COMBIMATRIX

Whole Genome Amplification - Preparation of Biotinylated Genomic DNA Target Samples for CustomArray™ and ElectraSense™ Microarrays (PTL015)

Whole Genome Amplification - Preparation of Biotinylated Genomic DNA Target Samples

Table of Contents

Table of Contents i
Introduction 1
Materials and Equipment Required 1
Procedure 2
Amplification 2
Fragmentation 2
Labeling 3

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Introduction

This protocol is a Whole Genome Amplification (WGA) method that allows the researcher to generate Biotinylated DNA targets for hybridization to the CombiMatrix CustomArray™ and ElectraSense™ platforms. It can be used for a variety of applications, including genotyping, SNP analysis, CGH, and genome tiling. This approach works best when preparing microarray targets from relatively small genomes (*e.g.* those of prokaryotic organisms). When working with large and complex genomes, it may be necessary to selectively amplify the regions of interest in order to reduce the general complexity of the target material.

The amplification of purified genomic DNA is carried out by an isothermal reaction based on Multiple Displacement Amplification (MDA) technology. In order to optimize the labeling and subsequent hybridization, the amplified DNA is digested with the restriction endonuclease CviJ I* (Chimerx) prior to labeling. In the presence of DMSO, CviJ I* cleaves 5'-GC-3', except 5'-YGCR-3' (R=purine; Y=pyrimidine). The procedure digests the DNA into fragments that are less than 1 kb. The fragmented DNA is then directly labeled with biotin using the Mirus *Label* IT® µArray™ Biotin Labeling Kit in a simple one-step chemical reaction. The kit covalently attaches biotin to guanine residues of DNA at a site that does not participate in base-pairing during hybridization.

This protocol has been developed to amplify and label purified genomic DNA specifically for use with CustomArray™ and ElectraSense™ microarrays. The recommended starting material is 1 ng - 10 ng of purified genomic DNA.

Materials and Equipment Required

- ❑ Purified Genomic DNA (to be amplified)
- ❑ Repli-g® kit (Qiagen Cat.#59043): contains DNA polymerase, buffers and reagents for 25 whole genome amplification reactions.
- ❑ KOH pellets
- ❑ 0.5 M EDTA (pH 8.0)
- ❑ TE Buffer
- ❑ Nuclease Free Water
- ❑ PicoGreen® dsDNA Quantitation Kit (optional, see Step 1): Invitrogen Cat. # P-7589; requires spectrofluorometer or fluorescence microplate reader
- ❑ CviJ I* (Chimerx Cat. #2126): 100 units restriction enzyme, includes 10X reaction buffer and DMSO.
- ❑ Mirus Label IT® µArray™ Biotin Labeling Kit (Cat.# 8010): contains reagents to perform 10 labeling reactions
- ❑ QIAquick PCR Purification kit (Qiagen Cat. #28106)
- ❑ 30°C heating block, or thermocycler (recommended)
- ❑ 37°C incubator or heating block
- ❑ UV Spectrophotometer, preferably a Nanodrop® ND-1000 Spectrophotometer
- ❑ 0.5 µl to 1000 µl micropipettors
- ❑ Sterile (nuclease-free) micropipette tips
- ❑ Sterile (nuclease-free) polypropylene 1.5 ml microcentrifuge tubes
- ❑ 0.2 ml PCR tubes
- ❑ Gloves (powder-free)
- ❑ Microcentrifuge
- ❑ SpeedVac® Concentrator System

Procedure

IMPORTANT! Follow all manufacturers' safety precautions for handling and disposal of reagents. Wear lab coat, safety goggles, and gloves while handling all reagents.

Amplification

CombiMatrix recommends the Repli-g® Kit Protocol for Whole Genomic Amplification using the Denaturation and Amplification protocol for **already purified genomic DNA** as amplification template. The recommended starting material is 1 -10 ng of purified genomic DNA, suspended in 2.5 μl of TE buffer.

The amplification takes 16 hours, at a temperature of 30°C. Since the amplification product will contain unused reaction primers, the DNA must purified prior to quantification using a UV spectrophotometer. We recommend purifying the DNA using a BioRad Micro Bio-Spin P-30 column, or Qiagen QIAquick PCR Purification kit according to the manufacture's protocol. Alternatively, the DNA yield can be determined without purification using the PicoGreen® dsDNA kit.

The expected yield from the Repli-g® reaction is 45μg ± 10% for 10ng of starting material. The quantity and quality of the amplified DNA will depend on the quality of the input DNA.

Keep the amplified DNA on ice if proceeding immediately with the fragmentation and labeling steps. Alternatively, the DNA can be stored at -20°C, avoiding repeated freezing and thawing.

Fragmentation

1. Add the following reagents to a 1.5 ml microcentrifuge tube on ice:

Reagent	Volume for 50 μl
Nuclease-free water	**to 50 μl**
10X CviJ I * Reaction Buffer (supplied with enzyme)	**5 μl**
DMSO (supplied with enzyme)	**15 μl**
amplified DNA	**2 μg**
CviJ I* enzyme (0.5 units/μl)	**2 μl**
Total Volume	**50 μl**

2. Mix well by pipetting, and spin the tube briefly in microcentrifuge. Incubate for 3 hours at 37°C.
3. The fragmented DNA must be purified from the reaction buffer before proceeding to the labeling reaction. CombiMatrix recommends using the QIAquick PCR Purification kit. Before eluting the DNA from the spin column, dilute the EB buffer in 1/10 nuclease-free water to 1mM Tris, pH 8.5, or use 1/10 TE buffer (1mM Tris pH 8.0, 0.1 mM EDTA). For optimal recovery, elute the DNA twice with 50 µl 1/10 EB or 1/10 TE and pool the resultant eluates.
4. Determine the absorbance at 260nm, using elution buffer as the blank. Use a clean 50 µl microcell cuvette, or use a Nanodrop® ND-1000 Spectrophotometer (which can take readings from 1 µl of sample). Calculate the concentration using 50 µg/ml for 1 OD_{260}.
5. Proceed directly to the Labeling Step.

Labeling

Combimatrix recommends labeling the fragments DNA using the Mirus *Label* IT® µArray™ Biotin Labeling Kit, according to the manufacture's protocol.

1. The *Label* IT® µArray™ Biotin Reagent is supplied as a dried pellet (which may not be visible). Warm the vial to room temperature and spin briefly to collect the contents before opening.
2. Add the indicated amount of Reconstitution Solution to the dried pellet (see table). To ensure reconstitution of the pellet, mix well by gently pipetting up and down, and then quick spin to collect all solution at the bottom of the tube.

Label IT® µArray™ Biotin Labeling Kit	Volume of Reconstitution Solution
MR 8010 (10 reactions, 1 µg each)	40 µl
MR 8050 (50 reactions, 1 µg each)	200 µl

Store unused reconstituted *Label* IT® µArray™ Biotin Reagent tightly capped at -20°C. For subsequent use, warm the vial to room temperature, and spin briefly before opening.

3. Use four microliters of the resuspended labeling reagent per one microgram of DNA to be labeled.
4. Prepare the following labeling reaction:

Fragmented DNA sample (1 µg):	up to 86 µl
10X Labeling Buffer M	10 µl
Nuclease-free water:	bring volume to 96 µl
Label IT® µArray™ Biotin Reagent	4 µl
Total Volume	100 µl

5. Incubate the labeling reactions at 37°C for 1 hour. If condensation appears at the top of the tubes during the incubation, perform a quick spin after 30 minutes of incubation.
6. Add 0.1 volume of Reagent D1 (10 µl to a 100 µl labeling reaction), mix well, and incubate for 5 minutes at room temperature. Immediately add 0.1 volume of Neutralization Buffer N1 (10 µl to same 100 µl labeling reaction), mix well, and incubate on ice for at least 5 minutes.
7. Purify the DNA using the Qiagen QIAquick PCR Purification kit according to the manufacturer's protocol.
8. Quantify the DNA by A_{260} absorbance (see Fragmentation Section, Step 4).
9. Store the samples at -20°C, or use immediately for microarray hybridization.

IMPORTANT! Keep the labeled DNA on ice if proceeding immediately with hybridization of CustomArray™ or ElectraSense™ microarrays. Alternatively, the labeled DNA can be stored at -20°C, avoiding repeated freezing and thawing. If stored properly the labeled DNA is stable for up to 6 months.

NOTE: CombiMatrix recommends using approximately 1 to 5 µg biotin-labeled WGA-DNA per CustomArray™ or ElectraSense™ 12K microarray. The samples can be dried down in a SpeedVac®, or ethanol precipitated, and then directly resuspended in microarray hybridization buffer, or in nuclease-free water.