

High-Throughput 96-well and 384-well nucleic DNA purification with MagSi-DNA clean^{FIX}

Walk-away Magnetic Bead-based PCR clean-up and Dye-Terminator Removal

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Figure 1: MagSi-DNA clean^{FIX} magnetic bead with BigDye[®] labeled DNA

INTRODUCTION

In many genomics applications, **purification of DNA fragments** is required to prevent downstream interference with analysis methods. Purification of PCR reactions often requires removal of dNTPs, primers, enzymes, salt by-products and various additives. Purification protocols involve different sample types, different volumes and often require multiple washing and centrifugation steps. Errors during sample preparation result in lost time and significant costs due to re-work and lost reagents.

MagSi-DNA clean^{FIX} offers a fast and efficient solution for purifying DNA fragments, removing all unwanted side products and reagents, such as single nucleotides, terminator dyes, primers and primer dimers, enzymes, buffers and salts. MagSi-DNA clean^{FIX} enables a **combination of PCR clean-up and dye terminator removal** from sequence reaction mixes with a **single product**. The kit uses magnetic particle technology, and is easily automated because no columns or centrifugation steps are involved. The kit uses a simple “Bind, Wash & Elute” procedure common to magnetic particle purification protocols.



Figure 2: JANUS Mini workstation

This application note describes how MagSi-DNA clean^{FIX} is used on JANUS[®] Automated Workstations for high throughput nucleic acid purification. The goal was to set-up a **384-well clean-up automated solution**, increasing throughput, reliability and data robustness while reducing reagent consumption and disposable costs. **PerkinElmer NV** and **MagnaMedics Diagnostics BV** created an **off-the-shelf solution** for automating the 384-well clean-up protocols.

This project was realized in collaboration with **IME Fraunhofer Institute** for Molecular Biology and Applied Ecology. The institute conducts research in the field of applied life sciences from a molecular level to entire ecosystems and offers a complete portfolio of **DNA sequencing** and related services. Fraunhofer IME, Division Molecular Biology (Aachen, DE) was selected for **sample preparation, readout and data analysis** needed in the development of automated high throughput DNA purification protocols, because of their extensive knowledge about PCR amplification and DNA sequencing of samples from various source materials, requirements of high-throughput DNA purification methods, and high quality analysis of the generated data.

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The **JANUS Automated Workstation** family was chosen as the platform for setting up the combined solution. The JANUS Automated Workstations come in a variety of sizes and system configurations, addressing various needs and requirements in terms of capacity and throughput. The **deck sizes** range to accommodate from 9 to 32 positions for SBS labware, while maintaining a high degree of flexibility towards deck layout and custom labware introduction with the use of deck support tiles.

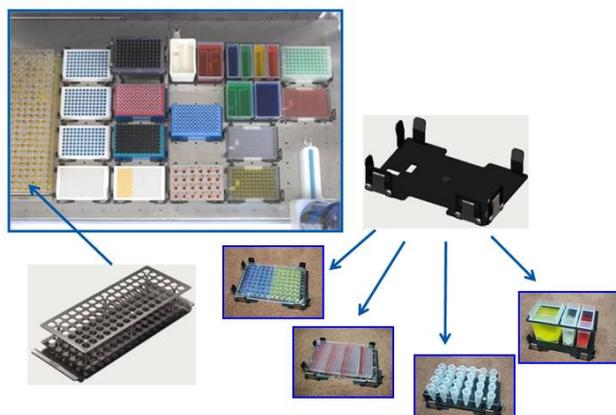


Figure 3: JANUS Deck Tile Concept



Figure 4: VersaTip Mixed-Tip mode

The system configurations can be built by implementing **three arms** on the JANUS Automated Workstation.

The **4-channel or 8-channel VariSpan™** pipetting arm provides the highest degree of flexibility towards accessing different kinds of labware

ranging from tubes and vials to 96-well and 384-well plates. This is realized by the variable span between the different tips. This arm holds the patented **VersaTip™** technology, allowing the same tip adaptor to be used in disposable tip mode or fixed tip mode while eliminating the use of disposables where possible.

A **Modular Dispense Technology (MDT) arm** can be fitted to the system. The MDT arm supports 96-channel and 384-channel pipetting heads. The patented technology allows on-the-fly changing of the heads: the system can switch from the 96-channel head to the 384-channel head without any user intervention. In terms of robustness and maintenance friendliness, the aluminium heads do not contain any glass syringes or o-rings.

The system can be equipped with a **gripper arm**, to enable automated traffic of plates on the deck and physical integration with other devices and instrumentation such as plate readers, shakers, incubators etc.

To demonstrate the performance of the MagSi-DNA clean^{FIX} and JANUS workstation in automated purification of DNA fragments, varying sample volumes of PCR and sequence reactions were purified using 96- and 384-well PCR plates. Purified PCR reactions were analyzed by gel

electrophoresis. Purified sequence reactions were analyzed with a ABI PRISM® 3730 Genome Analyzer (Applied Biosystems).

Materials and Methods

Instrumentation. The JANUS Automated Workstation consisted of a JANUS mini workstation (figure 4) fitted with an MDT arm. The Modular Dispense Head installed on the system was the P50 head, accommodating 20 µL and 50 µL tips. The deck was populated with the MDT Tipwash station, MDT Medium Support Tiles (figure 6) and the MM-Separator 96 SBS and MM-Separator 384 SBS (figure 5). Consumables included P20 and P50 MDT Non-Sterile Tips (PerkinElmer, Inc.), 96 well Full Skirt PCR Microplates (Axygen, Inc.) and 384 well PCR Microplates (Axygen, Inc.), and Thermo-Fast® 96-Well Detection Plates (ABGene, Thermo Scientific).

The purification kit and reagents included MagSi-DNA clean^{FIX} (MagnaMedics Diagnostics BV) for PCR clean-up as well as Dye-Terminator removal, BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems), ethanol absolute and 2-propanol (VWR), and USP-WFI (Lonza). The MagSi-DNA clean^{FIX} purification kit includes a magnetic particle mix for Dye-Terminator removal and an additional MagSi-DNA clean Buffer P for PCR clean-up. An alcohol mix solution containing 42.5% 2-propanol and 42.5% ethanol is prepared for dye-terminator removal.

Preparations. The MDT Tipwash Station on the JANUS Automated Workstation is supplied with demineralized water. Reagents are supplied in Tip-box lids and consumables are placed on standard JANUS labware (as shown in Figure 3). PCR sample plates and processing plates are positioned according to the desired set-up and throughput. In most cases, sample plates can directly be used for purification. However, transfer of samples to a clean plate for processing may improve purification performance. The magnetic separator for 96- or 384-well PCR plates is placed on a Medium Deck Support Tile (Figure 6, right), enabling easy set-up and definition in the software.

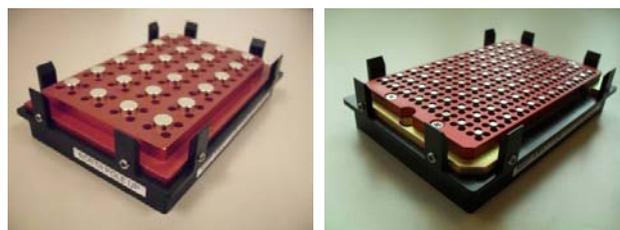


Figure 5: MM-Separator 96 SBS for 96-well plates (left) and MM-Separator 384 SBS for 384-well plates (right) – MagnaMedics Diagnostics BV

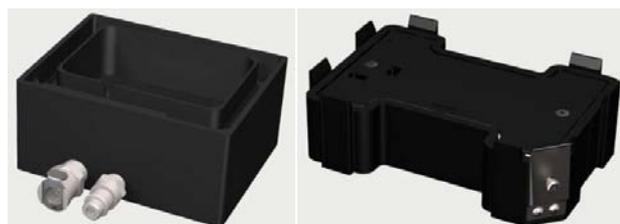


Figure 6: MDT TipWash (left), Medium Deck Support Tile (right)

Deck layout and protocols. Figure 7 shows the deck layout used to automate the MagSi-DNA cleanTM protocol. The deck demonstrates how a **JANUS Mini** with **9 deck positions** can be used to process up to **384 samples** in a **single run**, with sample volumes ranging from **5 to 20 µL**. Larger deck sizes (expansion to a JANUS standard deck or JANUS expanded deck) enable the clean-up processing of batches of plates, while maintaining the walk-away automation character of the application set-up. A similar deck layout is used for PCR clean up as well as dye terminator removal (1 deck position difference, not shown).



Figure 7: JANUS MagSi-DNA cleanTM
Deck layout for dye terminator removal
A. MDT Tipwash
B. Additional Waste Container
C. Clean-up Plate (off-magnet position)
D. Elution Plate
E. Clean-up Plate (on-magnet position)
F. Reagent Reservoir (elution buffer)
G. Reagent Reservoir (alcohol mix)
H. Reagent Reservoir (magnetic beads)

PCR clean-up

Sample preparation. PCR products of (~500bp) were prepared in 20 µL reactions with 2X PCR Master mix Solution (*i-MAX-II*) (iNTRON Biotechnology, Inc.) (*MaximeTM* PCR PreMix Kit). Samples were pooled and divided into 96 well PCR microplates.

JANUS PCR clean-up Protocol
◊ Aspirate and dispense 5 µL MagSi-DNA clean TM
◊ Aspirate and dispense 15 µL MagSi-DNA clean Buffer P
◊ Incubate for 3 minutes and move plate to magnet
◊ Move to magnet, remove supernatant
◊ Move plate from magnet, add 30 µL EtOH 70% and mix by pipetting
◊ Move to magnet, remove supernatant
◊ Repeat EtOH washing loop twice
◊ Move plate from magnet
◊ Air-dry for 6 minutes
◊ Add 30 µL WFI-USP and mix by pipetting
◊ Incubate for 2 minutes, move to magnet
◊ Transfer 25 µL purified DNA sample to collection plate

Figure 8: JANUS MagSi-DNA cleanTM Protocol for PCR clean-up of 10 µl PCR sample.

Results. Purified PCR Samples were analysed by gel electrophoresis with a 1.2% agarose gel and ethidium bromide staining. Figure 7 shows that primer dimers were efficiently removed, with high recovery of the PCR product (>70%).

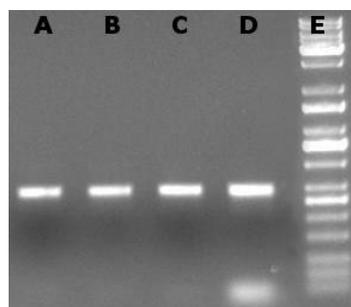


Figure 9: Gel electrophoresis of PCR samples. Lane A, B, C: samples purified with MagSi-DNA cleanTM. Lane D: unpurified PCR sample showing primer dimers. Lane E: Low Molecular Weight DNA Marker

Dye Terminator removal

Sample preparation. 10 µL single primer sequencing reactions were set up with 0.5 µL pGEM Plasmid template DNA, 3.2 pmol primer (AB product), 1.2 µL 5X BigDye Sequencing Buffer, 0.8 µL BigDye Terminator Premix (v3.1), 2 µL betaine 5 M, and nuclease free water. Samples were pooled and divided into wells of 96 and 384 PCR microplates, in volumes ranging from 5 to 20 µL.

JANUS Dye Terminator Removal Protocol
◊ Aspirate and dispense 5 µl MagSi-DNA clean TM
◊ Aspirate and dispense 20 µl Alcohol Mix
◊ Incubate for 3 minutes and move plate to magnet
◊ Move to magnet, remove supernatant
◊ Move plate from magnet, add 30 µL alcohol mix and mix by pipetting
◊ Move to magnet, remove supernatant
◊ Repeat alcohol mix washing loop twice
◊ Move plate from Magnet
◊ Air-dry for 6 minutes
◊ Add 30 µL WFI-USP and mix by pipetting
◊ Incubate for 2 minutes, move to magnet
◊ Transfer 25 µL purified reaction mix to collection plate

Figure 10: JANUS MagSi-DNA cleanTM Protocol for dye terminator removal of 10 µl sequencing reaction sample.

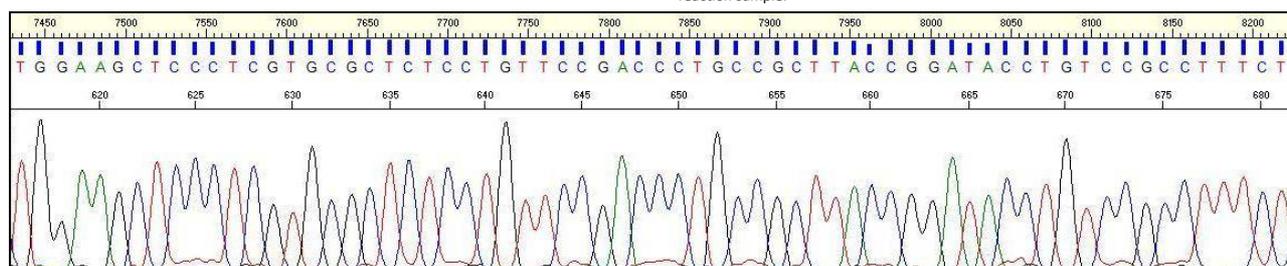


Figure 11: Sequence electroferogram after purification of pGEM Plasmid Template DNA

Results. Purified sequencing reactions were injected into an ABI 3730xl DNA Analyzer. Figure 12 and 13 show the results obtained from purification of varying sequencing reaction volumes. A typical electroferogram is shown in Figure 11, with quality resulting in QV20+ Score of >1000.

Figure 12: Quality scores and read lengths after purification of varying sequencing reaction volumes.

Sequencing reaction volume purified (pGEM Plasmid)	Trace Score	QV20+
5	43	1013
10	42	1024
20	44	1022

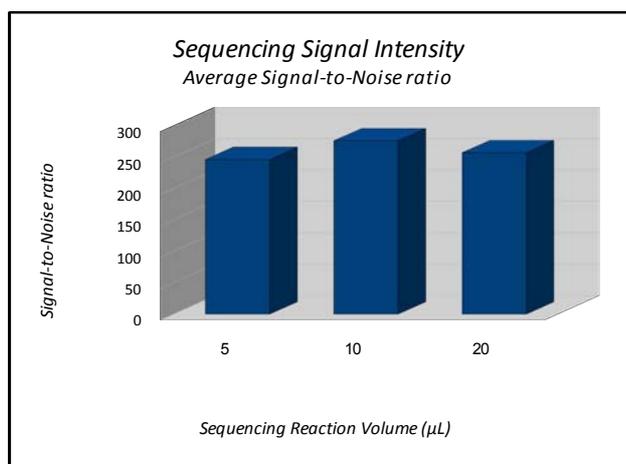


Figure 13: Signal intensities for varying sequencing reaction volumes.

Conclusions

The MagSi-DNA clean^{FIX} purification methods developed for the JANUS Automated Workstations provide an **automated walk-away solution for PCR clean-up and dye terminator removal** from sequence reactions. The precision and reproducibility of the JANUS workstations in combination with the customized magnets for MagSi-DNA clean^{FIX} allow fast magnetic separation and homogenisation of the samples.

The **PCR clean-up** protocol enables fast and efficient recovery of DNA fragments larger than 80 bp with >99% removal of primers and primer dimers. The **dye terminator removal** method consistently delivers high quality sequence data with Phred >20 scores above 700.

Both methods are **easily adaptable** for individual set-ups and sample throughputs. The purification system can be scaled up with a larger version of the JANUS Automated Workstation. Using the **JANUS expanded or integrator** version and implementing additional arms, a gripper, sufficient magnets and a stacker option, up to **12x96 plates** can be processed **each hour** continuously, enabling the highest sample throughput available in the market.

Figure 14: Protocol times for 1-12 PCR plates (96w) with various system configurations

System configuration	Throughput	Time (min)
JANUS Mini with 9 positions, MDT arm, magnetic separator	4x96 samples	35
JANUS Standard with 24 positions, two arms, two magnetic separators	8x96 samples	45
JANUS Integrator with 32 positions, three arms, three magnetic separators	12x96 samples	60

Custom application accessories are easily integrated with the modular deck design of the JANUS. Pipetting tools of the JANUS workstation can be linked with labware movement around the deck or into devices such as sequencers, readers or incubators to completely automate the application.

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