5 x 5 mL	**97067-782**	**Each**
HiTrap Capto Q ImpRes Resin	25 mL	**97067-938**	**Each**
HiTrap Capto Q ImpRes Resin	100 mL	**97067-936**	**Each**

Image Now! Who Has Time to Wait?

azure biosystems

The c300 Imager is Ideal for an Individual Lab's Western Blotting Needs

- Chemiluminescent performance that's affordable
- Push button imaging and easy quantitation
- Lab workhorse

The c300 is a powerful yet budget-concious system for fast-moving scientists who would like to image their chemilumenescent blot and protein/DNA gels now, and not when a colleauge down the hall is done using the shared imaging system. Addtionally, it's fully upgradable to a fluorescent imaging system if your experimental needs change.

Description	Cat. No.	Unit
c300: The Darkroom Replacer Imaging System	10147-220	Each

Simplifying Immune Oncology Research

CORNING

Novel 3D Tumor Model Streamlines High Throughput Immunotherapy Testing

Content provided by Corning

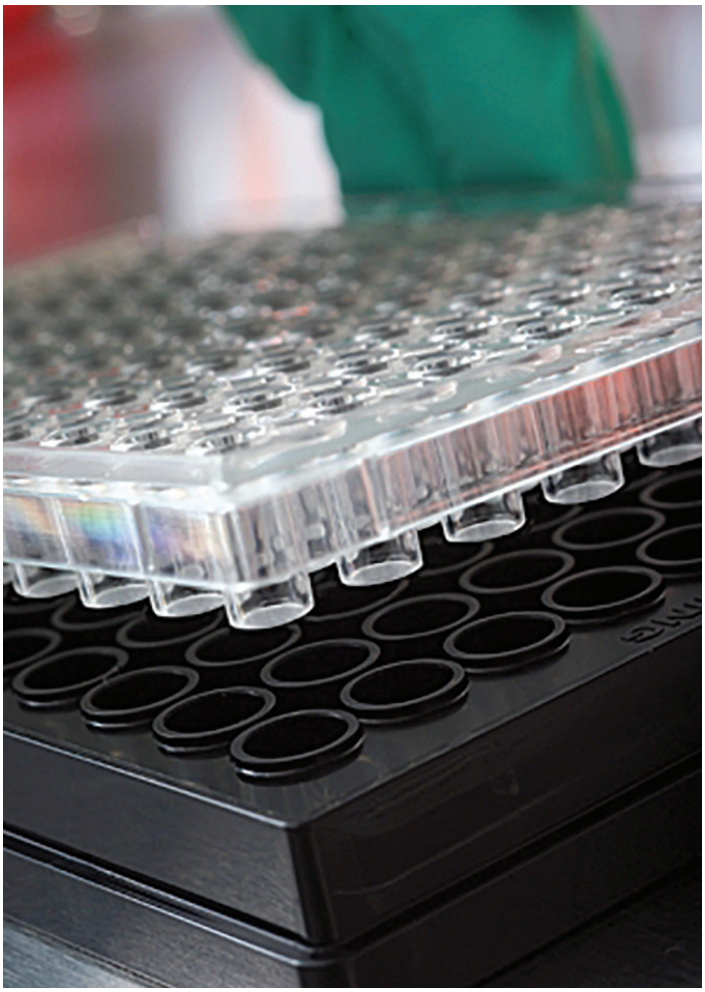
Immunotherapy has become a topic of increased interest for scientists studying cancer treatment. By using a patient's own immune cells, immunotherapy has shown promise in attacking cancer cells with best-case clinical results of total cancer remission. But immunotherapy is not a one-size-fits-all approach. Treatment strategy and efficacy can vary across patients and cancer types.

In order to more carefully study immunotherapy as a means to treat cancer, scientists need to be able to utilize efficient, *in vitro* screening models to examine targeted therapy options. This type of research has historically been conducted using cell culture models that allow scientists to study immune cell migration, and that can be easily adapted for high throughput screening. However, these models typically utilize two-dimensional (2D) cell culture monolayers which do not accurately reflect a tumor's complexity in the human body, leading to a growing trend to use more *in vivo*-like three dimensional (3D) models. While current 3D models are more *in vivo*-like, in order to incorporate the immune cell component, these 3D models require directly adding immune cells to the 3D spheroids. This may not be an accurate reflection of how immune cells migrate towards, and further invade a tumor within a patient suffering from cancer.

According to Hannah Gitschier, Corning Life Sciences Applications Lab Manager in Kennebunk, ME, "With immunotherapy, it is imperative that researchers have solid models to best recapitulate the *in vivo* microenvironment and interactions between the engineered immune cells and patient tumor samples. These models must be easy to use and transferrable to a high throughput screening environment, enabling rapid screening of conditions to best predict clinical outcomes. Traditionally, 2D assays were the method of choice given the high proliferation rate of cells in 2D culture, as well as the reproducibility of assays. However, with emerging technologies and the development of novel lab consumables, 3D assays are becoming easier to adapt and more relevant for researchers end goals."

Creating a Better Way

Hilary Sherman, a Corning Applications Scientist created a 3D culture model to observe immune cell and tumor cell interactions by combining the Corning® 96-well spheroid microplate with the



high throughput screening (HTS) Transwell®-96 tissue culture system. Coated with Corning's unique Ultra-Low Attachment surface, the spheroid microplate allows for highly reproducible, single multicellular tumor spheroids to form in each well. The HTS Transwell permeable support systems are commonly used in migration and invasion studies, with a flat bottom receiver plate for monolayer culture. This novel combination of both products allows researchers to observe changes to tumor behavior, and by adding immune cells to the Transwell insert, to observe their rates of migration through the membrane and towards the tumor spheroids in the receiver plate. By combining both technologies one can study how these two cell types interact in a more *in vivo*-like environment.

Adds Gitschier, "We frequently utilize both HTS Transwell systems and the spheroid microplates in our Applications lab. When

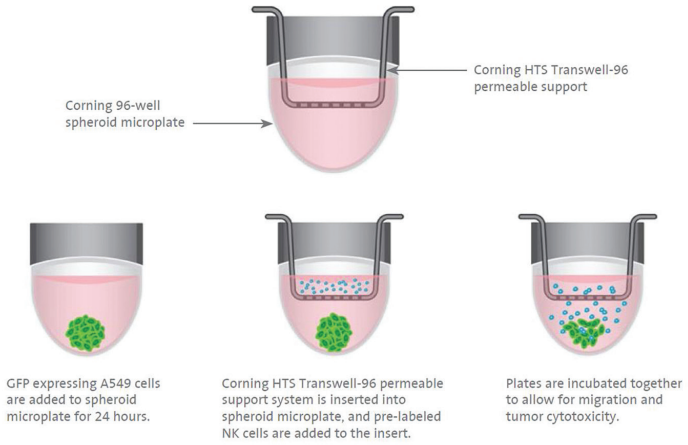


Figure 1. HTS Transwell 96-well permeable supports were placed into 96-well spheroid microplates for 3D immune cell migration and tumor invasion assays.

Hilary [Sherman] realized the two products fit together perfectly and could be used for many applications requiring a migration/invasion component, as well as a 3D cell culture model component, it was very clear this would be very useful. This product synergy will provide a more realistic model to study more *in vivo*-like cell behavior and interactions."

Rather than using the flat-bottom Transwell® receiver plate, Sherman placed the spheroid microplate under the HTS Transwell insert plate. This enabled her to more accurately observe tumor cell activity in a 3D system while facilitating migration of immune cells towards the tumor cell model. Doing so allows for the investigation of immune cell homing, tumor toxicity, and tumor immune evasion in an easy-to-use, reproducible, 3D high throughput assay.

Study Methods

To form the tumor spheroid models, cells from a cancerous lung tissue cell line (A549 cells) were seeded into 96-well spheroid microplates. The following day, Sherman placed the HTS Transwell 96-well permeable supports in the spheroid microplates. To assess immune cell homing and migration, natural killer (NK) cells were added into the inserts and were allowed to migrate for 24 hours. (See Figure 1.)

Implications on Future Research

It is well understood that in order to effectively research the body's response to targeted immunotherapy, a model must be used that successfully allows for the immune cells to reach the tumor cells of interest. Sherman's first-of-its-kind research model confirms that not only are the NK cells in this study able to reach the target tumor cells, but they are also able to infiltrate the 3D spheroid structure and lead to the desired cytotoxic outcome. This novel system is a more streamlined model to observe all necessary components of this immunotherapy research in a single, high throughput, reproducible *in vivo*-like format.

"This model allows researchers to study immune system–tumor interactions in a more comprehensive way that also has the benefit of being high throughput and easy to use. The system can also be used to study other immune and cancer cells as well as the ability to form more complex models by adding endothelial or glial cells to the Transwell membrane to create more advanced blood brain barrier models," says Gitschier.

Learn more about this new research model, request the Corning application note, CLS-AN-425 from your VWR Life Science Specialist.

Offering Solutions for Cell Culture Success

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Description	Size	Cat. No.	Unit
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Ultra-Low Attachment Surface Spheroid Microplate, Black/Clear	384-Well	10185-096	Case of 5
HTS Transwell Permeable Support Systems and Plates, PC, 0.3 µm	96-Well	89089-932	Case of 8
HTS Transwell Permeable Support Systems and Plates, PC, 0.4 µm	96-Well	89089-924	Case of 5
Corning® Matrigel® Matrix, Growth Factor Reduced	10 mL	47743-720	Case of 1
Corning® DMEM [+] 4.5 g/L Glucose, L-Glutamine, Sodium Pyruvate	1 L	45000-306	Pack of 6

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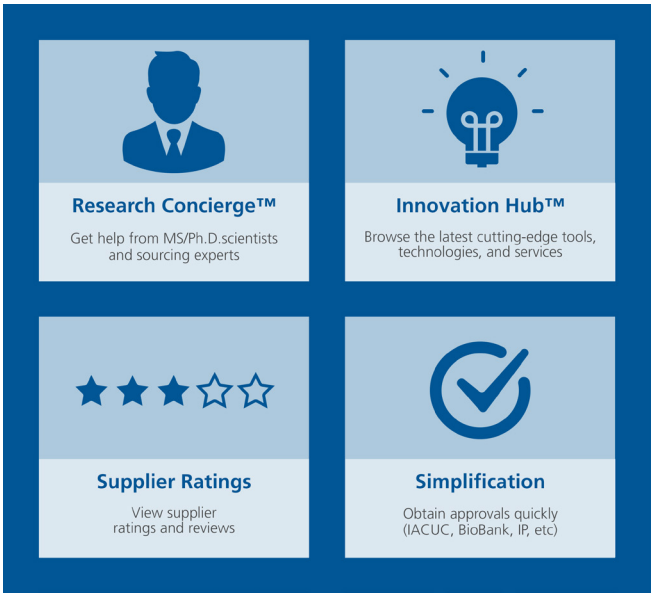

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Efficient Transient Gene Expression in Neurosphere with jetMESSENGER™ mRNA Transfection Reagent

By Barbara Demeneix, Karine Le Blay, and Sylvie Remaud [Muséum National d'Histoire Naturelle (MNHN), Paris, France], Maxime Dumont, Patrick Erbacher, Géraldine Guérin-Peyrou, and Alengo Nyamay'Antu (Polyplus-transfection®, Illkirch, France)

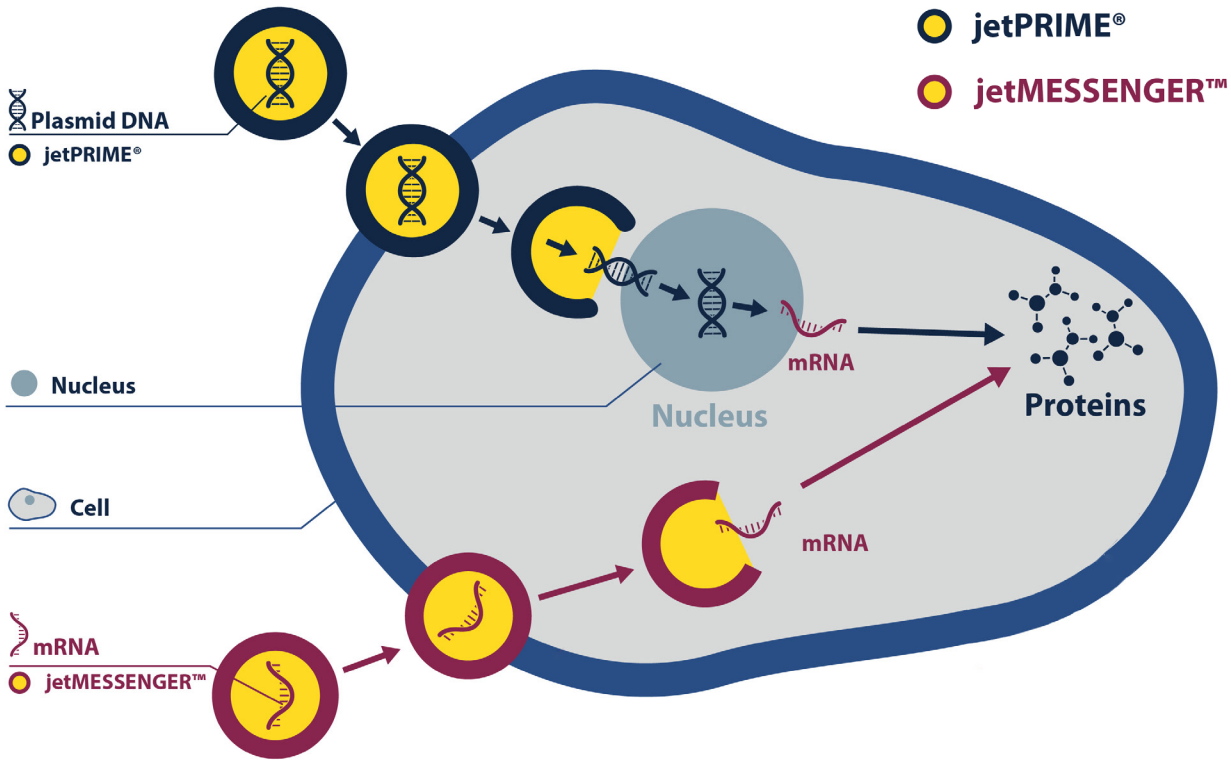

Fig. 1: Comparison between mRNA transfection using jetMESSENGER™ and DNA transfection using jetPRIME®.

Introduction

In light of their remarkable properties of both self-renewal and differentiation, adult neuronal stem cells are a unique cellular system that has demonstrated promising potential in developmental biology, regenerative medicine, and disease therapy. Cultivating adult neuronal stem cells *in vitro* as non-adherent free-floating spheroids is a frequently used 3D cell culture model to mimic the *in vivo* niche. Growing neural stem cells as neurospheres is an established approach to studying molecular mechanisms that drive self-renewal and differentiation. Gene expression strategies to examine the role of proteins in modulating these cellular mechanisms rely on the ability to induce ectopic or endogenous gene overexpression.

As neurospheres are composed of both stem cells and progenitor cells, it is important to find a gene delivery method that will target all cell types, irrespective of their self-renewal capacity and consequently of their division rate. As an alternative to viral-based transfection methods which could give rise to undesirable random genomic integration and subsequent potential pathogenicity, Polyplus-transfection® has developed an mRNA transfection reagent, jetMESSENGER, for difficult-to-transfect cells. Messenger RNA transfection is a growing alternative transfection method to plasmid DNA delivery which allows reproducible and highly efficient transfection in a wide variety of cells by circumventing the need for nuclear import of DNA, hindered in cells that are dividing slowly or have stopped altogether[1]. This process has many advantages over DNA transfection, including a high percentage

of transfected cells irrespective of their division rate and earlier and more controlled protein expression with no risk of genome integration. Concomitantly, toxicity and immune response usually induced by mRNA entry into the cytosol, has been reduced with the latest generation of modified mRNA.

Here, we propose a mRNA transfection based-method to reach high transfection efficiencies in neuronal stem cells grown in spheroids, based on our combined expertise on neuronal stem cells and the novel mRNA transfection reagent jetMESSENGER™ developed by Polyplus-transfection®. The aim of this study was two-fold. Firstly, to test mRNA as an alternative method to DNA transfection for neuronal stem cells. Secondly, to determine the transfection efficiency of the heterogeneous population of neurosphere-forming stem cells including neuroblasts, oligodendrocyte progenitor cells, as well as other cell types, such as astrocytes and glial cells (Fig 1.).

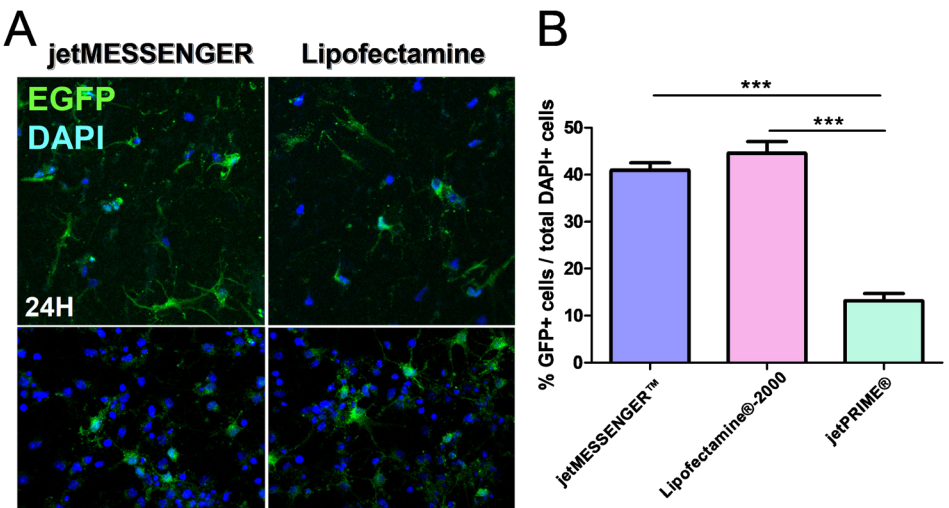

Fig. 2: Comparative transfection efficiency of mRNA versus DNA transfection reagents

Material & Methods

Neurospheres were transfected with either EGFP mRNA using jetMESSENGER™ (Polyplus-transfection®) or Lipofectamine®-2000 (Thermo Fisher Scientific®), or with DNA using jetPRIME® (Polyplus-transfection®) according to the manufacturers' recommendations. Neurospheres were gently dissociated and plated together at equal densities of 40,000 cells per well of a 24-well plate in complete medium. Twenty four hours post-seeding of cells, transfection was performed by adding 0.5µg of mRNA. The GFP encoding mRNA was used with respectively a mRNA: reagent ratio of 1:2.4 and 1:3 ratio for jetMESSENGER™ and Lipofectamine®. For DNA transfection, 0.5µg of EGFP plasmid DNA was complexed with 1.5µL of jetPRIME® per well. EGFP expression in transfected cells was examined by fluorescence microscopy from 24 hours and up to 7 days post-transfection.

Results

First, we analyzed the transfection efficiency of both jetMESSENGER™ and Lipofectamine®-2000 transfection reagents. To this end, transfected cells were fixed at different time points following transfection (from 24 hours to 7 days). For both transfection reagents, we detected EGFP expression 24 hours post-transfection. Both continued to provide high levels of EGFP expression up to 7 days post-transfection (Fig 2A).

To evaluate more precisely the transfection efficiency, we quantified the percentage of EGFP+ transfected cells 48 hours post-transfection. We observed a 4-fold increase in transfected cells using either jetMESSENGER™ (40.93 ± 1.57) or Lipofectamine®-2000 (44.56 ± 2.51) compared to jetPRIME (13.15 ± 1.59) (Fig 2B), showing that mRNA transfection is more efficient for transfection of

differentiated neuronal cells from neurospheres than DNA transfection. Since neurospheres contain a mix of cell types, we studied which cell type is preferentially targeted by each transfection reagent. To this end, immunohistochemistry using antibodies directed against markers expressed in NSCs (GFAP and SOX2) or astrocytes (GFAP) was performed. For each reagent, the EGFP signal was mostly detected in the cytoplasm of (i) NSC co-expressing SOX2 and GFAP and (ii) SOX2+ progenitors (Fig 3A).

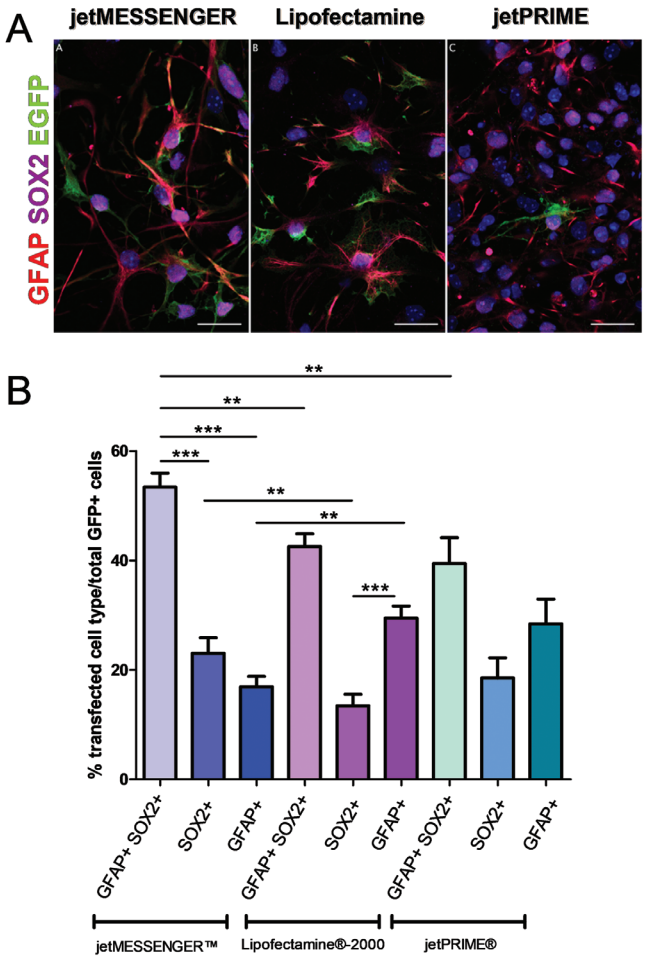

Fig. 3: mRNA transfection using jetMESSENGER™ targets preferentially both NSCs and progenitor-derived neurospheres.

jetMESSENGER™ transfects more efficiently both NSCs and progenitors (53.37 ± 2.55 and 23.04 ± 2.81) compared to either Lipofectamine®-2000 (42.55 ± 2.32 and 13.44± 2.09) or jetPRIME® (39.42± 4.73 and 18.53 ± 3.67). Moreover, jetMESSENGER™ privileges transfection of NSCs and progenitors as opposed to astrocytes, GFAP+ astrocytes showing only 16.92 ± 1.9 transfection efficiency as opposed to either Lipofectamine® 2000 (29.46 ± 2.2) or jetPRIME® (28.42± 4.49) (Fig 3B). Thus, jetMESSENGER™ shows more specific targeting of NSC/progenitors than Lipofectamine® 2000.

Conclusion & Perspectives

Here we show that mRNA transfection is an efficient technique to express exogenous proteins in neurospheres. Indeed, mRNA transfection leads to significantly higher transfection efficiency than DNA transfection, by a 3-fold increase.

To target different cell types within the neurosphere, such as neuronal stem cells and progenitors, mRNA delivery using jetMESSENGER™ was the most efficient approach.

In the future, we will use an mRNA delivery approach for our gene expression experiments in neurospheres using jetMESSENGER™ as it is the most efficient way to target our cells of interest, neuronal stem cells, and progenitors.

Reagent Size	Cat No.	Each
1 mL	**75806-306**	**10 mL**
0.75 mL	**75806-224**	**60 mL**
1.5 mL	**75806-226**	**2 x 60 mL**

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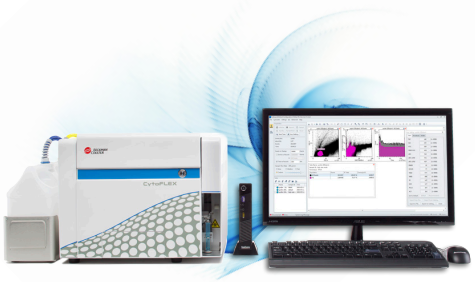

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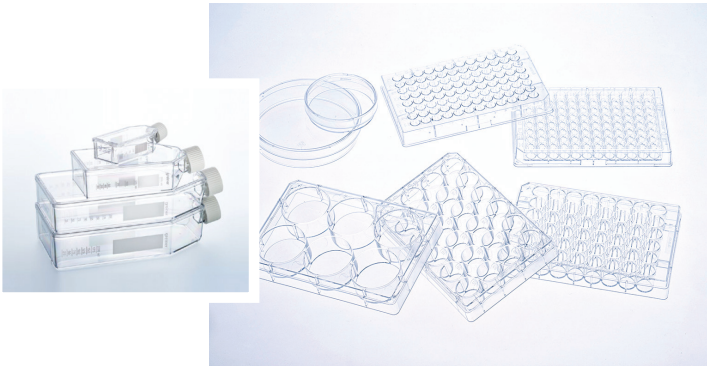

Descriptions	Cat. No.	Unit
250 mL Cell Culture Flask, Cell-Repellent Surface, PS, Sterile, White Filter Screw Cap	10836-541	Case of 15
100x20 mm Dish, Cell-Repellent Surface, PS, Clear, Sterile, with Lid	30618-024	Case of 5
6-Well Plate, Cell-Repellent Surface, PS, Clear, Sterile, with Lid	30618-022	Case of 5
24-Well Multiwell Plate, Cell-Repellent Surface, PS, Clear, Sterile, with Lid	10014-320	Case of 5
96-Well Plate, Cell-Repellent Surface, PS, Round U Bottom, Clear, Sterile, with Lid	30618-026	Case of 6
96-Well Plate, Cell-Repellent Surface, PS, Flat Bottom, Chimney Style, Clear, Sterile, with Lid	30618-028	Case of 6
96-Well Plate, Cell-Repellent Surface, PS, Flat Bottom, Black µClear, Sterile, with Lid	10014-318	Case of 32
384-Well Pate, Cell-Repellent Surface, PS, U Bottom, Clear, Sterile, with Lid	76199-198	Case of 8

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CELLCOAT® TC-Flask, 250 mL, 75 CM^2, Collagen I	75795-996	Pack of 5
CELLCOAT® TC-Plate, 6-Well, Poly-D-Lysine	75796-008	Pack of 5
CELLCOAT® TC-Plate, 12-Well, Poly-D-Lysine	75795-982	Pack of 5
CELLCOAT® TC-Plate, 24-Well, Poly-D-Lysine	75795-992	Pack of 5
CELLCOAT® TC-Plate, 6-Well, Collagen I	75795-998	Pack of 5
CELLCOAT® TC-Plate, 12-Well, Collagen I	75796-000	Pack of 5
CELLCOAT® TC-Plate, 24-Well, Collagen I	75796-002	Pack of 5

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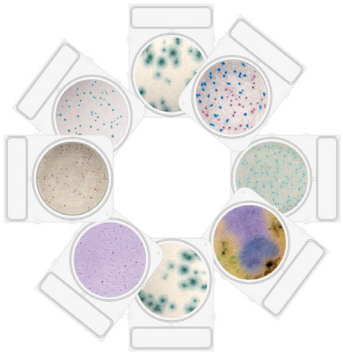

Description	Application	Cat. No.	Unit
Compact Dry EC	EC — *E. coli* and Coliforms	10145-954	Pack of 100
CompactDry ETB	ETB — *Enterobacteriaceae*	10753-986	Pack of 100
CompactDry LS	LS — *Listeria spp.*	10789-408	Pack of 100
CompactDry X-SA	X-SA — *Staphylococcus aureus*	10145-970	Pack of 100
CompactDry SL	SL — *Salmonella spp.*	10789-458	Pack of 100
CompactDry YM	YM — Yeast and Mold	10145-972	Pack of 100
CompactDry YMR	YMR — Yeast and Mold Rapid Test	76076-714	Pack of 100
CompactDry TC	TC — Total Plate Count	10145-968	Pack of 100

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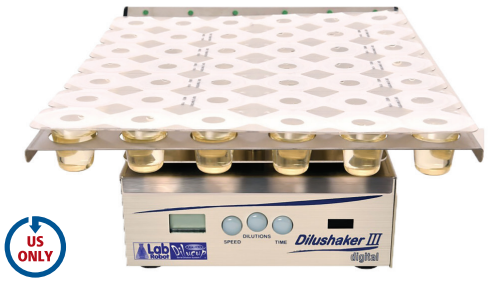

Description	Cat. No.	Unit
Dilushaker III Digital, 3 Rows, LED, 21 Cups, 7x3	75860-388	Each
Dilushaker III Digital, 6 Rows, LED, 42 Cups, 7x6	75860-392	Each
Dilucup Elegance Solutions		
Dilucup Elegance BPW, 32 Trays, 7 x 3 Cups, 9 mL	75860-416	Each
Dilucup Elegance BPW, 16 Trays, 7 x 6 Cups, 9 mL	75860-418	Each
Dilucup Elegance MRD, 32 Trays, 7 x 3 Cups, 9 mL	75860-406	Each
Dilucup Elegance MRD, 16 Trays, 7 x 6 Cups, 9 mL	75860-408	Each
Dilucup Elegance NaCl, 32 Trays, 7 x 3 Cups, 9 mL	75860-412	Each
Dilucup Elegance NaCl, 16 Trays, 7 x 6 Cups, 9 mL	75860-414	Each

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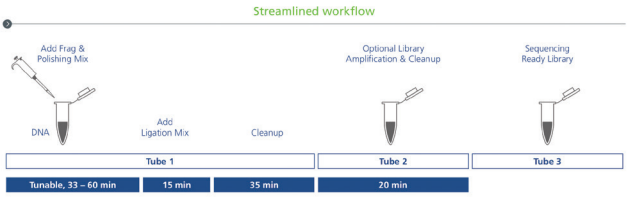
sparQ DNA Frag and Library Prep Kit provides rapid enzymatic fragmentation and construction of DNA libraries from double-stranded DNA for sequencing on Illumina NGS platforms. Using a simple two-step protocol, high-quality libraries can be completed in less than 3 hours with minimal hands-on time. The optimized chemistry coupled with high fidelity amplification results in better library yield, lower duplication bias, and consistent coverage across a broad range of GC content.



Description	Size	Cat. No.	Unit
sparQ DNA Frag and Library Prep Kit	24 Reactions	76183-242	Each
sparQ DNA Frag and Library Prep Kit	96 Reactions	76183-244	Each
sparQ Adapter Barcode, Set A	12 Single Index Barcodes for 96 Reactions	76169-154	Each
sparQ Adapter Barcode, Set B	12 Single Index Barcodes for 96 Reactions	76169-156	Each

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Description	Size	Cat. No.	Unit
AccuGreen High Sensitivity dsDNA Quantitation Kit (for Qubit)	100 Assays	75845-718	Each
AccuGreen High Sensitivity dsDNA Quantitation Kit (for Qubit)	500 Assays	75845-716	Each
AccuGreen Broad Range dsDNA Quantitation Kit (for Qubit)	500 Assays	76204-710	Each
AccuClear Ultra High Sensitivity dsDNA Quantitation Kit with DNA Standards	200 Assays	89493-596	Each
AccuClear Ultra High Sensitivity dsDNA Quantitation Kit with DNA Standards	1000 Assays	89427-078	Each
AccuBlue NextGen dsDNA Quantitation Kit with DNA Stds.	500 Assays	76204-714	Each
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AccuBlue Broad Range dsDNA Quantitation Kit with DNA Standards	1000 Assays	89139-010	Each

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High-Yield, Scalable Library Preparation with the NEBNext® Ultra™ II FS DNA Library Prep Kit

Improving Performance, Ease of Use and Reliability of Enzymatic DNA Fragmentation

Lynne Apone, Vaish Panchapakesa, and Karen McKay
New England Biolabs, Inc.

Introduction

The continued expansion of the use of next gen sequencing depends in large part on overcoming the limitations and bottlenecks associated with high-quality library preparation. The requirement for numerous steps and expensive equipment can result in sample loss, errors, and limited throughput. To address these issues, we have built upon our NEBNext Ultra II DNA library prep workflow to create a kit that includes a fragmentation system: the NEBNext Ultra II FS DNA Library Prep Kit. This kit integrates a new enzymatic DNA fragmentation reagent into the library prep workflow, such that fragmentation is combined with end repair and dA-tailing. The combination of these reactions eliminates the need for equipment to shear DNA mechanically and also reduces the number of sample transfers and losses. Subsequently, adaptor ligation is also carried out in the same vial, after which a single cleanup step is performed. For low input samples, PCR amplification is performed prior to sequencing.

Importantly, the enzymatic shearing of DNA with the FS kit does not introduce bias into the library, and the kit is suitable for input DNA from the full range of GC content. The reduced sample loss and increased efficiencies of the workflow enable use of lower input amounts, with a range of 100pg–0.5µg, and insert sizes of 100bp to 1kb can be generated.

Workflow

The NEBNext Ultra II FS DNA Library Prep Kit protocol, including fragmentation, is fast (~ 2.5 hours) and simple, and can accommodate 100pg–0.5µg of input DNA (Figure 1). Fragmentation, End Repair, and dA-Tailing reagents are combined, and there is no clean-up before adaptor ligation. The same fragmentation protocol is followed for all input amounts and for all GC contents. The kit also includes options for PCR-free workflows. The protocol is compatible with adaptors and primers from the NEBNext product line ("NEBNext Oligos") or from other sources.

	Fragmentation/ End Repair/ dA-Tailing	Adaptor Ligation	Clean Up/ Size Selection	Amplification	Clean Up	Total Workflow
Hands-On	2 min.	1 min.	5 min.	0–1 min.	0–5 min.	**8–14 min.**
Total	37–62 min.	16–31 min.	27–37 min.	0–34 min.	0–27 min.	**1.3–3.2 hr.**

Figure 1. NEBNext Ultra II FS DNA workflow.

Increased Library Yields

The use of enzymatic fragmentation can result in higher library yields than workflows incorporating mechanical shearing of DNA, due to both reductions in sample loss and decreased DNA damage. The NEBNext Ultra II FS kit further

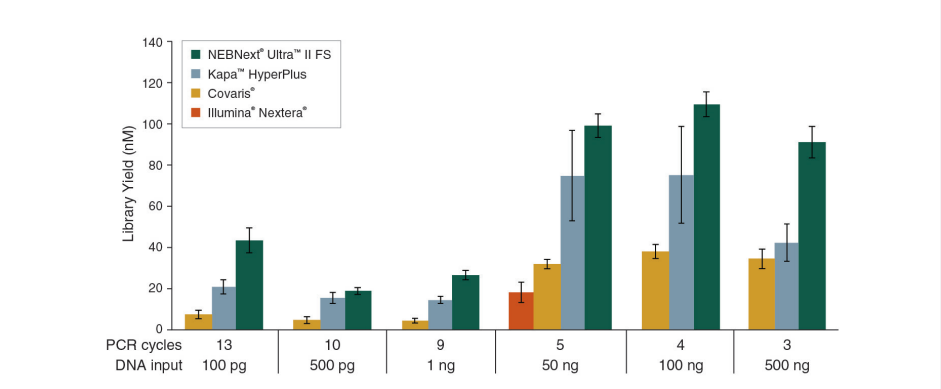

Figure 2. NEBNext Ultra II FS DNA Produces the Highest Yields From a Range of Input Amounts
Libraries were prepared from Human NA19240 genomic DNA using the input amounts and numbers of PCR cycles shown. For NEBNext Ultra II FS, a 20-minute fragmentation time was used. For Kapa™ HyperPlus libraries, input DNA was cleaned up with 3X beads prior to library construction, as recommended, and a 20-minute fragmentation time was used. Illumina® recommends 50ng input for Nextera®, and not an input range; therefore, only 50ng was used in this experiment. "Covaris®" libraries were prepared by shearing each input amount in 1X TE Buffer to an insert size of ~200bp using a Covaris instrument, followed by library construction using the NEBNext Ultra II DNA Library Prep Kit. Error bars indicate standard deviation for an average of 3–6 replicates performed by 2 independent users.

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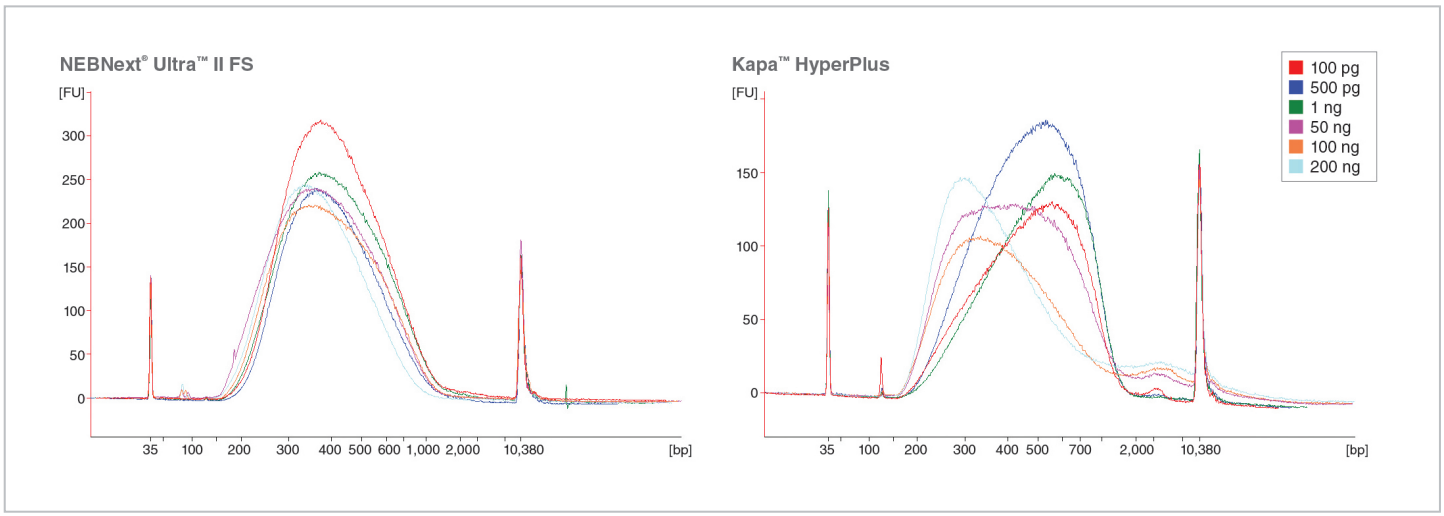

Figure 3. Consistent and Reliable Library Preparation with NEBNext Ultra II FS DNA
Libraries were prepared from Human NA19240 genomic DNA using the input amounts shown. NEBNext Ultra II FS libraries were prepared using a 20-minute fragmentation time. For Kapa HyperPlus, input DNA was cleaned up with 3X beads prior to library construction, as recommended, and a 20-minute fragmentation time. Library size was assessed using the Agilent Bioanalyzer. Low input (1ng and below) libraries were loaded on the Bioanalyzer without a dilution. High input libraries were loaded with a 1:5 dilution in 0.1X TE.

increases library yields through the integration of the fragmentation reagent with end repair and dA-tailing reagents (thereby minimizing loss during transfer steps), by not requiring a clean-up step before adaptor ligation, and through the high reaction efficiencies of each step in the workflow.

Achieving sufficient library yields for high quality sequencing from very low input amounts can be especially challenging with mechanical shearing of DNA, a situation compounded by the preference to amplify libraries using as few PCR cycles as possible. The NEBNext Ultra II FS kit overcomes this low-input challenge, and users can now obtain higher library yields with input amounts as low as 100pg of human genomic DNA, as shown in Figure 2 (below, left).

Robustness of DNA Fragmentation

Consistent and reliable fragmentation of DNA is critical for an enzymatic method to be adopted. Fragmentation using the NEBNext Ultra II FS kit is time-dependent, with fragmentation times amenable for manual or automated library preparation. Importantly, the final library size reflects the initial fragmented DNA, demonstrating that sufficient fragments of the desired size were produced during the fragmentation step.

Since in practice the exact quantity of DNA in a sample, and the exact GC content, may be unknown, it can be challenging when different fragmentation protocols are required for different input amounts and GC contents. Additionally, the ability to use input DNA in a range of buffers greatly simplifies the very start of the workflow, especially in situations where the input DNA buffer composition may be unknown or uncertain. The Ultra II FS kit addresses all of these issues by requiring a single fragmentation protocol for the full range of input amounts (100pg–0.5µg) (Figure 3) and for the full range of GC content (see next section). Additionally, DNA can be in water, Tris, 0.1X TE or 1X TE.

Uniform GC Coverage

While sufficient yield of a library is required for successful sequencing, the quality of a library is also critical, regardless of the

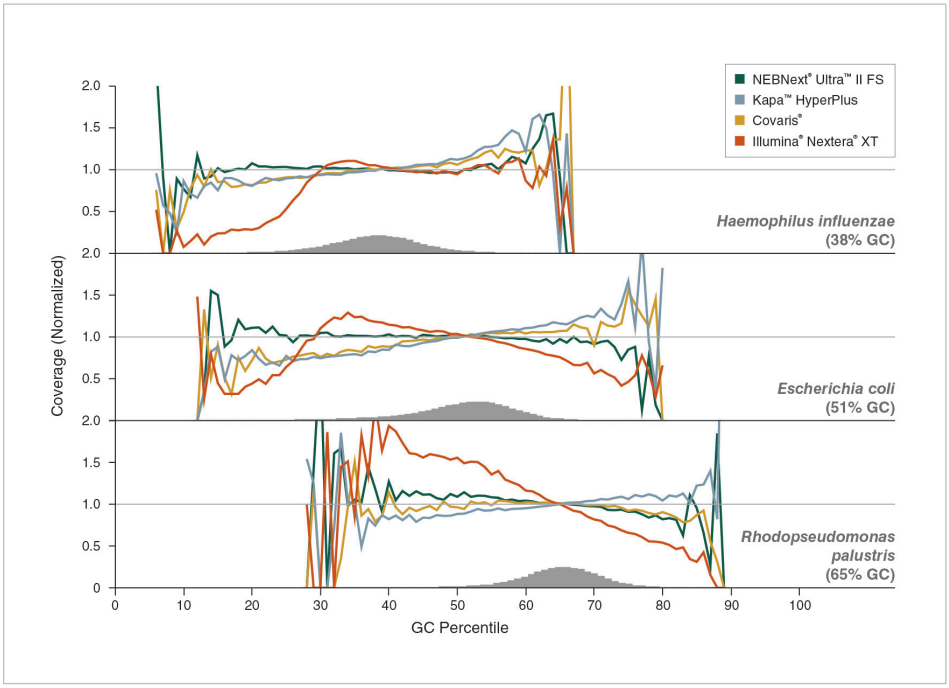

Figure 4. NEBNext Ultra II FS DNA Provides Uniform GC Coverage for Microbial Genomic DNA Over a Broad Range of GC Composition. Libraries were prepared using 1ng of a mix of genomic DNA samples from *Haemophilus influenzae, Escherichia coli* (K-12 MG1655), *Rhodopseudomonas palustris* and the library prep kits shown, with 9 PCR cycles for consistency across samples, and sequenced on an Illumina MiSeq. NEBNext Ultra II FS libraries were prepared using a 20-minute fragmentation time. For Kapa HyperPlus libraries, input DNA was cleaned up with 3X beads prior to library construction, as recommended, followed by a 25-minute fragmentation time. "Covaris" libraries were prepared by shearing 1ng of DNA in 1X TE Buffer to an insert size of ~200bp using a Covaris instrument, followed by library construction using the NEBNext Ultra II DNA Library Prep Kit. Reads were mapped using Bowtie 2.2.4 and GC coverage information was calculated using Picard's CollectGCBiasMetrics (v1.117). Expected normalized coverage of 1.0 is indicated by the horizontal grey line, the number of 100bp regions at each GC% is indicated by the vertical grey bars, and the colored lines represent the normalized coverage for each library.

input amount or GC content of the sample DNA. A high-quality library will have uniform representation of the original sample, including even coverage across the GC spectrum.

For enzymatic DNA fragmentation methods, randomness with regard to GC content can be of concern, and ensuring uniform GC coverage in libraries produced with the NEBNext Ultra II FS kit was of great importance. The Ultra II FS kit shows not only consistent uniformity of GC coverage at the full range of input amounts, but uniformity of GC coverage superior to mechanical shearing workflows and alternative enzymatic shearing methods (Figure 4, next page).

Library Quality

As noted above, an ideal library will represent completely and proportionally the sequence of the sample DNA, regardless of the input amount and GC content of the sample DNA. Especially when input amounts for a library are low, there is a risk that the resulting library will lack this diversity, and that some sequences will be over- or under-represented. This could be due to preferential amplification of some sequences during PCR of the final library, or non-random DNA fragmentation at the beginning of the library prep process. Comparison of the level of sequence coverage, in 10kb intervals, achieved for libraries with different input amounts is a useful measure of the diversity of a library. This is especially useful when the comparison is with a PCR-free library made with a higher input amount, as such a library is typically not affected by the factors that lead to bias in libraries. We see good correlation of a PCR-free library prepared with 100ng of Covaris-sheared human genomic DNA, and amplified libraries prepared with 1ng of Covaris-sheared DNA or with the NEBNext Ultra II FS kit.

PCR-Free Libraries

Construction of a library using a PCR-free workflow removes the risk of incorporation of bias during library amplification. However, the input amounts required to produce sufficient amounts of a high-diversity library without an amplification step are necessarily higher, and this can be limiting. With the NEBNext Ultra II FS protocol, by performing fragmentation, end repair and dA-tailing in the same reaction vial, sample loss due to transfer or clean-up steps after DNA fragmentation are eliminated, and yields are higher. Additionally, the use of Ultra II FS enzymatic DNA fragmentation rather than mechanical shearing reduces DNA damage, and consequently increases library yields, making the omission of the PCR step now feasible for lower nanogram level input amounts.

Variant Detection

Enzymatic DNA fragmentation enables increased library yields in comparison to mechanical shearing methods, in part due to the reduced DNA damage that occurs during shearing. Additionally, sequence markers indicative of oxidative damage to DNA that have been found in libraries constructed with mechanically-sheared

DNA, are absent in libraries constructed using the NEBNext Ultra II FS kit, and greater differences are seen with lower input amounts. This highlights the higher quality of libraries constructed with the Ultra II FS kit compared to Covaris-sheared DNA libraries, especially at low input amounts.

Conclusion

The NEBNext Ultra II FS DNA Library Prep Kit for Illumina provides a simple and reliable solution for DNA fragmentation integrated with library construction. The kit enables production of high quality libraries from a broad range of input amounts and GC contents with a single fragmentation protocol, greatly simplifying the scalability of library construction:

- Perform fragmentation, end repair, and dA-tailing with a single enzyme mix
- Obtain robust fragmentation with a single protocol, regardless of DNA input amount or GC content
- Generate high quality libraries from 100pg–0.5µg input DNA
- Use with DNA in standard buffers (TE, Tris-HCl) and water
- Save time with a streamlined workflow: (~ 2.5 hours), with < 15 minutes hands-on time
- Experience reliable fragmentation, even with very low input amounts
- Generate high yields with increased reaction efficiencies and minimized sample loss
- Vary incubation time to generate a wide range of insert sizes

References

For a complete list of references, visit ***vwr.com/vwrbiomarke.***

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Description	Size	Cat. No.	Unit
NEBNext® Ultra™ II FS DNA Library Prep Kit for Illumina®	24 rxns	103529-140	Each
NEBNext® Ultra™ II FS DNA Library Prep Kit for Illumina®	96 rxns	103529-168	Each
NEBNext® Ultra™ II FS DNA Library Prep with Sample Purification Beads	24 rxns	103529-166	Each
NEBNext® Ultra™ II FS DNA Library Prep with Sample Purification Beads	96 rxns	103529-164	Each
NEBNext® Ultra™ II FS DNA Module	24 rxns	103529-144	Each
NEBNext® Ultra™ II FS DNA Module	96 rxns	103529-142	Each

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