Axiom™ 2.0 gDNA Sample Preparation

Doc. Part No. 702987 **Pub. No.** MAN0017720 **Rev.** A.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Introduction

The Axiom™ 2.0 Assay can be used for genotyping applications (interrogating biallelic SNPs and indels) and for microbiome research applications (interrogating non-polymorphic sequences in both family-conserved and target-specific regions) using either a fully automated or manual assay workflow. Starting with genomic DNA, the samples are processed by performing either an automatic or manual target prep protocol followed by automated processing of the array plates in the GeneTitan™ Multi-Channel (MC) Instrument.

The following sources of gDNA have been successfully tested in the laboratories at Thermo Fisher Scientific for DNA that meets the requirements for the Axiom™ 2.0 Assay.

Source	Sample type	
Human	• Blood • Saliva	• Cell line • WGA pre-amplified DNA
Animal	• Blood • Semen • Nasal swabs	• Hair bulbs • Ear punch tissue
Plant	• Seeds • Leaves	

For microbiome research applications, the Axiom™ 2.0 Assay identifies microorganisms from samples using DNA and RNA extracted from microbial specimens on Axiom™ Microbiome Array Plate. The following sources of microbial gDNA have been successfully tested in the laboratories at Thermo Fisher Scientific for DNA that meets the requirements that are mentioned in this document:

- Stool

For detection of RNA viruses, RNA must be reverse-transcribed to yield input amenable to Axiom™ target preparation using the protocol outlined in the *Axiom™ Microbiome Solution User Guide* (Pub. No. 703408).

Success with other types of samples (for example, blood on FTA cards) depends on quality (degree of degradation, level of purity, and so on) and quantity of gDNA extracted. DNA derived from formalin-fixed paraffin-embedded (FFPE) blocks must not be used with this assay.

Workflow overview

Running the Axiom™ 2.0 Assay requires the following sets of steps:

1. Preparation of genomic DNA as described in this document.
2. Target preparation of the samples, performed using automated target preparation, described in the appropriate assay user guide or target preparation quick reference document.
3. Array processing, described in *GeneTitan™ MC Protocol for Axiom™ Array Plate Processing Quick Reference* (Pub. No. MAN0017718).

IMPORTANT! This document contains an abbreviated set of instructions. Carefully read all the instructions in the *Genomic DNA Preparation and Requirements* chapter of the appropriate user guide for more details on the protocol and sample requirements.

Requirements

Complete this step before proceeding with the DNA amplification stage for either automated or manual target preparation. The genomic DNA (gDNA) processed must meet the following requirements:

- Starting DNA must be double-stranded for accurate concentration determination.
- DNA must be of high purity and be free of DNA polymerase inhibitors.
- DNA must not be degraded.

All human Axiom™ arrays (except the Axiom™ Genome-Wide Pan-African Array Set) require a total of 100 ng. The Axiom™ Genome-Wide Pan-African Array Set requires a total of 300 ng, or 100 ng per array (there are three arrays in the Axiom™ Genome-Wide Pan-African Array Set). Diploid plants and animals require 150 ng per array and polyploid plants and animals require 200 ng per array. For Axiom™ Microbiome Array Plates, a total of 50 ng of gDNA or 17.5 µL of cDNA reaction + 2.5 µL reduced TE buffer starting material is required per array

Table 1 Genomic DNA sample input requirements

Sample type	Volume per well	Input mass per well	gDNA concentration
Human	20 µL	100 ng	5 ng/µL
Diploid plants and animals	20 µL	150 ng	7.5 ng/µL
Polyploid plants and animals	20 µL	200 ng	10 ng/µL
Stool	20 µL	50 ng	2.5 ng/µL

IMPORTANT! Prepare your genomic DNA sample plate in a clean room. The clean room must be separate from the laboratory where the assay is performed and must be free of DNA amplified in other procedures.

Reagents required

Reagent
Axiom™ Reference Genomic DNA 103 (positive control for human genotyping or microbiome arrays), Cat. No. 951957, –20°C
Reduced EDTA TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA) Cat. No. 75793
Positive control gDNA (if genotyping non-human samples)
Negative control for Axiom™ Microbiome: elution buffer or reduced EDTA TE Buffer

Equipment and consumables required

Quantity	Item
As required	Adhesive seals for plates[1]
1	Ice bucket, filled with ice
1 each	Pipettes: single channel P10 or P20 Optional: multichannel P10 or P20
As required	Pipette tips
1	Plate, deep-well See Table 2 to determine the required deep-well plate for your assay format
1	Plate centrifuge
1	Microtiter plate fluorimeter Quant-iT™ PicoGreen™ dsDNA Assay Kit (required only if no concentration measurements available for samples)
1	Vortexer

[1] For Axiom™ Microbiome applications, only use the MicroAmp™ Clear Adhesive Film (Cat. No. 4306311).

IMPORTANT! Different deep-well plates are required for automated and manual target preparation. In addition, the Microbiome Assay requires the use of a different deep-well plate for the 24-format and 96-format manual target preparation. Ensure that you have the correct deep-well plates available before starting target preparation.

Deep-well plate requirements

Table 2 Genomic DNA sample plate information for Axiom™ assay types.

Assay	Workflow	Format	Deep-well plate name	Manufacturer information
Genotyping	Manual	24	ABgene Storage Plate, 96-well, 2.2 mL, square well, conical	Thermo Scientific™ Cat. No. AB-0932
Genotyping	Manual	96	ABgene Storage Plate, 96-well, 2.2 mL, square well, conical	Thermo Scientific™ Cat. No. AB-0932
Genotyping	Automated NIMBUS	96	96 Round Deep-well Storage Microplate	Thermo Scientific™ 14-222-354 Axygen™ P-DW-20-C-S
Genotyping	Automated Biomek™ FX^P	96	Polypropylene, Deep-well Titer Plate, Sterile	Beckman Coulter™, Cat. No. 267007
Microbiome	Manual	24	Eppendorf 96 Deep-well Plate, 2,000 µL	Thermo Scientific™ Cat. No. 13-864-302 Eppendorf™ Cat. No. 951033481
Microbiome	Manual	96	Eppendorf 96 Deep-well Plate, 2,000 µL	Thermo Scientific™ Cat. No. 13-864-302 Eppendorf™ Cat. No. 951033481
Microbiome	Automated NIMBUS	96	96 Round Deep-well Storage Microplate	Thermo Scientific™ 14-222-354 Axygen™ P-DW-20-C-S

Prepare genomic DNA samples

1. Thaw samples and control.

   Thaw the Axiom™ Reference Genomic DNA 103 and positive control sample to room temperature. To thaw, either:

   - Place items on benchtop for one hour.
   - Thaw in a water bath:
     a. Fill a small plastic dish with ultra-pure water (such as Millipore™ water). Do not overfill. The level of the water must not overflow when the sample tubes or plates are placed in the bath.
     b. Place the DNA samples in the water bath and thaw for 30 minutes.
     c. Wipe water off the sample plate before removing the seal to avoid contamination of the samples.

2. Quantify, then dilute gDNA.

   a. Gently vortex (50% maximum), then centrifuge the gDNA samples and gDNA controls.
   b. Quantify each sample. The Quant-iT™ PicoGreen™ dsDNA Assay Kit is recommended.
   c. Using reduced EDTA TE buffer, dilute each sample to a concentration of:
      - 5 ng/µL for human DNA samples
      - 7.5 ng/µL for diploid plant and animal DNA samples
      - 10 ng/µL for polyploid plant and animal DNA samples
      - 2.5 ng/µL for stool samples
   d. Seal, vortex, then centrifuge.

3. Aliquot the diluted samples and the control.

   Aliquot diluted samples, positive (Axiom™ Reference Genomic DNA 103 for human and Microbiome arrays), and negative controls (Microbiome arrays only) to the appropriate deep-well plate:

   a. Diluted samples: Aliquot 20 µL of each diluted gDNA sample. For genotyping applications, this should be the equivalent of 100 ng to 200 ng of gDNA depending on the sample type that is hybridized. For Microbiome applications, a total of 50 ng of gDNA or 17.5 µL of cDNA is required per array.
   b. Controls:
      - Positive control: 20 µL of control gDNA. For genotyping arrays, we recommend including at least one positive gDNA control on each plate. For the Microbiome array, the Axiom™ Reference Genomic DNA 103 control must be used.
      - Negative control: For the Microbiome array, it is necessary to run one no template control (NTC) reaction. It is recommended that you run the same buffer that is used for elution during their gDNA extraction. Alternatively, Reduced EDTA TE Buffer can be run as a negative control
   c. Seal, then centrifuge.

4. Freeze or proceed. At this point you can:
   - Store the sample plate at –20°C, or
   - Proceed to DNA amplification for target preparation.

     Note: If proceeding immediately to DNA amplification, you can leave the gDNA sample plate at room temperature.

5. Create a GeneTitan™ Array Plate Registration file.

   Note: It is important to create and upload a GeneTitan™ Array Plate Registration file with your sample information before loading the array plate and hybridization tray in the GeneTitan™ Instrument. Create (but not upload) this file at the same time you prepare your plate of genomic DNA. When your samples are ready for hybridization, scan the array plate barcode and upload the file to GeneChip™ Command Console™ (GCC).

   GeneTitan™ Array Plate Registration file contains information that is critical for:

   - Data file generation during imaging.
   - Tracking the experimental results for each sample that is loaded onto an array plate.

   Detailed instructions for creating this file are in the appropriate assay user guide.

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  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

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Manufacturer:
Thermo Fisher Scientific Baltics UAB |
V.A. Graiciuno 8, LT-02241 |
Vilnius, Lithuania

Products:
Axiom™ 2.0 Reagent Kit

Manufacturer:
Affymetrix Pte Ltd |
7 Gul Circle #2M-01 |
Keppel Logistics Building |
Singapore 629563

Products:
Axiom™ Array Plates
Axiom™ myDesign™ Array Plates

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Revision history: Pub. No.

Revision	Date	Description
A.0	12 September 2018	Initial release in Thermo Fisher Scientific document control system. Supersedes legacy Affymetrix publication number 702987. Updated to the current document template, with associated updates to trademarks, logos, licensing, and warranty. Updated to reflect that Axiom Reference gDNA 103 has been removed from the reagent kit and has been made available for purchase separately.

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