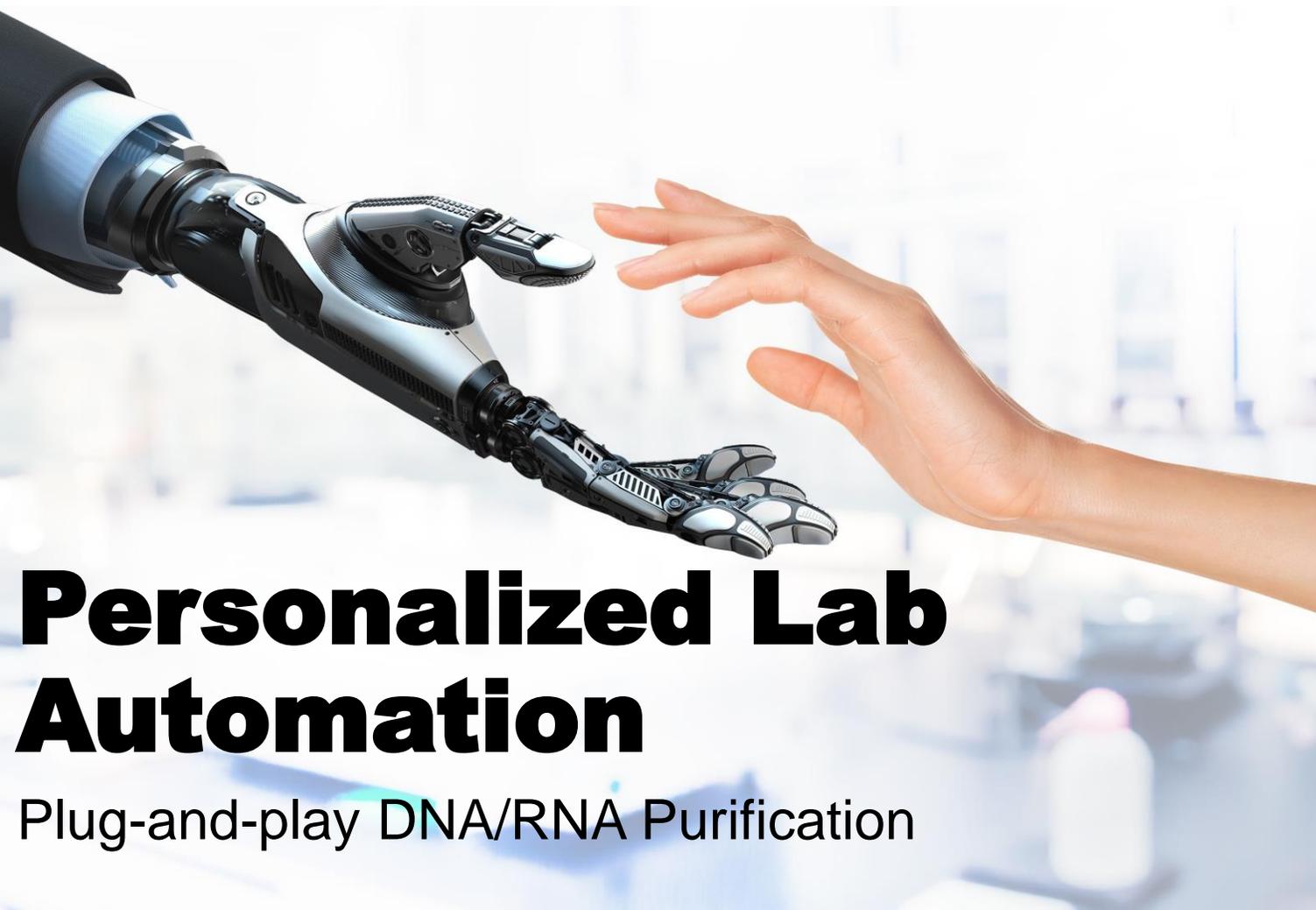




ZYMO RESEARCH

The Beauty of Science is to Make Things Simple®



Personalized Lab Automation

Plug-and-play DNA/RNA Purification

Pain Point: Custom Unique Workflows

Most desired workflows are unique and may require a flexible custom solution



Pain Point: No Scripting Experience



Lack of knowledge of how to script on robotic platforms

Zymo: Your Partner in Automated Sample Prep

- Free support service for every MagBead Customer
- We make automated NA extraction plug-and-play:
 - Custom Solutions
 - Flexibility
 - Scripting
 - Troubleshooting
 - Technical Support
 - Workflow Validation



Robotic Platform Expertise

Zymo Research and Hamilton Robotics Forge Partnership to Automate Epigenetics Research

More accurate, faster, and simpler workflows with automated methods are now available



Thermo Kingfisher

Tecan

Eppendorf



Application Notes

Hamilton, Tecan, & More!

Application Note



Direct-zol™ -96 MagBead RNA

Catalog Nos. R2100 - R2105

High-throughput, automated purification of DNA-free RNA directly from samples in TRIzol®, TRI Reagent®, or similar without phase separation.

Never Phase Separate Again!



Introduction

The Direct-zol™ -96 MagBead RNA facilitates purification of high quality (DNA-free) RNA directly from samples stored in TRIzol®, TRI Reagent®, or similar reagents. While exceptional in providing RNA stabilization and inactivating infectious agents, these reagents are complicated by phase separation, precipitation, and potential phenol carryover. The innovative, Direct-zol™ procedure from Zymo Research bypasses phase separation/precipitation requirements and eliminates phenol carryover. The Direct-zol™ -96 MagBead RNA meets the demands of scientists requiring high-quality RNA for sensitive analytical methods like miRNA profiling, RNA-seq, and viral detection.

AUTOMATED RNA ISOLATION FROM ZYMO RESEARCH

Application Note



EZ-96 DNA Methylation™ MagPrep Series

Catalog Nos. D5040-D5047

High-throughput, automated, magnetic bead-based bisulfite conversion of DNA with the Tecan Freedom EVO® for methylation analysis.

Introduction

The ability to detect and quantify DNA methylation efficiently and accurately has become essential for the study of cancer, gene expression, genetic diseases, and many other important aspects of biology. To date, a number of methods have been developed to detect/quantify DNA methylation including: high-performance capillary electrophoresis and methylation-sensitive arbitrarily primed PCR. However, the most common techniques used today still rely on bisulfite conversion.

Treating DNA with bisulfite chemically modifies non-methylated cytosine into uracil, methylated cytosine remains unchanged. Once converted, the methylation profile of the DNA can be determined using the desired downstream application. For single locus analysis, the region of interest is generally amplified following bisulfite conversion (i.e., bisulfite PCR) and then sequenced. However, recent advances in methylation detection allow the investigation of genome-wide methylation patterns, technologies include array-based methods, "pyrosequencing", reduced representation bisulfite sequencing (RRBS), and whole genome bisulfite sequencing.

To this point all bisulfite conversion products have been dependent on manual manipulation of spin plates and columns or been of limited throughput. By adapting the clean-up of bisulfite converted DNA to a magnetic bead based procedure and coupling it to the Freedom EVO® platform, Zymo Research has opened the door to high-throughput bisulfite conversion of samples for methylation analysis.

AUTOMATED BISULFITE CONVERSION FROM ZYMO RESEARCH

Application Note



Zyppy™ -96 Plasmid MagBead Miniprep

Catalog Nos. D4100, D4101, & D4102

High-throughput, automated, magnetic bead-based purification of high quality, endotoxin-free DNA directly from culture.

Automation Equipment

- Tecan Freedom EVO®
- Freedom EVOware®
- 8 channel Liquid Handling Arm (LHA), configured for Disposable Tips (DTs)
- Robotic Manipulation Arm (ROM)
- Te-Shake™ Shaker
- 96-well Magnetic Stand

AUTOMATED PLASMID ISOLATION FROM ZYMO RESEARCH

HAMILTON

Automation of the ZymoBIOMICS™-96 MagBead DNA Kit

High-throughput, magnetic bead-based purification of DNA, directly from any environmental sample or biological fluid for unbiased analysis of microbial communities on the MicroAmp® STARD™.

Features and Highlights

High-throughput, magnetic bead-based purification of high quality total DNA, directly from microbial communities in feces, soil, water, sediments, saliva, urine, body fluids, etc.



Figure 1: The ZymoBIOMICS™-96 MagBead DNA Kit provides superior yields when compared to Regener A. 96 MagBeads were used for purification of total DNA from environmental samples. 96 µl of each sample was added to 1 ml of 96 MagBeads and processed as per protocol.

Linear Recovery with unparalleled sensitivity of DNA isolated from trace or dilute sample sources.

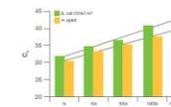


Figure 2: The ZymoBIOMICS™-96 MagBead DNA Kit provides superior yields when compared to Regener A. 96 MagBeads were used for purification of total DNA from environmental samples. 96 µl of each sample was added to 1 ml of 96 MagBeads and processed as per protocol.

HAMILTON

Automation of the EZ-96 DNA Methylation™ MagPrep Kits

High-throughput, magnetic bead-based procedure for bisulfite conversion of DNA for methylation analysis on the MicroAmp® STARD™.

Introduction

The need to detect and quantify DNA methylation efficiently and accurately has become essential for the study of cancer, gene expression, genetic diseases, and many other important aspects of biology. To date, a number of methods have been developed to detect/quantify DNA methylation including: high-performance capillary electrophoresis and methylation-sensitive arbitrarily primed PCR. However, the most common techniques used today still rely on bisulfite conversion.

Treating DNA with bisulfite chemically modifies non-methylated cytosine into uracil, methylated cytosine remains unchanged. Once converted, the methylation profile of the DNA can be determined using the desired downstream application. For single locus analysis, the region of interest is generally amplified following bisulfite conversion (i.e., bisulfite PCR) and then sequenced. However, recent advances in methylation detection allow the investigation of genome-wide methylation patterns, technologies include array-based methods, "pyrosequencing", reduced representation bisulfite sequencing (RRBS), and whole genome bisulfite sequencing.

To this point all bisulfite conversion products have been dependent on manual manipulation of spin plates and columns or been of limited throughput. By adapting the clean-up of bisulfite converted DNA to a magnetic bead based procedure and coupling it to the Freedom EVO® platform, Zymo Research has opened the door to high-throughput bisulfite conversion of samples for methylation analysis.



AUTOMATED BISULFITE CONVERSION FROM ZYMO RESEARCH

HAMILTON

Automation of the Direct-zol™-96 MagBead RNA

High-throughput, magnetic bead-based purification of DNA-free RNA directly from samples in TRIzol® without phase separation on the MicroAmp® STARD™.

Introduction

The TRIzol method for RNA extraction has been the gold standard. The powerful protein denaturant effectively stabilizes RNA and inactivates RNases and ribonuclease. However, the need for phase separation, precipitation, and potential phenol carryover can further complicate workflows. The magnetic bead-based Direct-zol procedure on the Hamilton MicroAmp STARD platform bypasses phase separation/precipitation and enables high-throughput, automated magnetic bead-based purification of high quality total RNA, directly from samples stored in TRIzol® or other acid guanidinium-thiocyanate based reagents. Direct-zol effectively isolates total RNA from a variety of sample sources including cells, tissues, serum, plasma, stool, and biological liquids for downstream applications, like miRNA profiling, RNA-seq, and viral detection.



Figure 3: The ZymoBIOMICS™-96 MagBead DNA Kit provides superior yields when compared to Regener A. 96 MagBeads were used for purification of total DNA from environmental samples. 96 µl of each sample was added to 1 ml of 96 MagBeads and processed as per protocol.

Linear Recovery with unparalleled sensitivity of DNA isolated from trace or dilute sample sources.



Figure 4: The ZymoBIOMICS™-96 MagBead DNA Kit provides superior yields when compared to Regener A. 96 MagBeads were used for purification of total DNA from environmental samples. 96 µl of each sample was added to 1 ml of 96 MagBeads and processed as per protocol.

HAMILTON

Automation of the Zyppy™-96 Plasmid MagBead Miniprep

High-throughput, magnetic bead-based automated purification of high quality endotoxin-free DNA directly from culture on the MicroAmp® STARD™.

Introduction

The success of plasmid DNA extraction can be highly variable, differing from one manual operator to the next and typically requiring long incubation times. This is a time-consuming procedure and increases the risk for low yields. The Zyppy™ procedure allows for high-throughput automation and requires no centrifugation or pelleting of cells. The technology features a modified alkaline lysis system that allows for the clean lysis of cells.

The MicroAmp STARD™ was configured with the Independent Handling Channel, Autoload optional, CO-RE™ Multi-Process Head (MPE), CO-RE Dispenser, Hamilton Header (HH), Zymo magnetic rack, and all related tips and magnet options.

The DNA concentration was analyzed using Thermo Scientific™ NanoDrop™ 2000 (UV-Vis Spectrophotometer). Multiple amplifications were performed using locus-specific primer pairs and the Fluidigm AccessAmp™ system. The resulting amplicons were pooled for harvesting and subsequent sequencing according to Fluidigm guidelines. After bioinformatic reviews were completed using the ZPL96 DNA Clean and Concentrate™ (Cat. #D4030) and then analyzed for missable possible sequencing using an Illumina® MiSeq and sequencing run. The protocol workflow is shown in Figure 1 (page 2).



AUTOMATED PLASMID ISOLATION FROM ZYMO RESEARCH



ZYMO RESEARCH

Customer Testimonials

“We are just so appreciative of all of your help in getting us **set up with the script, accommodating all of our requests** for customizing it to our LIMS needs, and everything else you have done for us.”

B.R.

“Thank you for **taking the time to go over the different protocols**. Thank you very much for the data package, scripts, and sample kit.”

R.M.

“These are very helpful suggestions. [The automation support team] has been extremely helpful and provided me with **excellent customer service, which I really appreciate.**”

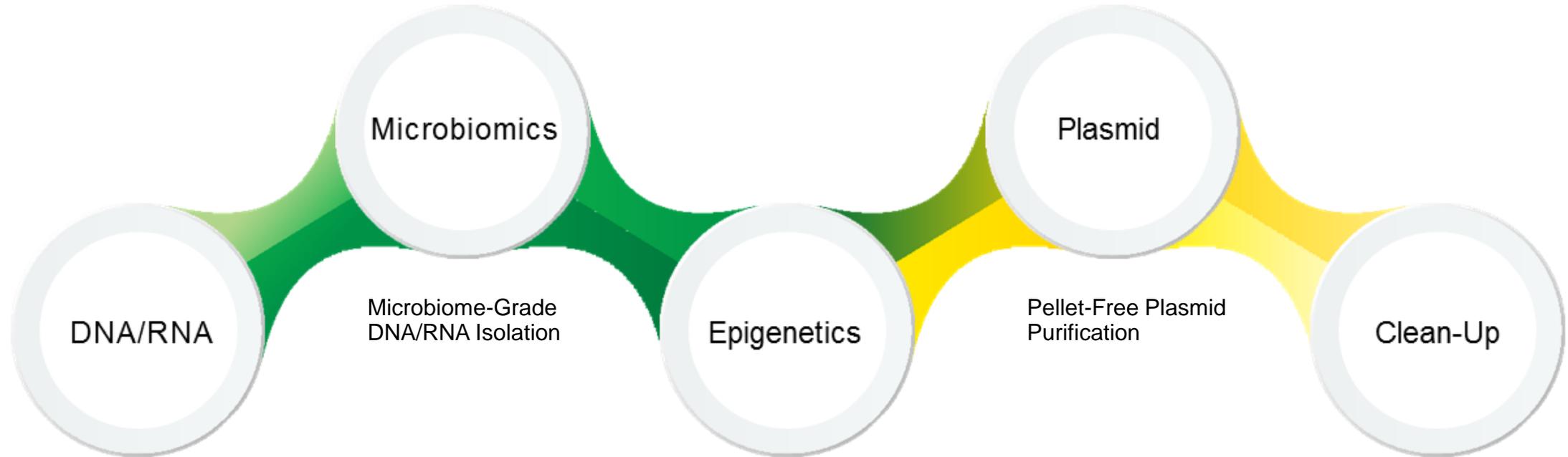
S.C.

“It was a **pleasure meeting all of you and putting a face to a name** after all this time. I look forward to our continued partnership.”

L.M.

Magnetic Bead Kits & Success Stories

Unique Sample Prep Technologies



DNA and/or RNA from any Sample
Viral & Pathogen DNA/RNA Extraction
RNA from any Sample in TRIzol®

Easy DNA Bisulfite
Conversion

Pellet-Free Plasmid
Purification

DNA/RNA Clean-Up
and Size Selection

Success Story: Nucleic Acids from Urine

Application:

Urine Diagnostic Lab: Match urine to sample provided to prevent urine substitutions

Needs

1. Extract total DNA/RNA (including cell-free) from buccal swabs and urine on the KingFisher Flex
2. Difficult to automate the extraction: high processing volumes and low biomass samples
3. Tested other commercially available kits with little to no success



Success Story: Nucleic Acids from Urine

Solution:

1. Custom protocol that transforms large volumes into small pellets sizes suitable for 96-well.
2. Designed a custom built-in RNase treatment (based on customers request).
3. Helped analyze and interpret results for customer (no experience working in genomics), and achieved validation of their new product service.



**Evaluated the *Quick-DNA/RNA MagBead* –
Best extraction of total nucleic acids**

Success Story: Genomic DNA

Application:

Extract genomic DNA from bacterial samples (E. coli) using the Quick-DNA MagBead Plus

Needs

1. Implement workflow on Tecan Freedom EVO 200 workstation using 96-well head
2. Reduce to smaller processing volumes (miniaturizing the reaction)



Success Story: Genomic DNA

Solution:

1. Provided customized script based upon customer's worktable/deck layout of instrument
2. Validated using less volumes in-house for customer's specific application needs
3. Optimized liquid classes to improve mixing speeds to get full resuspension of magnetic beads. Decreased drying time to improve elution efficiency
4. Improved yields from 7 ng/ul to 50 ng/ul



Success Story: Microbiomics

Application:

Extract mouse cecum tissue and stool DNA using the ZymoBIOMICS DNA MagBead for downstream 16S rRNA sequencing

Needs

1. Validate workflow on Eppendorf EpMotion 5075 TMX instrument and software
2. Concerns about cross contamination
3. Require comparable yields to Promega Maxwell Tissue DNA kit.



Success Story: Microbiomics

Solution:

1. Used checker-board plate design to validate no cross contamination occurred. Implemented controls by using the ZymoBIOMICS Microbial Community Standard
2. Optimized magnetic bead mixing aspiration speed and supernatant dispense volume parameters
3. Yields improved from 0.5-6 ng/ul to 26-30 ng/ul
Zymo achieved amazing quality in comparison.
Zymo: A260/280: 1.81, A260/230: 2.14
Promega: A260/280: 1.65, A260/230: 0.963



Success Story: Epigenetics/Methylation

Application:

Analyzing DNA methylation markers in cell-free DNA from plasma using the EZ DNA Methylation Lightning kit

Needs

1. Using the KingFisher Duo Prime (12-pin magnetic head version)
2. Transitioning from spin column format to magnetic bead chemistry to scale up for high-throughput processing
3. Small elution volumes for maximum yield



Success Story: Epigenetics/Methylation

Solution:

1. Provided initial KingFisher Duo script to get customer's workflow rapidly functioning
2. Optimized binding mixing speed, lowered drying time, and improved elution mixing. This accommodates the usage of small volumes as magnetic bead and liquid contact is maximized
3. Improved overall recovery from 66% to complete theoretical 100% rate.



Success Story: Plasmid Prep

Application:

Extract plasmid DNA using the Zyppy Plasmid MagBead – uses direct lysis with no extra centrifugation/pelleting steps

Needs

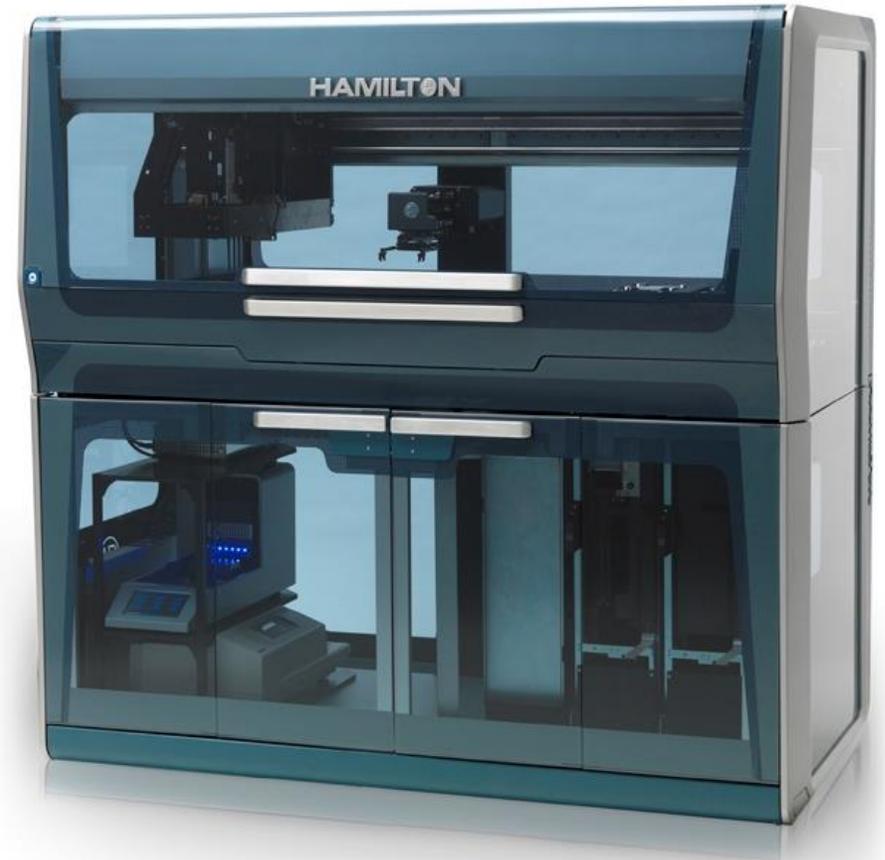
1. Validate chemistry on Hamilton VANTAGE instrument and Instinct V software
2. Decided to hire Hamilton Field Application Specialist to implement this workflow
3. Yield needs to be comparable to manual methods: 35 ng/ul.

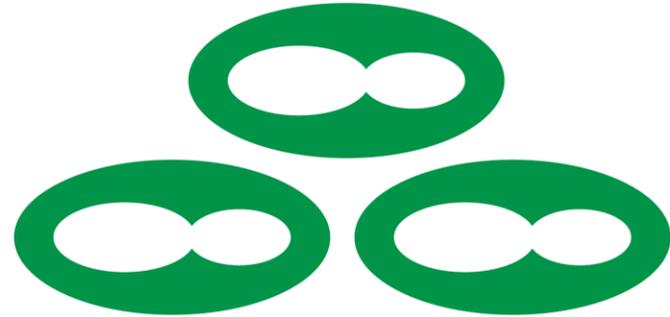


Success Story: Plasmid Prep

Solution:

1. Advised Field Application Specialist on critical steps (Ex. Alkaline Lysis, Neutralization) and specific mixing speeds, incubation times, temperature parameters, magnetic bead pelleting to translate into programming script steps.
2. Solved a problem that was ongoing for a while in 1 week. Increased plasmid DNA yield from 5 ng/ul to 40-50 ng/ul and resulted in satisfied customer.





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