APPLICATION NOTE

Comparison of Automated Nucleic Acid Purification Systems on High Molecular Weight (HMW) DNA Extraction Efficiency.

Summary

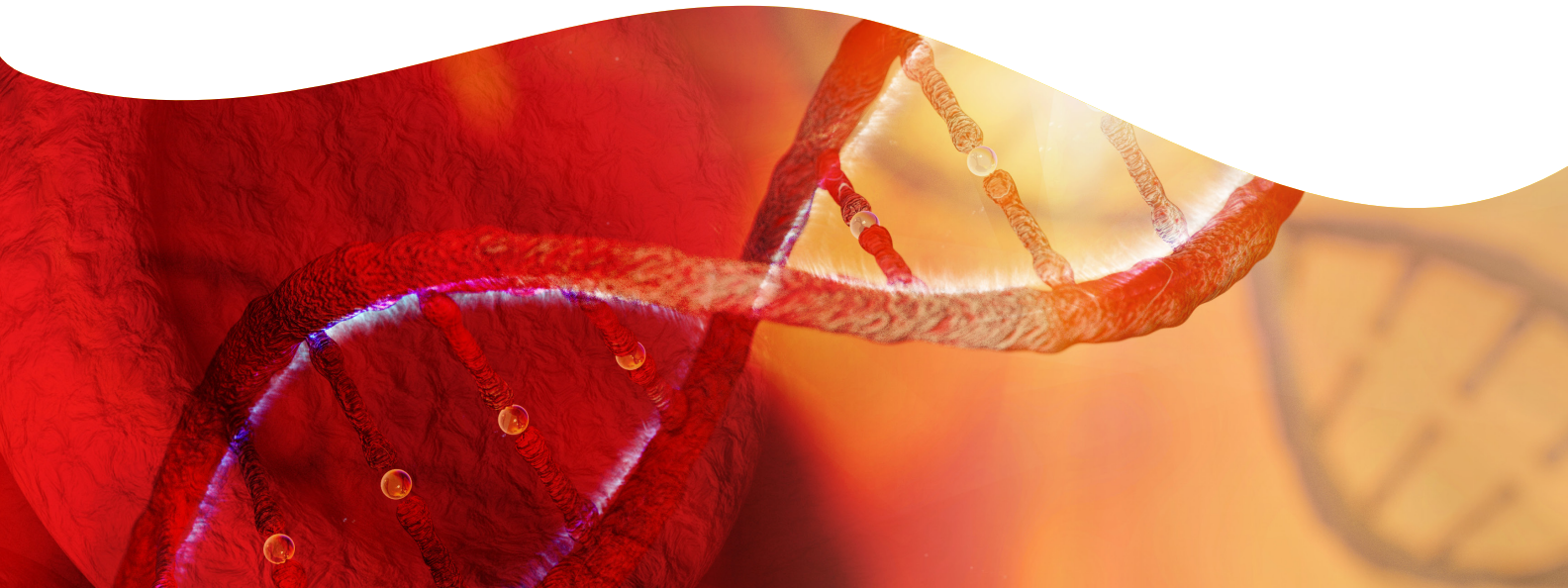
- Extraction performance from blood based on DNA yield, purity, and integrity (DNA length) was compared between two automated nucleic acid extraction systems
- chemagic™ 360 instrument extracted pure DNA at higher yields and integrity with both high and low volume blood extraction kits (CMG-1074, CMG-1091)
- A greater HMW DNA extraction efficiency was obtained with the chemagic™ system that can improve performance in long-read sequencing as well as long-term storage stability

Abstract

With automation being increasingly employed in laboratories performing nucleic acid purification, a more consistent quality of nucleic acids is generally expected. A high quality of nucleic acids should also be obtainable with automation to meet requirements of demanding downstream assays such as NGS, long read sequencing, and MLPA, while ensuring long-term storage stability. However, differences in mechanical processes and kit chemistries between commercial automation platforms may result in varying qualities of nucleic acid which can impact downstream results. Here, we evaluated two systems of magnetic bead-based automated nucleic acid purification using blood samples. Nucleic acid quality was assessed in terms of yield and purity. Integrity of DNA as seen by the extraction efficiency of high molecular weight (HMW) DNA was also assessed.

Introduction

As molecular genetic analyses are increasingly employed in research and clinical labs, there is a need for achieving quality nucleic acid isolation from diverse sample material. Automated nucleic acid extraction systems allow laboratories to achieve a higher throughput of sample processing with faster turnaround times, while reducing the risk of errors and minimizing exposure of personnel to hazardous reagents.



Despite the growing use of automation, the quality of nucleic acids obtained can vary between automated extraction systems, resulting in performance variations in downstream analysis. The quality of nucleic acids is represented by their yield, purity and integrity or fragment lengths. Though requirements of quality standards vary with the downstream assay, there has been an increasing shift towards whole genome sequencing, including the use of long-read sequencing. This calls for extraction of high molecular weight (HMW) DNA (lengths ≥ 50 kb) for use in various applications, including the construction of high-quality reference genomes for real-time surveillance of microbial/viral outbreaks or identifying structural variants and epigenetic defects associated with disease[1,2,3]. For biobanks and many laboratories, achieving a high integrity of nucleic acids is also advantageous to ensuring long-term storage stability.

This Application Note compares the performance of two automated nucleic acid purification systems which both incorporate the use of magnetic beads for DNA extraction from blood samples: 1) chemagic™ 360 instrument together with chemagic DNA extraction kits for low and high blood volumes 2) A common alternative system together with the associated kit (Kit A) from the same vendor for DNA extraction from low blood volumes. Yields, purity, and fragment lengths of extracted DNA were assessed.

Materials and methods

SAMPLE COLLECTION

Whole blood from five healthy donors was collected in 10 ml EDTA tubes (BD Vacutainer K3 (EDTA), Ref. 02.1066.001, Lot: #3031021). All blood tubes were stored at 4 °C for the specified time until DNA isolation. Prior to the nucleic acid extraction, the samples were brought to room temperature.

AUTOMATED NUCLEIC ACID EXTRACTION

Two extraction experiments were performed from the same samples for all conditions. Each extraction experiment comprised of 2-3 technical replicates – duplicates for the 4 ml sample volume condition using CMG-1074, and triplicates for the 400 µl sample volume condition using CMG-1091 and Kit A. All eluates were handled with wide bore tips and stored at -80°C until analysis.

Extraction with chemagic DNA extraction kits on chemagic 360 instrument

Nucleic acids were extracted on the chemagic 360 instrument from 0.4 ml or 4 ml human whole blood with the chemagic DNA Blood 400 Kit H96 (CMG-1091) or chemagic DNA Blood 4k Kit H24 (CMG-1074) respectively (Table 1). The chemagic™ separation technology involves specific capture of nucleic acids by chemagic M-PVA Magnetic Beads that are attracted to transiently magnetized metal rods, covered by sleeves. The magnetized rods transfer the chemagic™ M-PVA Magnetic Beads through the different process solutions and rotation of the demagnetized rod heads resuspend the particles during washing steps. A buffer dispensing system dispenses lysis, binding and wash buffers and no heating or centrifugation steps are required. Interchangeable rod heads can process extractions in 12-, 24- or 96- well formats, enabling processing of 10 µl to 18 ml sample volumes.

Figure 1. chemagic 360 instrument showing interchangeable rod head system

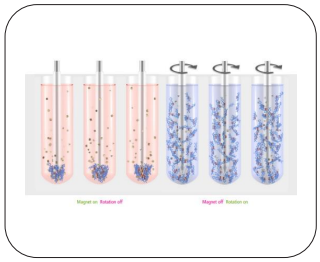


Figure 2. chemagic separation technology with rotating transiently magnetized rods

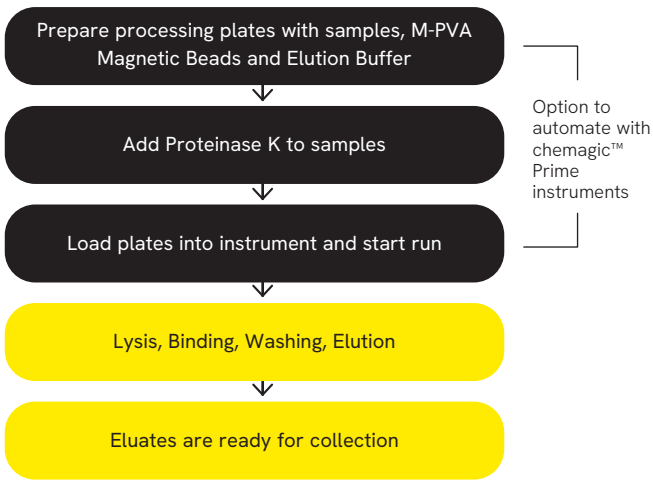


Figure 3. Processing steps with chemagic 360 instrument. Manual steps highlighted in black.

Table 1. Kits used with chemagic 360 instrument

Product #	Kit Name	chemagic Rod Head	Format	Preps/ Kit	Sample Volume
CMG-1091	chemagic DNA Blood 400 Kit H96	96	96-well	960	up to 400 µl
CMG-1074	chemagic DNA Blood 4K Kit H24	24	24-well	240	up to 4 ml

Extraction with alternative magnetic bead-based automated platform

For the compared platform, the standard blood extraction kit (Kit A) from the same provider was used (Table 2). The platform consists of permanent magnetic rods in a 96-well format covered in tip combs which associate with magnetic beads, moving them between different process solutions. Resuspension of beads are performed with an up-and-down motion to release beads and mix particles in reaction solution. There is no liquid dispensing system, and buffers and reagents must be added manually. A heating system is incorporated in the instrument for the Proteinase K digestion at 65 °C. This system also accommodates a 24-well format which was not tested here, enabling a sample volume range of 50 µl to 2 ml.

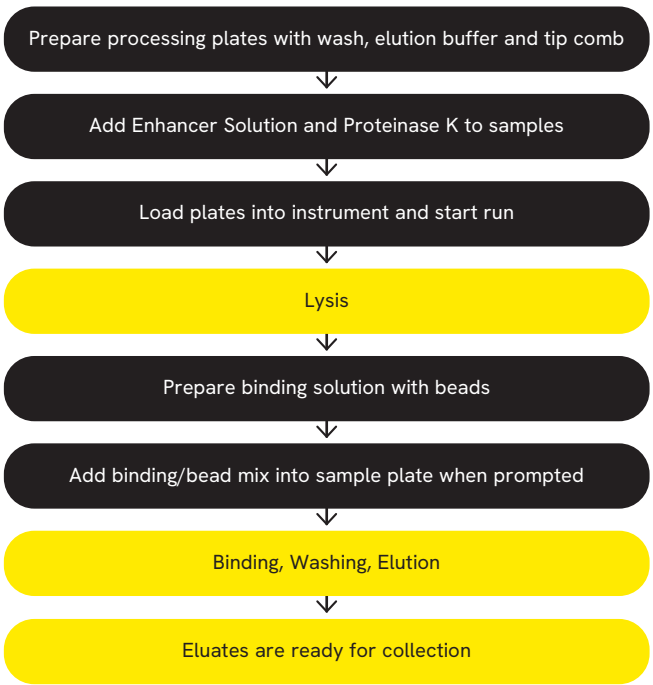


Figure 4. Processing steps with compared instrument. Manual steps highlighted in black.

Table 2. Kit A used on corresponding instrument platform

Kit Name	Rod Head	Format	Preps/Kit	Sample Volume
Kit A	96	96-well	200	200 – 400 µl

ANALYSIS OF EXTRACTED DNA

After DNA isolation, the DNA concentration of the purified eluates was determined with the Qubit™ dsDNA BR Assay Kit (Q32853, Thermo Fisher Scientific™, Waltham, MA, USA). In addition, the A_{260}/A_{280} and A_{260}/A_{230} purity ratios were determined with UV measurement (BioTek Epoch 160302E, Take3 measurement, Agilent Technologies, CA, USA). DNA fragment length distribution was analyzed with the Femto Pulse System (M5330AA, Agilent Technologies, CA, USA) using the Agilent Genomic DNA 165 kb Kit (FP-1002-0275, Agilent Technologies, CA, USA). The FP-1002-22 – Standard gDNA 165kb method was chosen to reach best resolution of high molecular weight peaks. The Femto Pulse results were analyzed with PROSize® 3.0 (version 3.0.1.5, Advanced Analytical Technologies, Inc., Ankeny, IA, USA).

Results

Higher DNA yields obtained with chemagic system

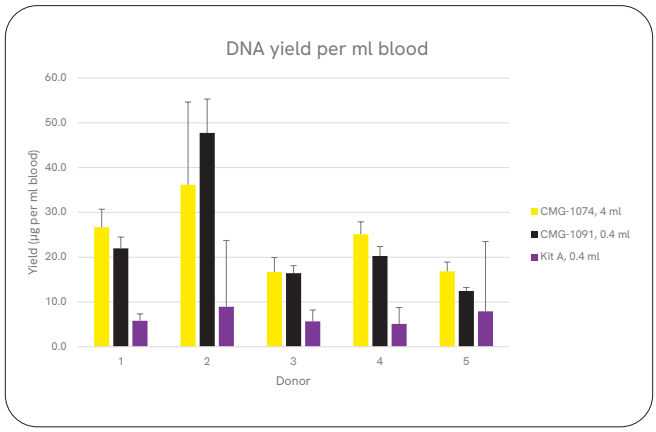


Figure 5. DNA yield represented by concentration of dsDNA in µg per ml blood extracted with the chemagic system compared to an alternative system. Two extraction experiments were performed on 1 day old blood from five donors using chemagic kits on chemagic 360 instrument for sample volume 0.4 ml (CMG-1091, 0.4 ml) and 4 ml (CMG-1074, 4 ml), and Kit A on alternative instrument for sample volume of 0.4 ml (Kit A, 0.4 ml). Mean values are shown ± s.d. of all measurements.

Figure 5 compares the yields obtained based on µg DNA extracted per ml blood as determined with the Qubit™ dsDNA BR Assay Kit. As yields of extracted DNA are highly donor-dependent, concentrations can vary strongly between donors. In all cases, the yields achieved with the chemagic system were higher than the compared system ranging between 16.8 – 36.2 µg/ml for CMG-1074 (4 ml sample volume), 12.5 - 47.8 µg/ml for CMG-1091 and 5.1 – 8.9 µg/ml for Kit A used with 0.4 ml sample volumes.

Expected DNA purities obtained with both systems

The A_{260}/A_{280} purities obtained with the chemagic kits on the chemagic 360 instrument were comparable to values obtained with Kit A on the corresponding system and were within the expected range of ~1.8 - 2.0 (Table 3). From all five donors, A260/A280 values ranged from 1.85 – 1.99 for 4 ml blood samples extracted with CMG-1074, 1.93 – 2.02 for 0.4 ml blood samples extracted with CMG-1091 and 1.89 – 2.08 for 0.4 ml blood samples extracted with Kit A. The A_{260}/A_{230} purities also measured were generally around ~2 or greater (1.84 – 2.56) for the chemagic kits on the chemagic 360 instrument while that obtained with Kit A on the alternative platform ranged from 1.63 – 2.02.

Table 3. DNA purity based on A_{260}/A_{280} absorbance ratios for DNA extracted with the chemagic system compared to an alternative system. Two extraction experiments were performed on 1 day old blood from five donors using chemagic kits on chemagic 360 instrument for sample volume 0.4 ml (CMG-1091, 0.4 ml) and 4 ml (CMG-1074, 4 ml), and Kit A on alternative instrument for sample volume of 0.4 ml (Kit A, 0.4 ml).

	Average A_{260}/A_{280}		
Donor	CMG-1074, 4 ml	CMG-1091, 0.4 ml	Kit A, 0.4 ml
1	1.91	1.96	1.96
2	1.85	1.93	2.02
3	1.93	2.00	1.89
4	1.96	2.02	1.96
5	1.99	1.97	2.08

Greater High Molecular Weight (HMW) DNA extraction efficiency with chemagic system

The fragment length distribution was analyzed with the Femto Pulse system applying the Agilent Genomic DNA 165 kb Kit, which offers the advantage of accurate separation of fragments larger than 100 kb. Exemplary electropherogram traces are shown for Donor 4 for DNA extracted from one day old blood with the compared platform (figure 6).

Blood extraction with both chemagic kits on the chemagic 360 instrument produced visibly longer DNA fragments than the compared system. For blood samples stored for one day at 4°C, the chemagic system obtained DNA from small to large sizes with a major peak typically around ~100 - 160 kb in size depending on the sample. The alternative system tended to show greater tailing toward the smaller size range with major peaks typically at size ~ 40 kb.

Blood when stored at 4°C for a further four days showed greater DNA fragmentation and a major peak accumulating at ~6 - 8 kb was seen for all tested conditions for donor 4 (figure 7). However, for chemagic extracted samples, a large proportion of HMW DNA was still visible with major peaks being detected at ~40 kb. Kit A in contrast showed only one major peak at 6-8 kb for DNA extracted from blood stored over five days.

To quantify the extraction efficiency of HMW DNA, the genomic quality number (GQN) was determined, where percentage of fragments over a threshold of 50 kb in length was calculated (Figure 8). It was clear that both chemagic kits on the chemagic 360 instrument extracted a significantly higher proportion of DNA fragments over 50 kb compared to the alternative system. The GQN 50 kb ranged from 61 - 68% for CMG-1074 (chemagic 4000), 55 - 70% for CMG-1091 (chemagic 400), and 22 - 42% for Kit A. This is indicative of a ~1-2 -fold greater extraction efficiency of HMW DNA for the chemagic system over the compared system.

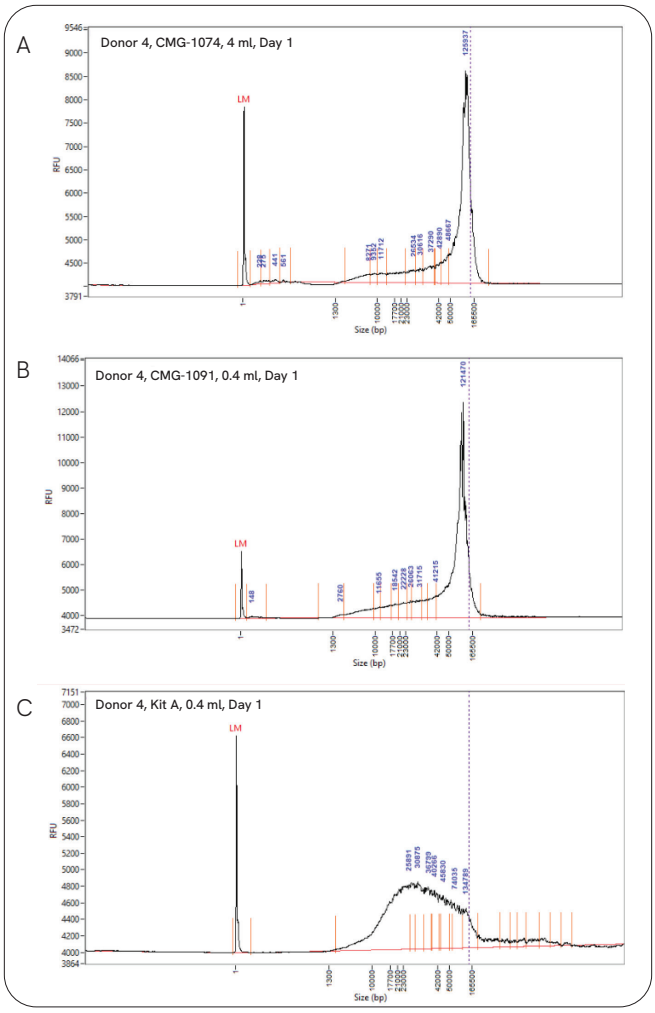


Figure 6. Exemplary Femto Pulse traces from Donor 4 for DNA extracted from 1-day old blood with the chemagic system compared to an alternative system. Conditions show A) DNA extracted with CMG-1074 on 4 ml blood on chemagic 360 instrument, B) DNA extracted with CMG-1091 on 0.4 ml blood on chemagic 360 instrument, and C) DNA extracted with Kit A on 0.4 ml blood on compared instrument

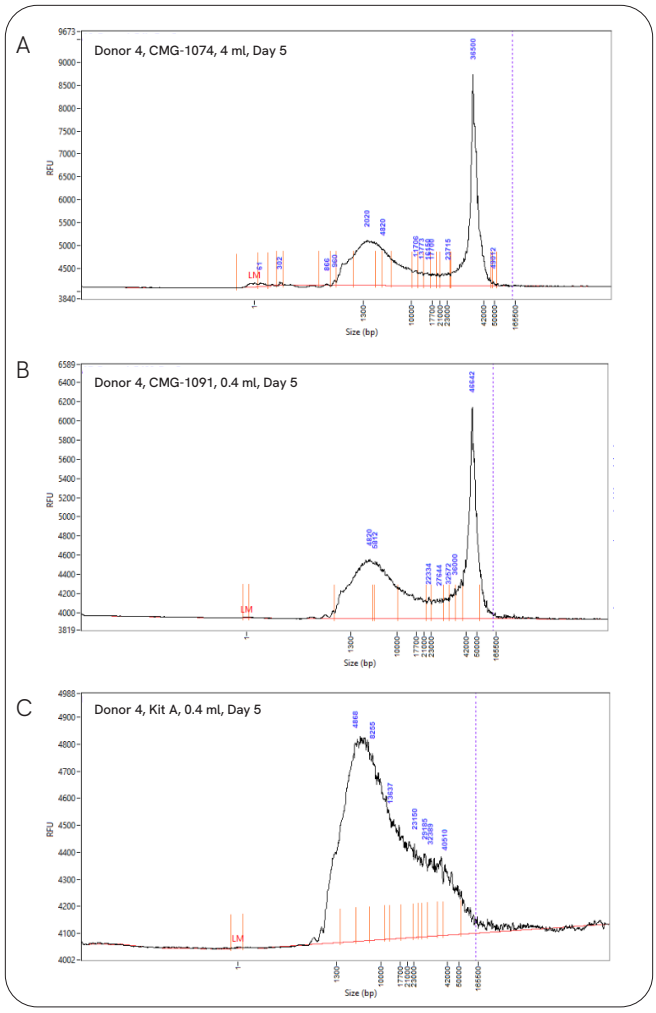


Figure 7. Exemplary Femto Pulse traces from Donor 4 for DNA extracted from 5-day old blood with the chemagic system compared to an alternative system. Conditions show A) DNA extracted with CMG-1074 on 4 ml blood on chemagic 360 instrument, B) DNA extracted with CMG-1091 on 0.4 ml blood on chemagic 360 instrument, and C) DNA extracted with Kit A on 0.4 ml blood on compared instrument

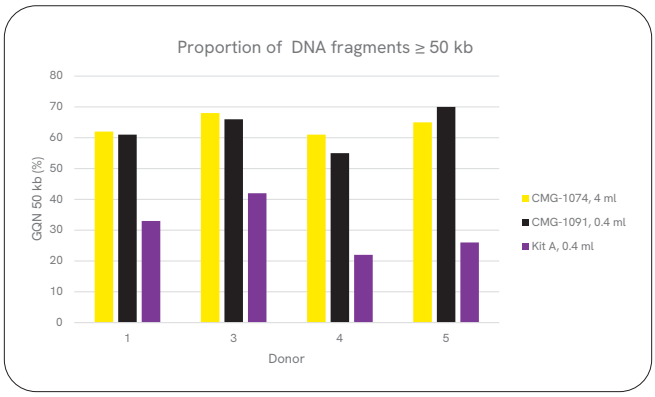


Figure 8. Proportion of DNA fragments ≥ 50 kb as depicted by Genomic Quality Number (GQN 50 kb) in % for DNA extracted with the chemagic system compared to an alternative system. One DNA sample from each of the four donors were subject to Femto Pulse analysis (donor 2 was excluded due to high variance in yield readings). Extractions were performed on 1-day old blood using chemagic kits on chemagic 360 instrument for sample volume 0.4 ml (CMG-1091, 0.4 ml) and 4 ml (CMG-1074, 4 ml), and Kit A on alternative instrument for sample volume of 0.4 ml (Kit A, 0.4 ml).

Discussion

The quality of nucleic acid is integral to the success of downstream molecular assays and can be impacted by variations in sample preparation methods. This application note shows that the choice of automated nucleic acid isolation platforms has a significant impact on the quality of DNA extracted from whole blood.

The chemagic system of automated purification with accompanying kits provided higher DNA yields of good purity when compared with the alternative system and Kit A when processing whole blood. Furthermore, extracted DNA integrity or fragment length, was significantly longer with the chemagic system. Extraction with both low and high-volume blood DNA chemagic kits on the chemagic 360 instrument

yielded DNA where a greater proportion of fragments were ≥ 50 kb in length compared to the alternative system and corresponding kit. This may be attributed to the reduced shearing forces produced by rotation of demagnetized rods on the chemagic system as opposed to the vigorous up and down motion used by permanent magnetic rods on the compared system. Furthermore, there are no heating steps in the chemagic workflow which can serve to further fragment DNA. As a result, chemagic systems have been used in conjunction with long read sequencing technologies from Oxford Nanopore[2,4,5,6] and PacBio[1,3] without the need for laborious manual sample processing steps and with standard extraction kits.

The high quality of DNA achieved with chemagic™ automated nucleic acid isolation together with the reliable high-end liquid handling automation one can incorporate for up- or downstream processing has also made it the choice for biobanks performing global population health studies where long-term storage and non-defined molecular assays make high quality a pre-requisite[2,7,8].

Conclusion

In conclusion, the chemagic kits used on the chemagic instruments provide an efficient and reliable solution for the automated extraction of small to large DNA fragments. The key benefit of greater HMW DNA extraction efficiency at high purity and yields provides multiple advantages both in terms of improving success in downstream assays and extending long term storage stability. With on-board lysis and an optimal buffer dispensing system, the fewer hands-on steps required by the chemagic system further helps to minimize workload as well as risk of errors.

References

1. Lang, K., Wagner, I., Schöne, B., Schöfl, G., Birkner, K., Hofmann, J. A., Sauter, J., Pingel, J., Böhme, I., Schmidt, A. H., Lange, V. (2016). ABO allele-level frequency estimation based on population-scale genotyping by next generation sequencing. BMC Genomics, 17(1), 1–11. https://doi.org/10.1186/s12864-016-2687-1

2. Beyter, D., Ingimundardottir, H., Oddsson, A., Eggertsson, H. P., Bjornsson, E., Jonsson, H., Atlason, B. A., Kristmundsdottir, S., Mehringer, S., Hardarson, M. T., Gudjonsson, S. A., Magnusdottir, D. N., Jonasdottir, A., Jonasdottir, A., Kristjansson, R. P., Sverrisson, S. T., Holley, G., Palsson, G., Stefansson, O. A., … Stefansson, K. (2021). Long-read sequencing of 3,622 Icelanders provides insight into the role of structural variants in human diseases and other traits. Nature Genetics, 53(6), 779–786. https://doi.org/10.1038/s41588-021-00865-4

3. Steiert, T. A., Fuß, J., Juzenas, S., Wittig, M., Hoeppner, M. P., Vollstedt, M., Varkalaite, G., Elabd, H., Brockmann, C., Görg, S., Gassner, C., Forster, M., & Franke, A. (2022). High-throughput method for the hybridisation-based targeted enrichment of long genomic fragments for PacBio third-generation sequencing. NAR Genomics and Bioinformatics, 4(3). https://doi.org/10.1093/NARGAB/LQAC051

4. Schmidt, J et al.(2022). Genotyping of familial Mediterranean fever gene (mefv)—single nucleotide polymorphism—comparison of nanopore with conventional Sanger sequencing. PLOS ONE, 17(3). https://doi.org/10.1371/journal.pone.0265622

5. Watson, C. M et al. (2019). Cas9-based enrichment and single-molecule sequencing for precise characterization of genomic duplications. Laboratory Investigation 100(1), 135–146. https://doi.org/10.1038/s41374-019-0283-0

6. Watson, C. M et al. (2021). Long-read nanopore sequencing enables accurate confirmation of a recurrent PMS2 insertion-deletion variant located in a region of complex genomic architecture. Cancer Genetics, 256–257, 122–126. https://doi.org/10.1016/J.CANCERGEN.2021.05.012

7. Valentin, N., Camilleri, M., Carlson, P., Harrington, S. C., Eckert, D., O'Neill, J., Burton, D., Chen, J., Shaw, A. L., & Acosta, A. (2017). Potential mechanisms of effects of serum-derived bovine immunoglobulin/protein isolate therapy in patients with diarrhea-predominant irritable bowel syndrome. Physiological Reports, 5(5). https://doi.org/10.14814/PHY2.13170

8. Kousathanas, A., Pairo-Castineira, E., Rawlik, K., Stuckey, A., Odhams, C. A., Walker, S., Russell, C. D., Malinauskas, T., Wu, Y., Millar, J., Shen, X., Elliott, K. S., Griffiths, F., Oosthuyzen, W., Morrice, K., Keating, S., Wang, B., Rhodes, D., Klaric, L., … Ganna, A. (2022). Whole-genome sequencing reveals host factors underlying critical COVID-19. Nature 2022 607:7917, 607(7917), 97–103. https://doi.org/10.1038/s41586-022-04576-6

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