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APPLICATION NOTE

KingFisher Sample Purification System

Next generation sequencing sample preparation on the KingFisher platform

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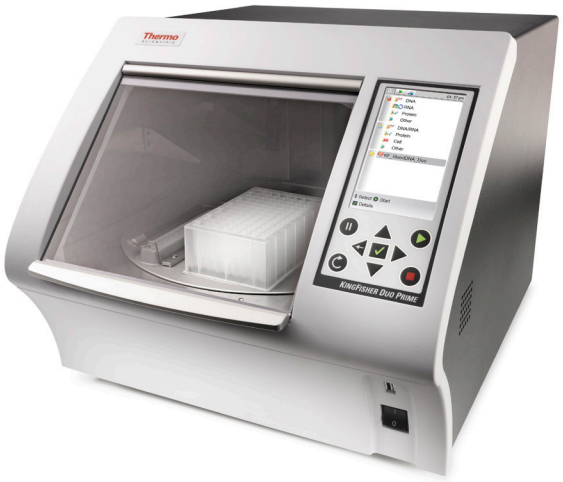

Abstract

Next generation sequencing (hereafter NGS) refers to a high-throughput nucleic acid sequencing technology that is significantly faster and more cost effective compared to former sequencing methods. The technology has revolutionized molecular biology and genomics research and holds great promise for the future of personalized medicine.

Thermo Scientific™ KingFisher™ instruments employ magnetic particle technology for biomolecule purification. Instrument function is based on the transfer of magnetic bead bound samples through successive sets of solutions that are necessary for the extraction protocol. KingFishers are well suited for various NGS sample preparation steps. Since only the bead-sample complex is transferred, the amount of carryover wash buffers or other impurities remains minimal.

KingFisher ready-made protocols with easy-to-use, intuitive BindIt software allow users to quickly and easily set up new protocols or make modifications to existing scripts.

Introduction

At present, several alternative platforms exist for NGS, yet the basic sample preparation is uniform and contains the following steps:

1. Nucleic acid extraction (DNA or RNA)
2. DNA fragmentation
3. Adapter sequence ligation
4. Size selection of the fragments
5. PCR amplification (optional)
6. Quantification of the obtained library

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Despite the advances in sequencing technology, manual NGS sample preparation is still labor intensive and time consuming, and includes numerous purification steps. The many benefits of automating the sample purification include reduced hands-on work and improved consistency between the processed specimens, all of which translates into enhanced laboratory output.

NGS sample preparation workflow assisted by the KingFisher instrument, presented in Figure 1, is based on the methodology employed by Blueprint Genetics, a molecular genetics laboratory that provides genetic diagnostics and clinical interpretation for hereditary diseases.

NGS sample preparation begins with nucleic acid extraction from the studied specimen. The extraction can be done either manually or with various automated instruments, including the KingFishers. At Blueprint Genetics, genomic DNA is isolated from whole blood or saliva. The obtained DNA is fragmented into smaller pieces mechanically using a focused-ultrasonicator (Covaris). The desired sized fragments are then isolated on a KingFisher Duo Prime with magnetic beads (Beckman AMPure SPRIselect). Uneven single-stranded ends of DNA (sticky ends) present on the fragments are repaired concurrently (NEBNext® Ultra™ DNA Library Prep Kit, New England BioLabs).

Specific adapters—short strands of nucleotides with known sequences and specialized barcode portions—are next ligated to both ends of the fragments. Any leftover adapters are removed in the following clean-up steps on the KingFisher Duo Prime. Library enrichment refers to PCR amplification of the samples and is

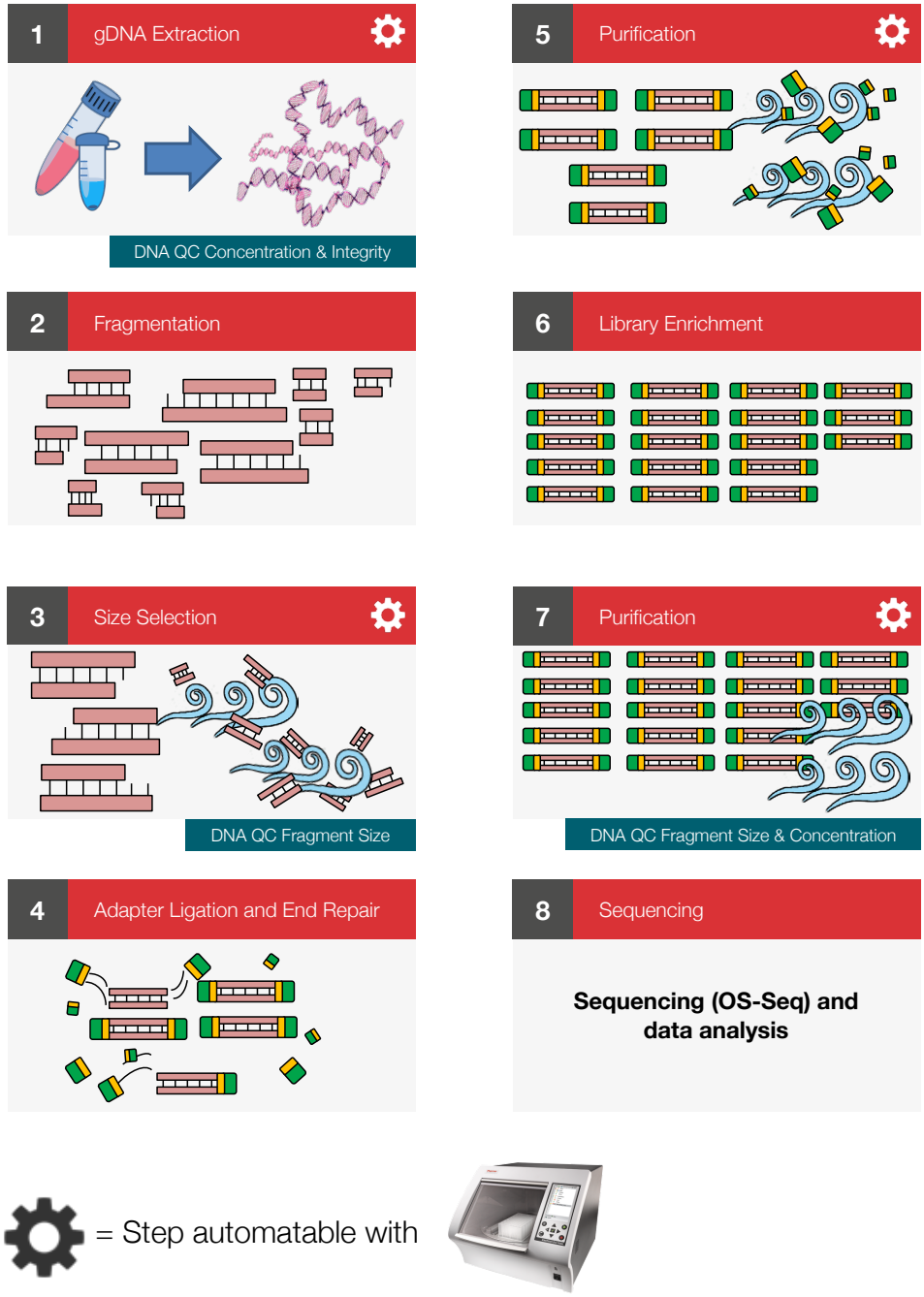

Figure 1. NGS sample preparation workflow.

1) Genomic DNA extraction from blood or saliva.

2) DNA fragmentation.

3) Selection of correct sized fragments with magnetic beads on the KingFisher instrument.

4) End repair and adaptor ligation to 5' and 3' ends of the fragments.

5) Sample purification on a KingFisher instrument to remove unused adapters.

6) PCR amplification of the sample.

7) Post-PCR purification on a KingFisher instrument.

8) Sample pooling, sequencing and analysis.

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performed to ensure adequate DNA yield for the sequencing. The last purification step removes unligated adapters, possible adapter-dimers and unused primers, as well as additional PCR reaction components. The step is carried out with a dedicated post-PCR KingFisher Duo Prime to guarantee that no amplicon contaminants are spread.

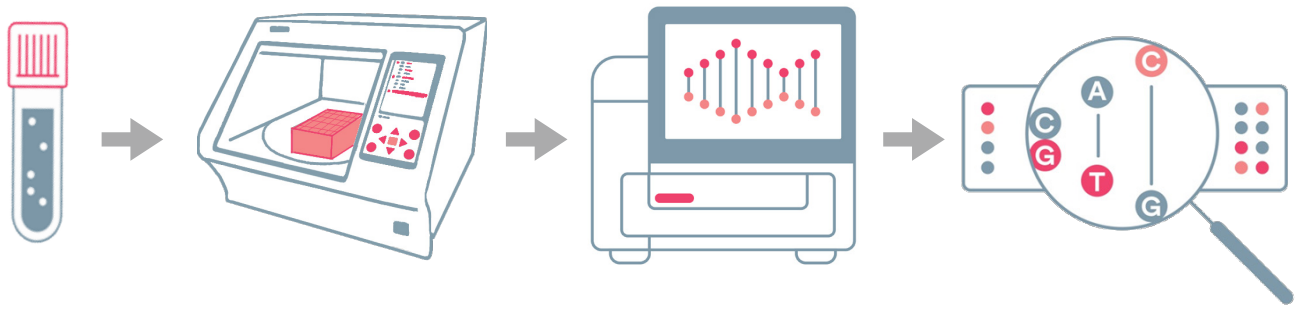
Finally, the samples are pooled and sequenced. The barcode sequences allow for multiple sample analyses on a single run. DNA quality control of fragment size, purity and concentration is performed several times during the process (Figure 1). At Blueprint Genetics the sequencing is accomplished by applying a targeted sequencing approach called Oligonucleotide-Selective Sequencing (OS-Seq™), (Myllykangas et al., 2011). High-quality sequencing data from the clinically significant target genes is analyzed on Blueprint Genetics' automated bioinformatics pipeline, and a team of geneticists and clinicians interprets the results to prepare a clinical statement.

Summary

KingFisher instruments are like a reliable extra set of hands for several NGS sample preparation steps. When purity and minimal sample-to-sample variation are requisite, automation with the KingFishers helps to procure top-grade samples with less hands-on time.

References:

1. Myllykangas, Buenrostro, Natsoulis, Bell and Ji. Efficient targeted resequencing of human germline and cancer genomes by oligonucleotide-selective sequencing. Nature Biotechnology 2011;11:1024-7.



NGS work flow including sample purification with the KingFisher Duo Prime

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