ion torrent

Extractions made easy: Fully automated extraction and quantitation of nucleic acid using Genexus™ purification system and ready to use consumables

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INTRODUCTION

Use of genetic information is fast becoming the standard of clinical and translational research. The process begins with isolating nucleic acid (NA) from a variety of sample types and quantifying to be used in downstream applications such as NGS. The process can be challenging in most labs due to lack of expertise and processes in place. As a solution here we report the Genexus™ purification instrument and ready to use consumables to extract and quantify NA using a fully automated workflow with minimal user touch points.

Four purification kits were developed which contain REACH regulations compliant reagents to extract NA from various sample types using the Genexus™ purification system. DNA, RNA or sequential extraction of both DNA and RNA from FFPE (formalin-fixed paraffin embedded) tissues can be achieved using the Genexus™ FFPE DNA and RNA purification kit. cfTNA (Cell free total nucleic acid) can be purified from plasma using the Genexus™ cell free total nucleic acid purification kit. Genomic DNA or RNA can be extracted from whole blood, peripheral blood lymphocytes, bone marrow aspirates, cells, saliva and fresh/frozen tissues using Genexus™ multi-sample DNA purification kit or Genexus™ total RNA purification kit respectively. An interactive user interface with touch screen allows intuitive run setup with customizable sample information, elution volumes and ability to select onboard quantitation. If selected, extracted nucleic acid is then quantified onboard using a relevant Qubit™ assay.

Ion Torrent™ Genexus™ System

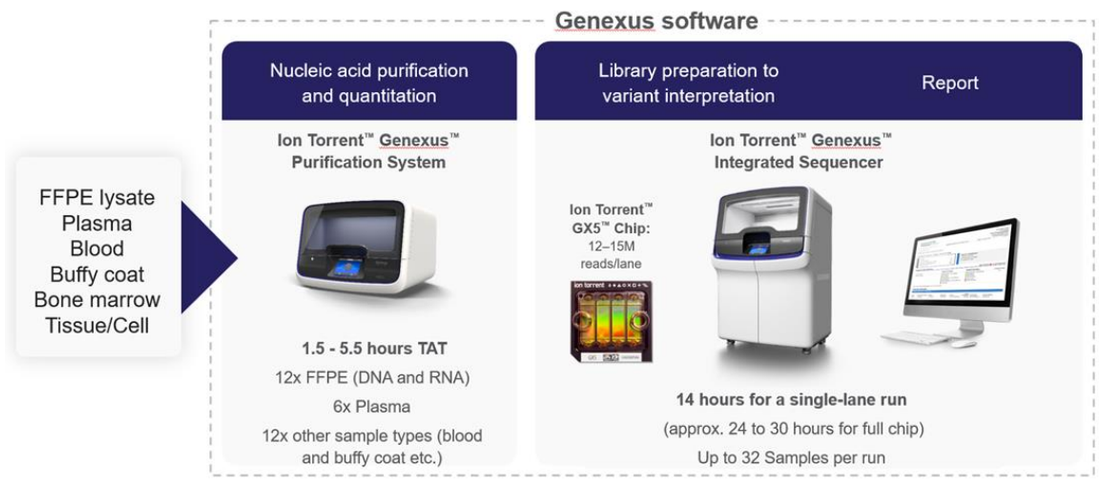

Figure 1. Ion Torrent™ Genexus™ System and workflow.

Genexus™ system is comprised of the Purification System and Genexus™ Integrated Sequencer. Genexus™ Software allows the integration of two instruments to perform end-to-end NGS workflows starting from run planning to generating a report. Genexus™ purification instrument can also be used as a standalone instrument to perform NA extractions and quantifications.

The Genexus™ purification system uses Genexus™ purification kits along with Genexus™ quantitation kit and consumables which contain pre-filled reagents. All consumables are loaded on to instrument guided locations on the deck and are tracked by the automated barcode scanning.

Ion Torrent™ Genexus™ Purification System

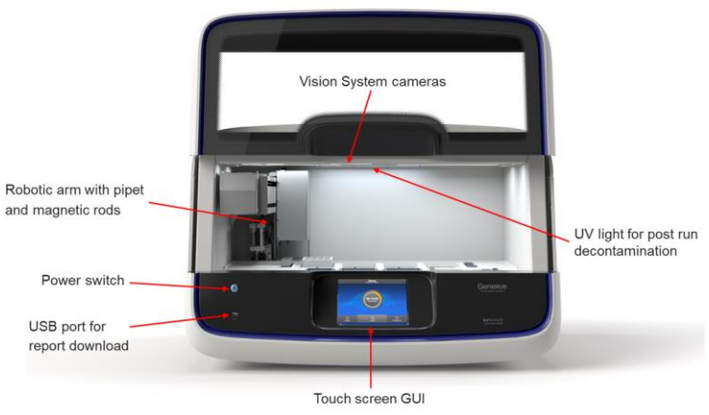

Figure 2. Ion Torrent™ Genexus™ Purification system. Leveraging industry-leading Applied Biosystems™ MagMAX™ sample preparation and Invitrogen™ Qubit™ quantitation technologies, the Genexus™ Purification System automates high-quality sample preparation for downstream applications such as NGS.

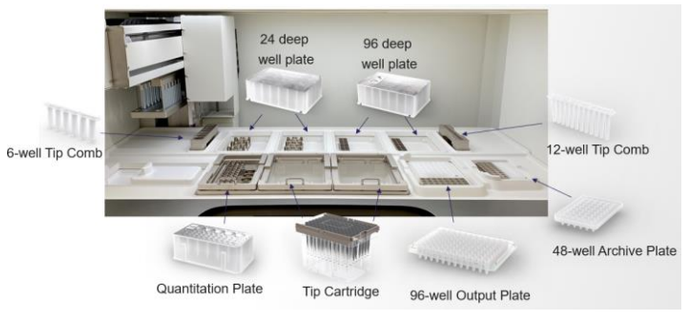

Figure 3. Genexus™ Purification System interior deck components and stations

RESULTS

Equivalent DNA and RNA purification performance between Genexus™ and KingFisher™/MagMAX™ systems from FFPE samples

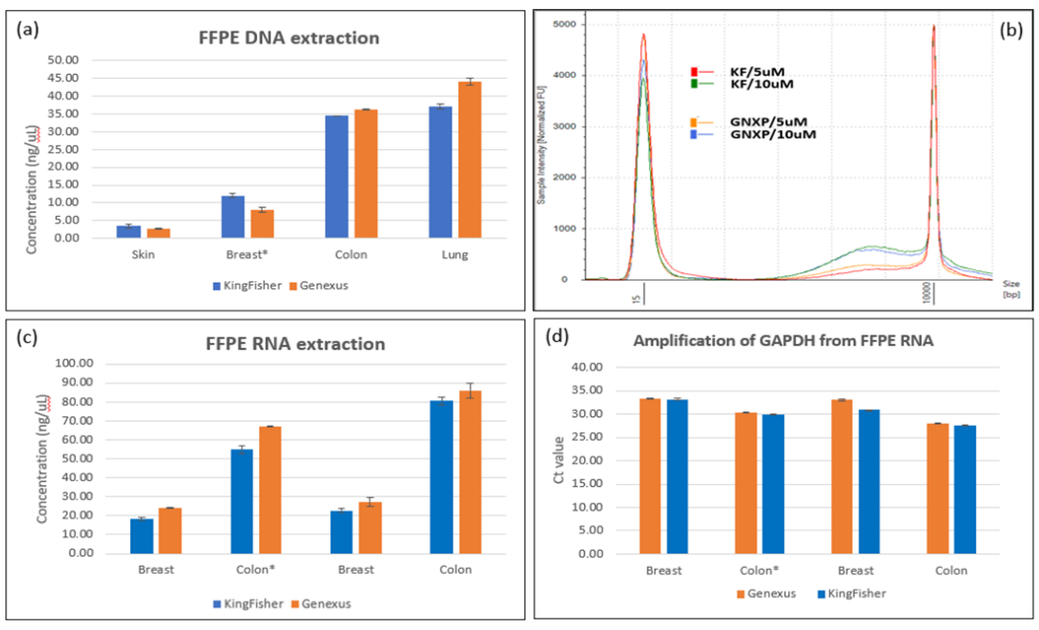

Figure 5. Comparison of (a) DNA concentration, (b) DNA fragment profile, (c) RNA concentration, (d) qRT PCR amplification of GAPDH target using NA extracted by Genexus™ purification system and KingFisher™ DUO prime instruments using Genexus™ FFPE DNA/RNA purification kit and MagMAX™ FFPE DNA/RNA Ultra kit respectively.

Equivalent cfTNA purification performance between Genexus™ and KingFisher™/MagMAX™ systems from liquid biopsy samples

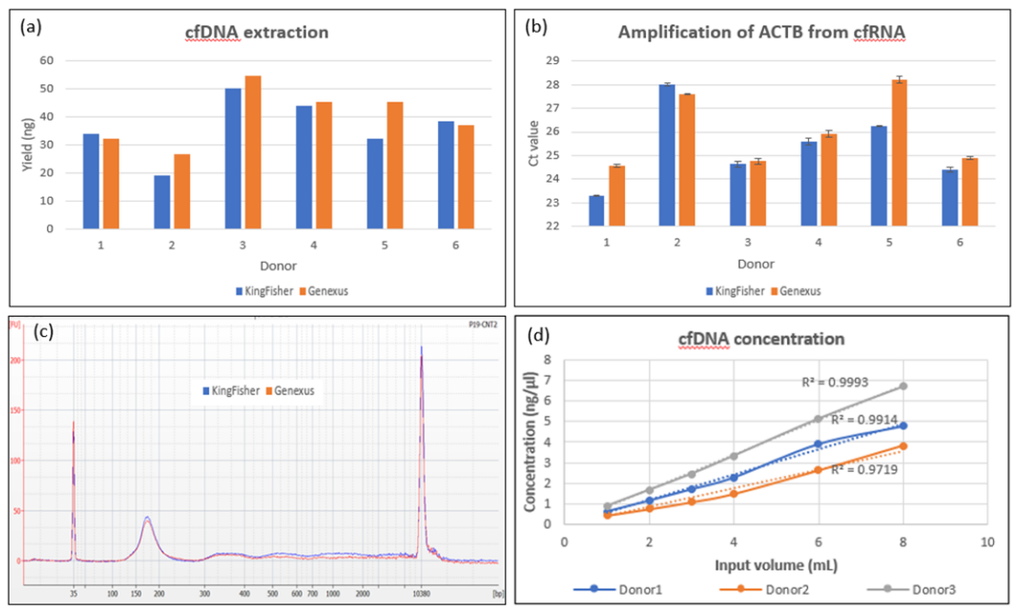

Figure 6. Comparison of (a) cfDNA concentration, (b) qRT PCR amplification of ACTB target (c) cfDNA fragment profile using cfTNA extracted by Genexus™ purification system and KingFisher™ DUO prime instruments using Genexus™ cell free total nucleic acid purification kit and MagMAX™ cell free total nucleic acid isolation kit respectively. (d) cfTNA can be efficiently extracted from sample input volumes ranging from 1 mL to 8 mL using Genexus extraction system as indicated by $R^2 > 0.97$.

Equivalent gDNA purification performance between Genexus™ and KingFisher™/MagMAX™ systems from tissue samples

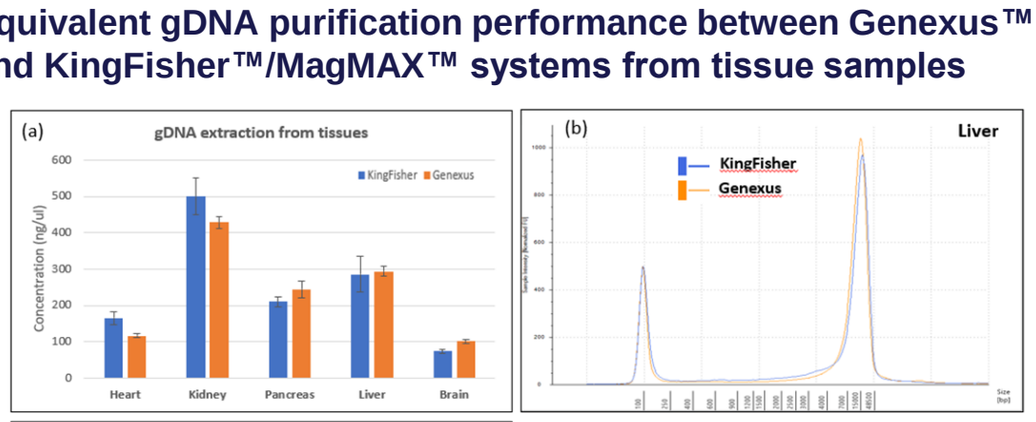

Figure 7. Comparison of (a) gDNA concentration, (b) gDNA fragment profile (c) qPCR amplification of ACTB target using gDNA extracted by Genexus™ purification system and KingFisher™ DUO prime instruments using Genexus™ multisample DNA purification kit and MagMAX™ DNA Multi-Sample Ultra 2.0 kit respectively.

Equivalent total RNA purification performance between Genexus™ and KingFisher™/MagMAX™ systems from whole blood samples

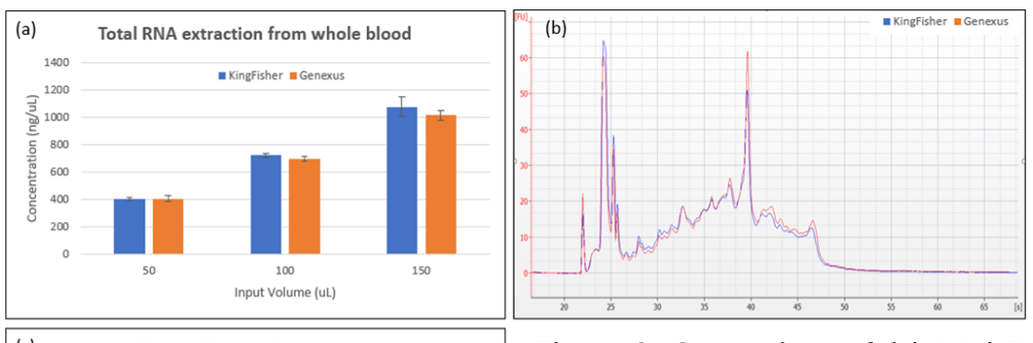

Figure 8. Comparison of (a) total RNA concentration, (b) total RNA fragment profile (c) qRT PCR amplification of ACTB target using total RNA extracted by Genexus™ purification system and KingFisher™ DUO prime instruments using Genexus™ total RNA purification kit and MagMAX™ mirVana Total RNA Isolation kit respectively.

Accurate On-board quantification of nucleic acid using Invitrogen™ Qubit™ technology and reagents

Workflow	Assay	Average GNXP:Manual ratio	SD	Percent difference %
FFPE	DNA HS	0.98	0.16	2.37
FFPE	RNA BR	0.96	0.07	3.41
cfTNA	DNA HS	0.89	0.06	10.61
gDNA	DNA BR	0.96	0.25	3.54
Total RNA	RNA BR	0.96	0.06	3.63

Figure 9. On-board quantitation accuracy of Genexus™ Purification system. On-board quantitation data were compared to corresponding manual qubit assay data and percent difference was calculated.

CONCLUSIONS

This report demonstrates the use of the Genexus purification system to extract and quantify NA from a variety of samples types and across multiple input levels to be successfully used in downstream applications. Ready to use consumables, automated extraction and quantitation allows the system to be used with minimum user interaction and training.

REFERENCES

- Ion Torrent™ Genexus™ System - https://www.thermofisher.com/us/en/home/life-science/sequencing/next-generation-sequencing/ion-torrent-next-generation-sequencing-workflow/ion-torrent-next-generation-sequencing-run-sequence/ion-torrent-genexus-system.html

ACKNOWLEDGEMENTS

Thanks to all the individuals involved in the development of the Genexus™ purification system, reagents and consumables.

DISCLAIMER

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