Axiom™ gDNA Sample Prep for Genome-Wide BOS 1 Array Plate

Running the Axiom Assay requires the following sets of steps:

1. Preparation of genomic DNA Prep as described in this QRC.
2. Target Prep of the samples, performed using either:
   - Automated Target Prep, described in the *Axiom™ 2.0 Assay Automated Target Prep Protocol QRC* (P/N 702962).
   - Manual Target Prep, described in the *Axiom™ 2.0 Manual Target Prep Protocol QRC* (P/N 702989).
3. Array Processing, described in the *GeneTitan® MC Protocol for Axiom 2.0 Array Plate Processing QRC* (P/N 702988).

IMPORTANT: This QRC contains an abbreviated set of instructions. You must carefully read all the instructions in *Chapter 2, Genomic DNA Preparation and Requirements* of the *Axiom™ 2.0 Assay Automated Workflow User Guide, (*P/N 702963) or the *Axiom™ 2.0 Assay Manual Workflow User Guide* (P/N 702990) for more details on the protocol and sample requirements.

NOTE: All chapter and appendix references are to the *Axiom™ 2.0 Assay Automated Workflow User Guide, (*P/N 702963) or the *Axiom™ 2.0 Assay Manual Workflow User Guide* (P/N 702990) unless otherwise specified.

Requirements

This step needs to be done before proceeding with the DNA amplification stages for either automated or manual target prep.

The genomic DNA (gDNA) you will process using the Axiom Assay should meet the following requirements:

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

A total of 200 ng gDNA per sample is required.

IMPORTANT: We recommend that you prepare your genomic DNA sample plate in a clean room. The clean room should be separate from the laboratory where the Axiom Genotyping Assay is performed and should be free of DNA amplified in other procedures.

Reagent	Supplier	Part Number	√
User-supplied			
Reduced EDTA TE Buffer (10 mM Tris-HCl pH 8.0, 0.1 mM EDTA)	Affymetrix	75793	

Equipment and consumables required for gDNA Sample Prep

√	Quantity	Item
	As required	Adhesive seals for plates
	1	Ice bucket, filled with ice
	1 each	Pipettes: Single-channel P10 or P20 Optional: multi-channel P10 or P20
	As required	Pipette tips
	1	Plate, deep well: ▪ Automated Target Prep: Beckman Deep Well Titer, polypropylene; P/N 267007 ▪ Manual Target Prep: ABgene 96 Square Well Storage; AB-0932
	1	Plate centrifuge
	1	Microtiter plate fluorimeter Quant-iT™ PicoGreen® dsDNA Kit from Life Technologies (required only if no concentration measurements available for samples)
	1	Vortexer

IMPORTANT: Different deep well plates are required for automated and manual target prep. Please ensure you have the correct deep well plates available prior to starting the target prep protocol.

1. Thaw Samples and Control

Thaw the components listed below to room temperature:

- gDNA samples

To thaw, either:

- Place items on benchtop for one hour.
- Thaw in a water bath:
  - **A.** Fill a small plastic dish with Millipore water. Do not overfill as the level of the water should 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 a half-hour (30 min).
  - **C.** Wipe water off of the sample plate prior to removing the seal to avoid contamination of the samples.

2. Quantitate and Dilute gDNA

1. Gently vortex (50% maximum) and spin the gDNA.
2. Quantitate each sample. The Quant-iT™ PicoGreen® dsDNA Kit is recommended.
3. Dilute each sample to a concentration of 10 ng gDNA/µL using reduced EDTA TE buffer.
4. Seal, vortex and spin.

3. Aliquot the Diluted Samples and the Control

Aliquot diluted samples to the appropriate deep well plate as follows:

1. 20 µL of each diluted gDNA sample (this should be the equivalent of 200 ng of gDNA).
2. Seal and spin.

4. Freeze or Proceed

At this point you can:

- Store the sample plate at –20 °C, or
- Proceed to DNA Amplification for Target Prep

NOTE: You can leave the gDNA sample plate at room temperature if proceeding immediately to DNA Amplification.

5. Create a Batch Registration File

NOTE: It is very important to create and upload a GeneTitan® Array Plate Registration file with your sample information prior to loading the Array Plate and hyb tray in the GeneTitan Instrument. We recommend that you create (but not upload) this file at the same time you prepare your plate of genomic DNA. When your samples are ready for hybridization, you will scan the array plate barcode and upload the file to Affymetrix® GeneChip® Command Console® (AGCC).

GeneTitan Array Plate Registration files contain information that is critical for:

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

Detailed instructions for creating this file are located in *Appendix D, Registering Samples in Affymetrix GeneChip® Command Console*.

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Not for use in Diagnostic Procedures.

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