Nucleic acid isolation

Centrifugation-free plasmid DNA extraction: a comparison of magnetic bead–based extraction methods for minipreps

Introduction

Plasmid purification has recently moved into the spotlight with the development of mRNA vaccines for SARS-CoV-2. Aside from numerous virology applications, plasmid DNA (pDNA) is crucial in multiple cell and gene therapy (CGT) pipelines. Prior to large-scale production, obtaining high-quality pDNA in the preclinical development stages is necessary to the success of transformative medicine.

In preclinical vaccine and CGT development, the process of isolating pDNA from plasmids is limited by the requisite number of centrifugation steps needed during the plasmid extraction process. Centrifugation is a common technique used to separate pDNA from the host cell's own genomic DNA. However, this technique is a rate-limiting step, especially in early-stage developmental workflows. Eliminating centrifugation may help relieve back-end bottlenecks and enable greater processing efficiencies, especially in high-throughput screening campaigns.

Here we have compared the results of three magnetic bead–based extraction mini kits designed for pDNA isolation and purification. An analysis of manual and automated extraction techniques is discussed.

- Invitrogen™ ChargeSwitch™ Plasmid ER Mini Kit (Cat. No. CS10100)
  - Requires centrifugation to pellet bacterial culture and clear lysate
  - Can be automated on liquid handling platforms and Thermo Scientific™ KingFisher™ systems
  - Uses Invitrogen™ ChargeSwitch™ Magnetic Beads to instantly elute pDNA
  - Enables purification from 1–5 mL of pelleted bacterial culture
  - Endotoxicity of ≤50 EU/µg pDNA
  - Isolates ≤20 µg of pDNA in 50–100 µL of elution buffer*

- Invitrogen™ ChargeSwitch™ NoSpin Plasmid Micro Kit (Cat. No. CS10201)
  - Centrifugation-free miniprep kit suited for high-throughput, automated workflows
  - No pelleted bacterial culture necessary
  - Can be automated on liquid handling platforms and KingFisher systems
  - Uses novel Invitrogen™ ChargeSwitch™ MagnaClear™ Beads to allow for direct capture of bacterial cells from culture or clear lysate, and to provide buffer-dependent surface charge to elute pDNA instantly
  - Enables purification from 1 mL of bacterial culture
  - Endotoxicity of ≤100 EU/µg pDNA
  - Isolates ≤5 µg of pDNA in 20–100 µL of elution buffer*
- Zyppy™-96 Plasmid MagBead Miniprep Kit (Zymo Research, Cat. No. D4100)
  - Centrifugation-free miniprep kit suited for automated workflows
  - Uses colored buffers for visual indication of cell lysis and neutralization
  - Requires wash buffer preparation and a heating element
  - Enables purification from 750 µL of bacterial culture
  - Endotoxicity of ≤50 EU/µg pDNA; yet claims to maintain endotoxin-free pDNA
  - Isolates ≤5 µg of pDNA in 40 µL of elution buffer

* ChargeSwitch magnetic bead kits (Cat. No. CS10201 and CS10100) can be scaled up for more yield. Typically, reagents can be scaled in a 1:1 fashion with minimal optimization needed.

Both the ChargeSwitch NoSpin Plasmid Micro Kit and Zyppy-96 Plasmid MagBead Miniprep Kit allow for the isolation of pDNA directly from bacterial culture. The ChargeSwitch NoSpin Plasmid Micro Kit uses Invitrogen™ ChargeSwitch™ MagnaClear™ technology, which enables bacterial cell flocculation that leads to pellet formation without centrifugation. The ChargeSwitch Plasmid ER Mini Kit is a "traditional" magnetic bead kit, requiring centrifugation and serving as a standard for comparison.

For evaluation purposes, manual extraction methods were performed according to the manufacturer's instructions for each kit. An automated extraction was performed using the ChargeSwitch NoSpin Plasmid Micro Kit and the Thermo Scientific™ KingFisher™ Flex Purification System.

Additionally, we investigated the endotoxicity of both the ChargeSwitch Plasmid ER Mini Kit and the Zyppy-96 Plasmid kit. We also observed the main pDNA isoforms after extraction for all three kits to assess nucleic acid stability.

Materials and methods

Bacterial culture

A single Invitrogen™ One Shot™ TOP10 Chemically Competent *E. coli* colony (Cat. No. C404003) was selected from a freshly streaked plate that was grown overnight, and inoculated in Luria-Bertani (LB) medium containing ampicillin. This starter culture was incubated for 8 hours at 37°C with shaking at 225 rpm. For extraction using ChargeSwitch kits, 200 µL of this starter culture was inoculated into 200 mL of LB medium and incubated at 37°C for 12–16 hours with vigorous shaking. Per the Zyppy-96 kit instruction manual, starter culture was inoculated into respective wells of a 96-well block (provided by Zymo Research) containing 750 mL of LB with ampicillin and incubated for 24 hours at 37°C with shaking at 225 rpm. The optical density ($OD_{600\ nm}$) reached 3.8 as measured by a Thermo Scientific™ NanoDrop™ Spectrophotometer.

Manual extraction

Methods—pDNA isolation and purification

Reagent preparation was carried out according to user guides for each kit evaluated and calibrated; handheld pipettors were used for all pipetting steps. pDNA was isolated from 1 mL of bacterial culture using the ChargeSwitch NoSpin Plasmid Micro Kit or the ChargeSwitch Plasmid ER Mini Kit, whereas pDNA from 750 µL of bacterial culture was isolated using the Zyppy-96 Plasmid kit. A sample size of n = 8 was processed for each kit. Samples extracted using the ChargeSwitch NoSpin Plasmid Micro or the ChargeSwitch Plasmid ER Mini Kit were processed in 1.5 mL Applied Biosystems™ Nonstick RNase-Free Microfuge Tubes (Cat. No. AM12450), and an Invitrogen™ DynaMag™-2 Magnet was used for magnetic separation. Sample extractions using the Zyppy-96 kit were performed using consumable plates provided with the kit and an Applied Biosystems™ Pharma Magnetic Stand-96 (Cat. No. A31543). Elution volume was 100 µL for both ChargeSwitch kits and 40 µL for the Zyppy-96 kit.

pDNA analysis

Sample preparations were analyzed using the NanoDrop Spectrophotometer to measure nucleic acid yield (A_{260}) and purity (A_{260}/A_{280} and A_{260}/A_{230}). Agarose gel electrophoresis was used to visually assess pDNA isoforms (supercoiled, nicked, linear, and single-stranded pDNA). The EndoSafe™ Limulus Amebocyte Lysate (LAL) system (Charles River Laboratories, Cat. No. PTS201) was used to quantify endotoxin levels of pDNA obtained using the ChargeSwitch Plasmid ER Mini Kit or the Zyppy-96 kit, since both kits claim endotoxin reduction.

Results

pDNA yield

The expected pDNA yields for these kits, according to the respective user manuals, are as follows:

- Zyppy-96 Plasmid MagBead Miniprep Kit: up to 10 µg
- ChargeSwitch Plasmid ER Mini Kit: up to 20 µg
- ChargeSwitch NoSpin Plasmid Micro Kit: up to 5 µg

The ChargeSwitch Plasmid ER Mini Kit enabled the highest average yield (10.7 µg pDNA). The ChargeSwitch NoSpin Plasmid Micro Kit enabled an average pDNA yield of 7.8 µg, and the Zyppy-96 Plasmid kit enabled an average yield of 5.7 µg. The standard deviation (SD) followed the same trend, with the ChargeSwitch Plasmid ER Mini Kit displaying the lowest SD at 0.25, followed by the ChargeSwitch NoSpin Plasmid Micro Kit (0.45), and then the Zyppy-96 Plasmid kit (0.76) (Figure 1).

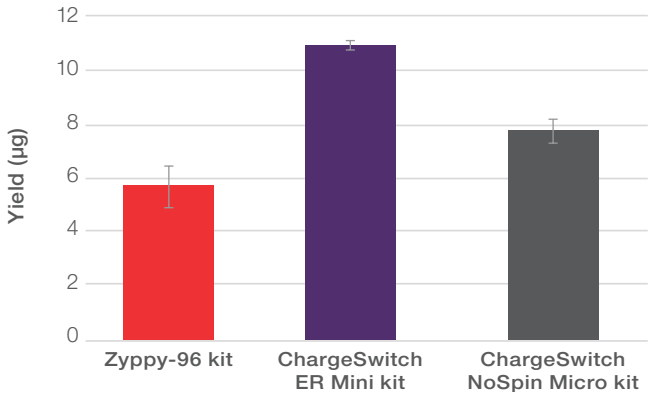

Figure 1. Average pDNA yield from manual extraction using the Zyppy-96 Plasmid MagBead Miniprep Kit, the ChargeSwitch Plasmid ER Mini Kit, or the ChargeSwitch NoSpin Plasmid Micro Kit.

As a head-to-head comparison between both pellet-free magnetic bead–based extractions, the experiment was repeated. This experiment was repeated with the intention of (1) demonstrating the superior yields of the ChargeSwitch NoSpin kit to the Zyppy-96 kit, despite ChargeSwitch NoSpin kit not being free of endotoxins and (2) demonstrating the consistency in the higher yields attained by the ChargeSwitch NoSpin kit compared to the Zyppy-96 kit. Using the same methodology, a second extraction was carried out to evaluate pDNA yields of the Zyppy-96 Plasmid and ChargeSwitch NoSpin kits (n = 8 per kit). The ChargeSwitch NoSpin Plasmid Micro Kit produced almost twice the yield (7.2 µg pDNA) as compared to the Zyppy-96 Plasmid kit (4.1 µg pDNA) (Figure 2).

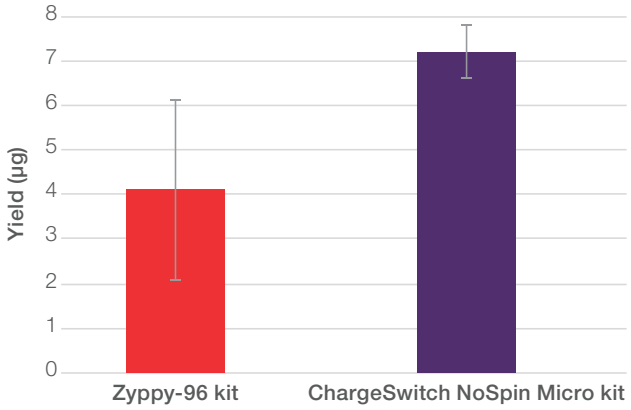

Figure 2. Average yield for manual extraction using the Zyppy-96 Plasmid kit or the ChargeSwitch NoSpin Plasmid Micro Kit. The ChargeSwitch NoSpin Plasmid Micro Kit yielded an average of 7.2 µg of pDNA (SD = 0.6), while the Zyppy-96 Plasmid kit yielded an average of 4.1 µg of pDNA (SD = 2).

pDNA purity

Sample purity was measured using A_{260}/A_{280} and A_{260}/A_{230} absorbance from samples in the second extraction experiment (Figure 2, n = 8 per kit). Average absorbance ratios for samples for each kit are in Figure 3. Pure dsDNA has an A_{260}/A_{280} of 1.85–1.88, while pure RNA has an A_{260}/A_{280} of near 2.1. A reduction in the A_{260}/A_{280} ratio may suggest the presence of protein contamination. The A_{260}/A_{230} ratio is a sensitive indicator of contaminants that absorb at 230 nm, including chaotropic salts, non-ionic detergents, proteins, and phenols. In common laboratory practice, DNA samples with A_{260}/A_{280} and A_{260}/A_{230} > 1.8 are considered “clean” and are suitable for most downstream applications.

The average A_{260}/A_{280} for the samples extracted using the Zyppy-96 kit (n = 8) did not meet the >1.8 threshold, falling slightly below at 1.79 (SD = 0.039). Conversely, the samples extracted using the ChargeSwitch NoSpin Micro kit (n = 8) exceeded the >1.8 absorbance value, with an average A_{260}/A_{280} of 1.93 (SD = 0.044), suggesting more double-stranded pDNA as compared to the samples extracted using the Zyppy-96 kit. The average A_{260}/A_{230} for the samples isolated using the Zyppy-96 kit was 0.98 (SD = 0.073), suggesting possible interference from residual organic contaminants in the buffer. Conversely, the average A_{260}/A_{230} for the samples extracted using the ChargeSwitch NoSpin Micro kit was 1.97 (SD = 0.197), indicating the samples processed using the ChargeSwitch NoSpin kit meet purity standards needed in common downstream processing workflows.

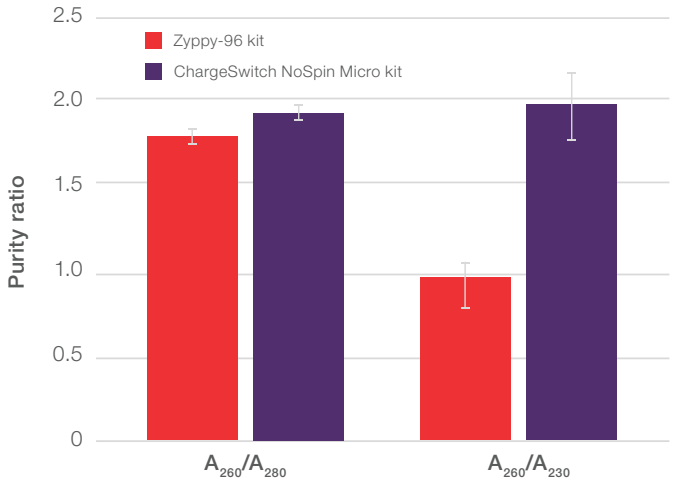

Figure 3. Average purity measurements from manual extractions. The average A_{260}/A_{280} for samples extracted using the Zyppy-96 kit was 1.79 (SD = 0.039), while the average A_{260}/A_{280} for samples extracted using the ChargeSwitch NoSpin kit was 1.93 (SD = 0.044), clearly indicating sufficient pDNA in the samples obtained using the ChargeSwitch NoSpin kit. The average A_{260}/A_{230} for samples extracted using the Zyppy-96 kit was 0.98 (SD = 0.073),** indicating the presence of possible contaminants that absorb at 230 nm, while the average A_{260}/A_{230} for samples extracted using the ChargeSwitch NoSpin kit was 1.97 (SD = 0.197).

** 1 ng of lipopolysaccharides (LPS) = 1.8 EU/mg.

Endotoxicity

Endotoxicity was measured using the EndoSafe LAL test system. According to industry standards, preparations with endotoxin levels of <0.1 EU/µg are regarded as "endo-free" (endotoxin-free), and are also commonly referred to as advanced transfection-grade. Preparations with endotoxin levels between 0.1 EU/µg and 1.0 EU/µg are considered "low-endo" (low endotoxin) and are characterized as transfection-grade, while endotoxin levels >1.0 EU/µg are molecular-grade.

The ChargeSwitch NoSpin Plasmid Micro Kit asserts no claims toward endotoxin reduction.† Hence, it was assumed that those samples had endotoxin levels of ≥1.0 EU/µg, and therefore, they were not evaluated using the EndoSafe LAL system. However, the ChargeSwitch Plasmid ER Mini Kit asserts endotoxin reduction, with levels at <50 EU/µg per preparation. The Zyppy-96 Plasmid kit claims an "endotoxin-free" kit preparation, yet the product literature states that an endotoxin level ≤50 EU/µg per preparation is expected.

With an average endotoxin level of 0.51 EU/µg, the ChargeSwitch Plasmid ER Mini Kit meets the criterion for an endotoxin-reduced extraction (n = 2, Table 1). Conversely, the Zyppy-96 Plasmid kit failed to meet the endo-free extraction claim and its specification of endotoxin levels of ≤50 EU/µg, with an average of 114.45 EU/µg per sample (n = 2, Table 1). These results suggest the Zyppy-96 Plasmid kit would be more appropriately described as a molecular-grade purification kit.

Table 1. Average endotoxicity measurements using the ChargeSwitch Plasmid ER Mini Kit and Zyppy-96 Plasmid kit.

Kit	Sample no.	EU/µg (per sample)	EU/µg (average)
ChargeSwitch Plasmid ER Mini Kit	Sample 1	<0.09	<0.51
	Sample 2	<0.93	
Zyppy-96 Plasmid MagBead Miniprep Kit	Sample 1	<131.07	<114.45
	Sample 2	<97.82	

† If low endotoxin levels are desired for the ChargeSwitch kit, users may supplement an endotoxin reducing reagent; however, this addition has not yet been validated by Thermo Fisher Scientific.

pDNA isoforms

The most desired pDNA isoform is the supercoiled form, due to its excellent stability and favorable antigenicity. A supercoiled shape allows DNA to enter cells more easily, resulting in more colonies post-transformation. Other isoforms may also exist such as the nicked form, which is a relaxed (non-supercoiled) open circular DNA. Linear pDNA forms when both DNA strands are broken at or near the same point. The presence of host cell genomic DNA, nicked pDNA, linear pDNA, or single-stranded pDNA (sspDNA) is considered an extraction impurity that may adversely impact downstream workflow processing. To assess the presence of pDNA isoforms, a 1% agarose gel electrophoresis was run for 8 samples from each kit (Figure 4). The results indicated that ≤50% of pDNA from each sample obtained from the Zyppy-96 kit was supercoiled; the remaining sample consisted of genomic, nicked, or single-stranded forms of DNA. In contrast, the samples extracted using the ChargeSwitch No Spin or ChargeSwitch Plasmid ER kits showed that supercoiled pDNA was the predominant isoform in >50% of the samples, with limited amounts of nicked or genomic DNA.

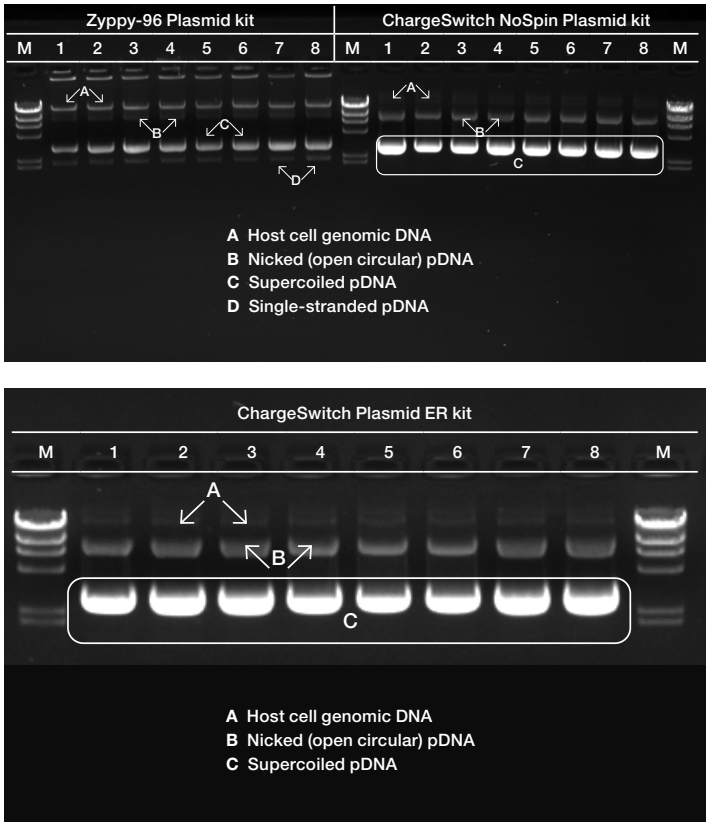

Figure 4. Electrophoresis of a 1% agarose gel to assess the presence of pDNA isoforms. Samples extracted using the Zyppy-96 Plasmid kit contained genomic DNA, nicked pDNA, supercoiled pDNA, and single-stranded pDNA. The samples extracted using the ChargeSwitch NoSpin or Plasmid ER kits contained supercoiled pDNA as the predominant pDNA isoform.

Automated extraction

Methods—pDNA isolation and purification

An automated extraction of samples using the ChargeSwitch NoSpin Plasmid Micro Kit was performed using the KingFisher Flex Purification System (Cat. No. 5400630). Seven plates were filled, created, and labeled, according to the reagents and plate names listed in Table 2. Five wells of a Thermo Scientific™ KingFisher™ 96 Deep-Well Plate, labeled "Plasmid Sample Plate", were filled with 1 mL of bacterial culture in LB medium, followed by the addition of 30 µL of vortexed ChargeSwitch MagnaClear Beads. Then, two clean Thermo Scientific™ KingFisher™ 96 tip combs were inserted into two empty Thermo Scientific™ KingFisher™ 96 Standard Plates, labeled "Tip Comb Plate 1" and "Tip Comb Plate 2". Additionally, two KingFisher 96 Deep-Well Plates were labeled "Wash Plate 1" and "Wash Plate 2". A KingFisher 96 Standard Plate was filled with 100 µL of ChargeSwitch Elution Buffer and labeled "Elution Plate". Plates were loaded onto the KingFisher Flex System using the protocol "CS10201_CS_NoSpin_Flex_Automated.bdz". Since a liquid handler was not available for this experiment, the protocol "CS10201_CS_NoSpin_Flex_Automated.bdz" was written with two user-prompted pauses for the manual addition of 150 µL of chilled Invitrogen™ ChargeSwitch™ Precipitation Buffer and 30 µL of ChargeSwitch Magnetic Beads to each well.

For comparison, manual extractions using the ChargeSwitch NoSpin Plasmid Micro Kit were completed for the same number of samples according to the user guide.

A NanoDrop Spectrophotometer was used to measure yield (A_{260}) and purity (A_{260}/A_{280} and A_{260}/A_{230}) of samples extracted using the ChargeSwitch NoSpin kit from both sets—manual extraction and automated extraction using the KingFisher Flex Purification System. Electrophoresis of a 1% gel was performed to visually assess the presence of pDNA isoforms in samples from each set. Endotoxicity was not measured for the ChargeSwitch NoSpin Plasmid Micro Kit, as this kit does not include "low endotoxin" or "endotoxin-free" claims.

Table 2. Plates and reagents needed for automated extraction using the ChargeSwitch NoSpin Plasmid Micro Kit and the KingFisher Flex Purification System.

Plate no.	Plate label	Plate type	Reagent	Volume/well
1	Plasmid Sample Plate	KingFisher 96 Deep-Well Plate	Overnight bacterial culture in LB medium	1 mL
			ChargeSwitch MagnaClear Beads	30 µL
2	Plasmid Processing Plate	KingFisher 96 Deep-Well Plate	ChargeSwitch Resuspension Buffer (R4) + ChargeSwitch RNase A	150 µL
			ChargeSwitch Lysis Buffer (L9)	150 µL
			ChargeSwitch Precipitation Buffer (N5) (dispensed manually or with liquid handler)	150 µL
			ChargeSwitch Magnetic Beads (dispensed manually or with liquid handler)	30 µL
3	Tip Comb Plate 1	KingFisher 96 Standard Plate	Tip Comb (96) in empty plate	—
4	Wash Plate 1	KingFisher 96 Deep-Well Plate	ChargeSwitch Wash Buffer (W11)	900 µL
5	Wash Plate 2	KingFisher 96 Deep-Well Plate	ChargeSwitch Wash Buffer (W12)	900 µL
6	Elution Plate	KingFisher 96 Standard Plate	ChargeSwitch Elution Buffer (E5)	100 µL
7	Tip Comb Plate 2	KingFisher 96 Standard Plate	Tip Comb (96) in empty plate	—

Results

pDNA yields—automated vs. manual extractions

In comparison to manually processed samples, samples extracted using the ChargeSwitch NoSpin kit and KingFisher Flex Purification System generated an average 2.9-fold increase in pDNA yield (Figure 5). The average pDNA yield of ChargeSwitch NoSpin samples using automated extraction was 13.2 µg compared to 4.5 µg for manually extracted samples (n = 5).

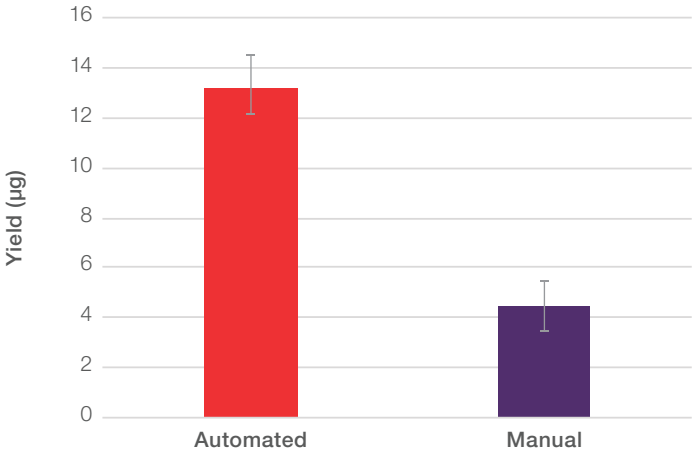

Figure 5. Average pDNA yields for samples processed with the ChargeSwitch NoSpin Plasmid Micro Kit using automated or manual extraction.

Purity—automated vs. manual extractions

A_{260}/A_{280} and A_{260}/A_{230} ratios were measured for samples extracted using the ChargeSwitch NoSpin Plasmid kit with manual or automated extraction (Figure 6). The A_{260}/A_{280} was equivalent for both sample sets at >1.8, indicating dsDNA was the main product in both samples. The A_{260}/A_{230} for the samples that underwent automated extraction was slightly better than for the manually extracted samples; however, both extraction methods produced ratios of >1.8, suggesting all samples were contaminant-free and suitable for downstream processing.

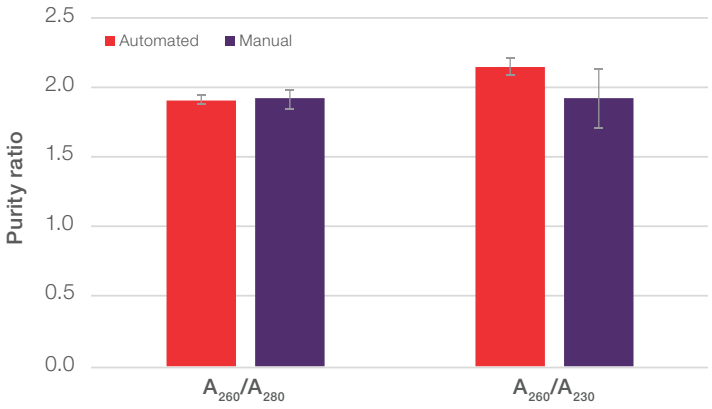

Figure 6. Average purity measurements for the samples processed with the ChargeSwitch NoSpin Plasmid Micro Kit using automated or manual extractions.

DNA isoforms—automated vs. manual extractions

To assess the integrity of pDNA extracted using the ChargeSwitch NoSpin kit using either automated or manual methods, samples were separated in a 1% agarose gel. Manually extracted samples were loaded into lanes 1 through 3, and samples that were extracted using the KingFisher Flex system were loaded into lanes 4 through 7 (Figure 7). Both automated and manually extracted samples contained supercoiled pDNA as the predominant isoform, followed by nicked pDNA.

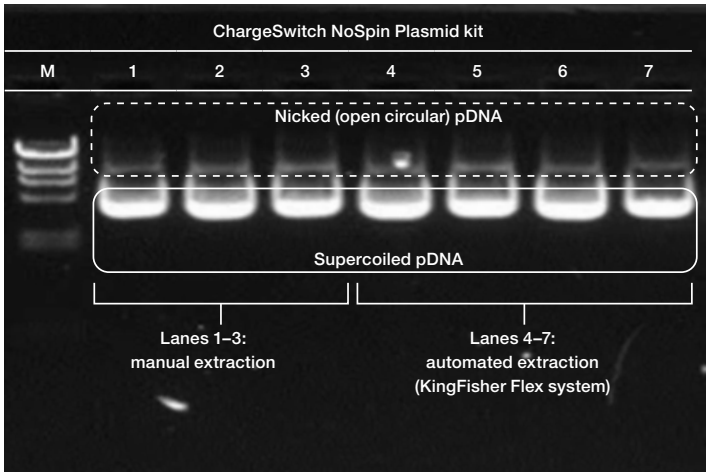

Figure 7. Comparison of DNA isoforms generated using the ChargeSwitch NoSpin Plasmid Micro Kit with manual or automated extraction.

Discussion

Both the ChargeSwitch NoSpin and Zyppy-96 MagBead Plasmid kits offer pellet-free extraction methods suited for high-throughput workflows. Eliminating time-consuming centrifugation steps during extraction is one way to increase workflow efficiency. However, the value associated with eliminating centrifugation is only realized if a system constraint has been removed as well. Here, the ChargeSwitch NoSpin kit in conjunction with a KingFisher Flex system provides the best solution, as the automation system performs the extraction, thereby freeing up automated liquid handlers or physical resources for other workflow tasks, including plate preparation. By using KingFisher Flex system automation, plate preparation can be performed concurrently with extraction, thereby increasing throughput. The main limiting factor is the number of KingFisher systems one may choose to integrate to maximize efficiency.

While the Zyppy-96 MagBead kit claims to be the "fastest" pellet-free kit, we found this statement to be untrue. With respect to manual extractions, both the Zyppy-96 and ChargeSwitch NoSpin kits were comparable, taking approximately 90 minutes. However, when evaluating overall speed for automated extractions, the ChargeSwitch NoSpin kit was faster. A calculation of the timings specified in the Zyppy-96 kit user manual indicates an automated extraction of 96 samples using the Zyppy-96 kit would take approximately 2 hours, including buffer preparation and pipetting times. This differs from the workflow for the ChargeSwitch NoSpin kit that took less than 1 hour using the automated KingFisher system (44 minutes for the KingFisher Flex system protocol and 10 minutes for manual pipetting).

Even though pDNA yields can vary due to differences in plasmid copy number, growth conditions, and bacterial strains, we observed higher average yields with ChargeSwitch NoSpin and Plasmid ER kits when compared to the Zyppy-96 kit. Automating the ChargeSwitch NoSpin kit protocol on the KingFisher Flex Purification System almost tripled average yield compared to manual processing.

Traditional magnetic bead–based extraction techniques, completed manually or using a liquid handling robot, rely on isolating magnetically bound pDNA through liquid transfer. Conversely, extractions using a KingFisher system focus on moving magnetically bound beads, in lieu of liquid, and potentially reducing bead loss associated with traditional techniques, thereby increasing overall pDNA yields. Other factors that limit kit efficiency include bacterial growth conditions and buffer preparation times. The Zyppy-96 kit instructs users to grow cultures in a 96-well block. Doing so presents a possible constraint, as the aeration of bacteria is restricted to the volume and size of the vessel, which may negatively impact yields. The ChargeSwitch NoSpin kit does not impose any size restrictions on culture vessels and requires less time overall for bacterial culture growth. Unlike the Zyppy-96 kit, the ChargeSwitch NoSpin kit contains all buffers needed for the extraction, with the only preparation being the addition of pre-aliquoted RNase A to the Invitrogen™ ChargeSwitch™ Resuspension Buffer. As opposed to the Zyppy-96 kit, the ChargeSwitch NoSpin kit does not require heating of lysis buffer, which simplifies the extraction process by reducing the total time needed for bacterial cell lysis.

Both the ChargeSwitch NoSpin and Zyppy-96 kits demonstrated ideal levels of "pure" DNA at the A_{260}/A_{280} ratio. However, at the A_{260}/A_{230} ratio, the ChargeSwitch NoSpin kit was superior to the Zyppy-96 kit. The A_{260}/A_{230} ratio is a sensitive measurement of potential contaminants that absorb at 230 nm. Common contaminants that absorb at this wavelength include chaotropic salts, non-ionic detergents, and phenolic compounds. Ideal purity for the ChargeSwitch NoSpin kit at A_{260}/A_{230} was expected, due to the chemical composition of the ChargeSwitch buffers.

With regard to endotoxin levels, we found the Zyppy-96 kit did not meet the criterion to be classified as an endotoxin-free or low-endotoxin extraction method. Endotoxins are a source of concern because they can induce nonspecific activation of immune responses in cells, resulting in (1) suboptimal transfection and toxicity in many cell lines, (2) a decrease in protein expression in sensitive cells, and (3) adverse health effects. Endotoxin levels of samples prepared with the Zyppy-96 kit were comparable to molecular-grade plasmid purification kits at an average of 114.5 EU/µg pDNA. The ChargeSwitch Plasmid NoSpin Micro Kit is a molecular-grade kit and does not make claims for endotoxin reduction, so endotoxin levels were not measured and were assumed to be >1.0 EU/µg pDNA. An endotoxin-reducing agent could be added to the cleared lysate during the ChargeSwitch beads dispensing step to achieve low- or no-endotoxin extraction.

Summary

Both magnetic bead–based ChargeSwitch Plasmid kits from Thermo Fisher Scientific outperformed the automated Zyppy-96 Plasmid kit from Zymo Research. Liquid handling automation in conjunction with the KingFisher Purification System provides the most seamless extraction scenario. However, the KingFisher Purification System alone still allows for efficient and convenient plasmid extraction, only requiring two manual dispense steps. Users who wish to automate plasmid mini preps as easily as possible can incorporate the ChargeSwitch Plasmid NoSpin Micro Kit and a Thermo Scientific™ KingFisher™ magnetic bead processor to achieve approximately the same yields as magnetic bead kits that require up-front manual manipulation and centrifugation.

Learn more at **thermofisher.com/nospin**