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Nuclefy Genomic DNA Purification Kit

Spin Column-Based Nucleic Acid Extraction

Introduction

The Nuclefy Genomic DNA Purification Kit is designed for rapid and reliable sample lysis, RNA removal and purification of intact genomic DNA (gDNA) from a variety of biological sample types. The protocol utilizes specially formulated buffer systems allow processing of blood, tissue, and cultured cells. Supplemental protocols allow processing of clinically-relevant samples such as saliva and buccal swabs, as well as bacteria, yeast, and insects. In addition, it can also be used for classic gDNA cleanup.

Purified gDNA has high quality metrics, including A260/280 > 2.0, high DIN scores and minimal residual RNA. The purified gDNA is suitable for numerous downstream applications such as qPCR, SNP analysis, genotyping, microarray analysis, and library preparation for NGS sequencing.

Ideal for Extraction from:

- Whole blood (non-nucleated)
- Nucleated red blood cells
- Tissue
- Insects
- Gram-negative bacteria
- Gram-positive bacteria and archaea
- Yeast
- Saliva
- Buccal swab
- Cultured cells
- gDNA cleanup

Sample Lysis

Three specially formulated lysis buffers are provided, which fulfill requirements for optimal processing of various sample types. Each kit includes:

- **Blood Lysis Buffer:** Rapid degradation of protein components including hemoglobin in combination with a protective property for high nuclease activity
- **Tissue Lysis Buffer:** Optimized for rapid digestion of all common tissue types
- **Cell Lysis Buffer:** Capable of overcoming the viscosity commonly present in cell samples

RNA Removal

The buffer systems used are developed for optimal and selective binding of gDNA. To counteract the inevitable co-isolated RNA purification, an optional RNase A digestion can be performed. Approximate RNA content without RNase treatment: 1% for blood, 1-4% for tissue, up to 10% for cells.

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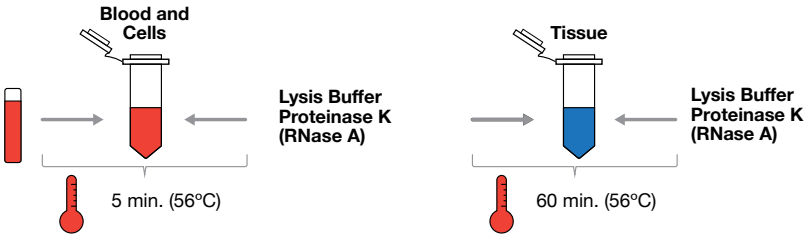
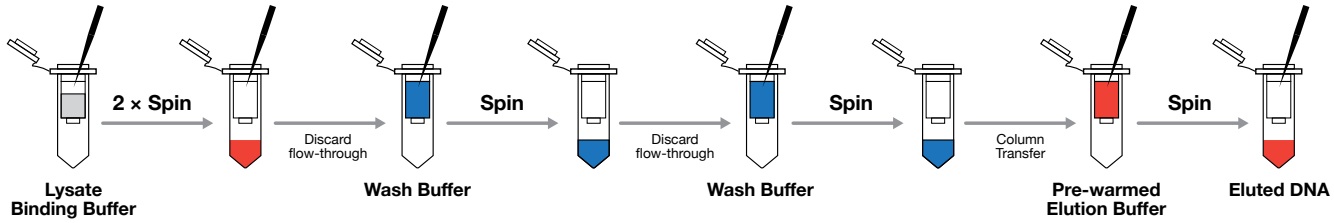
Binding and Washing

A chaotrophic salt-based binding buffer is used for selective binding of gDNA with minimal RNA binding capability. To ensure maximal recovery/efficiency, binding to the column's silica membrane takes place with sequential—low speed to maximum speed—spin steps.

Part 1: Sample Lysis



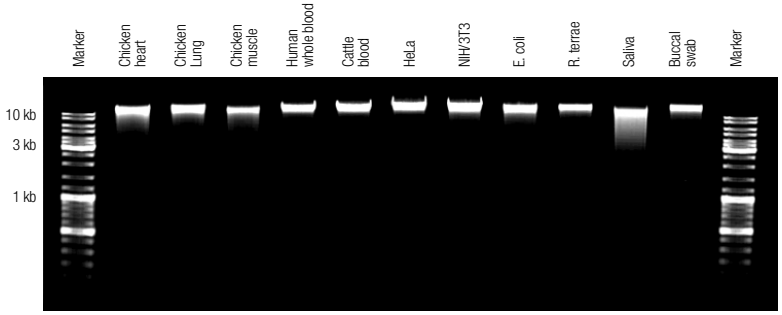
Part 2: Binding and Elution



The Nuclefy Genomic DNA Purification Kit generates excellent input material regarding DNA integrity and purity for downstream applications such as quantitative PCR and NGS libary preparation.

DNA Integrity

Genomic DNA samples were analyzed for DNA integrity by loading 50 ng on a 1% agarose gel. Genomic DNA purification was performed following the appropriate purification protocals. Three representative samples of DNA for tissue (chicken heart, lung and muscle), human whole blood, cattle blood, HeLa, and NIH/3T3 cells, *E. coli*, *R. terrae*, saliva as well as buccal swab are shown.



Integrity of gDNA isolated from represenativ sample types by agarose gel electrophoresis.

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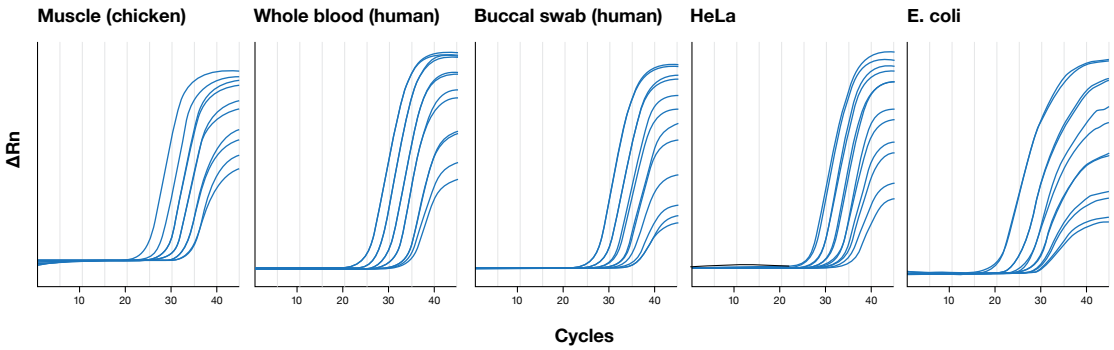
Quantitative PCR

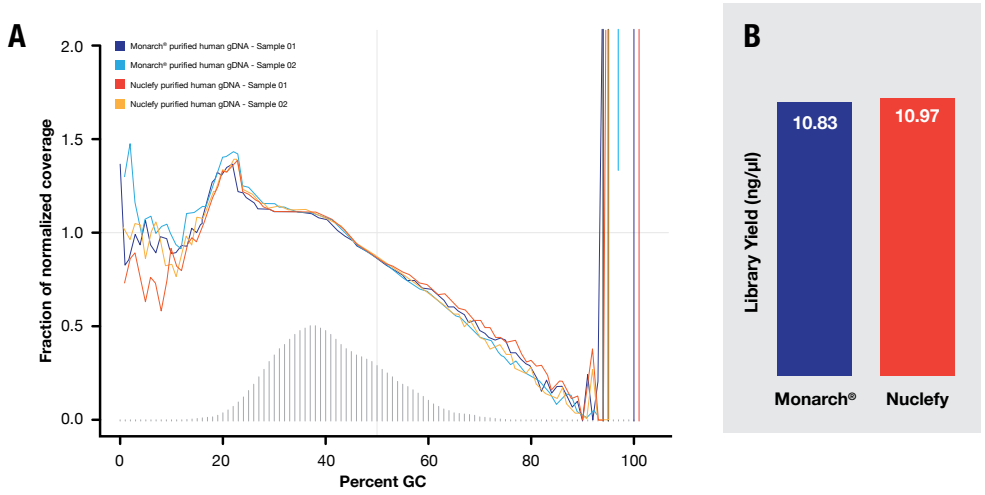
Purified genomic DNA from several representative sample types was diluted to produce a for log range of input template concentrations. The results were obtained using primers targeting poultry- (chicken muscle), human- (whole blood, buccal swab, HeLa cells) as well as bacteria-specific (E. coli) genomic sequences in combination with qPCR assay with the PhoenixDx® qPCR Mastermixes (Procomcure Biotech #PCCSKU12022) and cycled on an Applied Biosystems™ QuantStudio 5 qPCR thermal cycler. The results confirm that the eluted genomic DNA is highly pure and free from inhibitors, optimal for qPCR.



qPCR data using species-specific designed assays.

NGS Library Preparation

Genomic DNA was isolated from whole human blood with Nuclefy Genomic DNA Purification Kit (Procomcure Biotech # PCCSKU16073) and Monarch® Genomic DNA Purification Kit (NEB #T3010). Duplicate libraries were prepared from 200 ng human genomic DNA using the NEBNext Ultra II FS DNA Library Prep Kit for Illumina (NEB #E7805). Libraries were sequenced on an Illumina MiSeq®. Reads were mapped using BWA and GC coverage was calculated using Picard's CollectGCBiasMetrics (v2.26.2). Library yields of the samples were assassed on a Caliper Labchip GX II using a High Sensitivity DNA Kit.



A. GC bias plot. **B.** Library yield.