USER GUIDE

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BioPrime® Array CGH Genomic Labeling System

For generating fluorescently labeled genomic DNA to use in microarray screening

Catalog Numbers 18095-011 and 18095-012

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For Research Use Only. Not for use in diagnostic procedures.

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Contents

Kit Contents and Storage

Kit Sizes and Modules

This manual supports the following kit configurations:

Catalog No.	# of Reactions	Modules
18095-011	30	Labeling and Purification
18095-012	30	Labeling only

Shipping and Storage

The Labeling Module is shipped on dry ice and should be stored at –20°C. The Purification Module is shipped and should be stored at room temperature.

Labeling Module

Store at –20°C.

Component	Components/Concentration	Amount
Exo⁻ Klenow Fragment	40 U/µL, Klenow fragment	31 µL
2.5X Random Primers Solution	750 µg/mL oligodeoxyribonucleotide primers (random octamers)	700 µL
10X dCTP Nucleotide Mix	1.2 mM dATP, dGTP, dTTP and 0.6 mM dCTP in 10 mM Tris (pH 8.0), 1 mM EDTA	155 µL
10X dUTP Nucleotide Mix	1.2 mM dATP, dGTP, dCTP and 0.6 mM dTTP in 10 mM Tris (pH 8.0), 1 mM EDTA	155 µL
Control DNA (Salmon Sperm)	10 µg/µL	100 µg
Stop Buffer	0.5 M EDTA (pH 8.0)	500 µL
Sterile Water	—	1 mL

Kit Contents and Storage, Continued

Purification Module

Store at room temperature.

Component	Amount
PureLink® Spin Columns with Collection Tubes	30 each
Binding Buffer (B2) (combine with 100% isopropanol; see **Preparing Binding Buffer B2 with Isopropanol**)	9 mL
Wash Buffer (W1) (combine with 100% ethanol; see **Preparing Wash Buffer W1 with Ethanol**)	10 mL
Elution Buffer (E1) (10 mM Tris-HCl, pH 8.5)	3 mL
Amber Recovery Tubes	30 tubes

Preparing Binding Buffer B2 with Isopropanol

Binding Buffer B2 supplied with the Purification Module must be mixed with 100% isopropanol prior to use. Add the amount of isopropanol below directly to the bottle of Binding Buffer B2, and mark the checkbox on the bottle to indicate that you have added the isopropanol.

	Amount
Binding Buffer B2	9 mL (entire bottle)
100% Isopropanol	6 mL
Final Volume	15 mL

Store the buffer with isopropanol at room temperature.

Preparing Wash Buffer W1 with Ethanol

Wash Buffer W1 supplied with the Purification Module must be mixed with 100% ethanol prior to use. Add the amount of ethanol below directly to the bottle of Wash Buffer W1, and mark the checkbox on the bottle to indicate that you have added the ethanol.

	Amount
Wash Buffer W1	10 mL (entire bottle)
100% Ethanol	40 mL
Final Volume	50 mL

Store the buffer with ethanol at room temperature.

Materials Required

Materials Needed

The following materials are supplied by the user:

- 250 ng–1 µg genomic DNA (for BAC arrays) or 4 µg genomic DNA (for cDNA arrays); amount is array-dependent
- Microcentrifuge
- Vortex Mixer
- Aerosol-resistant pipette tips
- Amber 1.5-mL microcentrifuge tubes
- Incubators or water baths set at 95ºC and 37ºC
- Ice
- CyDye® fluorescent dCTP or dUTP, or fluorescent dCTP/dUTP from another manufacturer
- 100% isopropanol and 100% ethanol (for preparing the purification buffers; see page 5)

Description of the Kit

Purpose of the Kit

Comparative genomic hybridization (CGH) is a microarray-based method for analyzing the whole genome to detect variations in gene copy number between samples (Pollack et al, 1999, 2002). In CGH, two genomic DNA samples are labeled with different fluorophores and hybridized to a microarray. The ratio of the fluorescent intensities of the fluorophores is measured for each gene on the array. This ratio provides a relative measure of the difference in gene copy number between the samples.

The BioPrime® Array CGH Genomic Labeling System uses random primers and a mutant form of the Klenow fragment of DNA polymerase I (Exo⁻ Klenow) to differentially label genomic DNA samples with fluorescently labeled nucleotides for analysis using CGH.

Probes generated with this system can differentially detect differences in gene copy number from as little as 250 ng of genomic DNA. The kit is compatible with fluorescent nucleotides from a variety of manufacturers, including Cy3®- and Cy5®-labeled dNTPs and Alexa Fluor®-labeled dNTPs.

How to Use

Using the kit, you anneal your genomic DNA with random octamers. The primers are extended in a polymerization reaction using a high concentration of Exo⁻ Klenow and fluorescently labeled nucleotides, resulting in high nucleotide incorporation and at least a 7–10-fold amplification of starting material. You then purify the labeled samples using spin columns to remove unincorporated nucleotides and proceed to microarray hybridization.

Advantages of the System

- Exo⁻ Klenow polymerase lacks both 5′-3′ and 3′-5′ exonuclease activity, producing higher yields of labeled sample than standard Klenow for increased sensitivity.
- Exo⁻ Klenow polymerase incorporates fluorescently modified nucleotides more effectively than standard Klenow, enabling you to obtain stronger hybridization intensities and greater reproducibility of results.
- Simplified random priming protocol takes less than three hours.

Workflow Overview

Add each genomic DNA sample to be compared to a separate tube.

Add random primers to each sample.

Heat each mixture briefly to denature the DNA, then cool to anneal the primers.

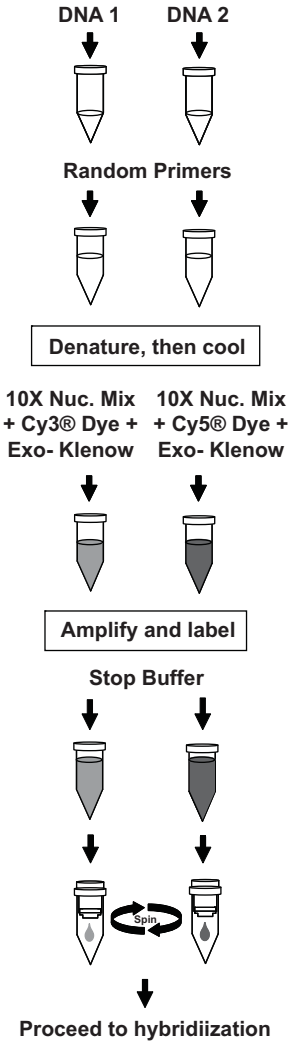
Add the appropriate 10X Nucleotide Mix, the appropriate dye, and Exo⁻ Klenow to each tube.

Incubate at 37ºC for 2 hours to amplify and label the DNA.

Add Stop Buffer to stop each reaction.

Add Binding Buffer B2 to each labeled sample, then spin, wash, and elute using the PureLink® spin columns.

Proceed to hybridization.



Description of the Kit, Continued

Dye Compatibility

This kit has been developed using Cy3®- and Cy5®-labeled nucleotides from GE Healthcare. Fluorescently labeled nucleotides from other manufacturers (e.g., Alexa Fluor®-labeled dNTPs from Molecular Probes) are also compatible with this system.

Control DNA

Control DNA (salmon sperm DNA) is included in the kit to help you determine the efficiency of the labeling procedure. We recommend that you perform the complete labeling procedure using the Control DNA if you are a first-time user of the system.

Equations for calculating the efficiency of the labeling procedure using the Control DNA are provided on page 15.

Methods

Preparing the DNA

Starting Material

This kit is optimized for use with 250 ng–1 µg genomic DNA (for BAC arrays) or 4 µg genomic DNA (for cDNA arrays) as starting material. In general, use the amount of starting material recommended by your array manufacturer.

Isolating Genomic DNA

Isolate genomic DNA using your method of choice. The PureLink® Genomic DNA Purification Kit (Cat. no. K1810-01) is a complete kit for the isolation of genomic DNA. See page 19 for ordering information. A wide range of ChargeSwitch® Genomic DNA purification kits is also available from Life Technologies.

DNA Digestion/ Sonication

Genomic DNA may be either intact or treated by enzymatic digestion or sonication, depending on the requirements of your array manufacturer. Note that DNA that has been fragmented by enzymatic digestion or sonication generally yields better results in array CGH.

General Handling of DNA

When handling DNA, use sterile conditions to ensure that no DNases are introduced. All equipment that comes into contact with DNA should be sterile, including pipette tips, microcentrifuge tubes, snap-cap polypropylene tubes, and pipettes. Be sure pipettor barrels are clean and treated with ethanol.

Checking DNA Quantity and Quality

Genomic DNA may be run on an agarose gel to check for quantity and quality. Bufferless E-Gel® Pre-cast Agarose Gels are available from Life Technologies for fast and easy electrophoresis. See page 19 for ordering information.

Storing DNA

After isolating the DNA, we recommend that you proceed directly to **Labeling**, page 11. Otherwise, store the isolated genomic DNA at 4°C.

Labeling

Introduction

This section provides a protocol for labeling genomic DNA with fluorescently labeled nucleotides. The protocol uses CyDye™ fluorescent nucleotides, and is compatible with fluorescent nucleotides from other manufacturers.

Dye Information

This system has been developed using the following CyDye® fluorescent nucleotides:

Cy3®-dCTP (GE Healthcare Life Sciences, #PA53021)
Cy3®-dUTP (GE Healthcare Life Sciences, #PA53022)
Cy5®-dCTP (GE Healthcare Life Sciences, #PA55021)
Cy5®-dUTP (GE Healthcare Life Sciences, #PA55022)

It is also compatible with fluorescent nucleotides from other manufacturers.

Important

Fluorescent nucleotides are sensitive to photobleaching. When preparing the reaction:

- Use amber microcentrifuge tubes as specified.
- Be careful to minimize exposure of the fluorescent nucleotides and labeled DNA to light.

Materials Needed

The following materials are supplied by the user:

- 250 ng–1 µg genomic DNA (for BAC arrays) or 4 µg genomic DNA (for cDNA arrays); amount is array-dependent
- Microcentrifuge
- Amber 1.5-mL microcentrifuge tubes
- Incubators or water baths set at 95ºC and 37ºC
- Ice
- CyDye® fluorescent dCTP or dUTP, or fluorescent dCTP/dUTP from another manufacturer

Preparing the Control DNA

Use 1 µg of Control DNA per reaction. The Control DNA is provided at a concentration of 10 µg/µL; to avoid pipetting 0.1 µL, we recommend diluting 1 µL of the control 1:10 in sterile distilled water prior to use.

Labeling, Continued

Incubation Methods

The incubation steps may be performed in a heat block, air incubator, or thermocycler with a heated lid. Incubate the reaction protected from light.

Nucleotide Mixes

Select the type of nucleotide mix based on the type of dye-labeled nucleotides you are using. Use the 10X dCTP Nucleotide Mix with labeled dCTP and 10X dUTP Nucleotide Mix with labeled dUTP.

Labeling Procedure

Follow the steps below to incorporate the fluorescent nucleotides in the genomic DNA.

1. Add each genomic DNA sample to be compared to a new **amber** 1.5-mL microcentrifuge tube and suspend in sterile distilled water to a final volume of 21 µL.

   Control reactions: Use 1 microgram (µg) of the Control DNA included in the kit (supplied at 10 µg/µL) per reaction.

2. Add 20 µL of 2.5X Random Primers Solution to each sample.

3. Incubate at 95ºC in an incubator or water bath for 5 minutes, and then immediately cool on ice for 5 minutes.

4. On ice, add the following to each tube to differentially label each sample:

	DNA 1	DNA 2
10X dCTP or dUTP Nucleotide Mix	5 µL	5 µL
Cy3®-dCTP or –dUTP	3 µL	—
Cy5®-dCTP or –dUTP	—	3 µL
Exo⁻ Klenow Fragment	1 µL	1 µL

5. Mix gently and perform a quick spin down (5 seconds).

6. Incubate at 37ºC for 2 hours.

7. Add 5 µL of Stop Buffer to each tube and place on ice. The reaction can be stored at –20ºC overnight, if necessary.

Proceed to **Purification Procedure** on page 13.

Purification

BioPrime® Purification Module

Cat. no. 18095-011 includes a purification module with PureLink® spin columns and buffers. Follow the procedure in this section to purify your labeled DNA using this module.

Other Purification Methods

Cat. no. 18095-012 does not include a purification module. Use your preferred method of purification, and then proceed to page 15. When assessing the labeling efficiency using a spectrophotometer, be sure to blank the spectrophotometer using the elution buffer from your purification system.

Note: The PureLink® PCR Purification System can be ordered separately if you are using Cat. no. 18095-012. Ordering information is provided on page 19.

Purification Procedure

Use the following steps when using the Purification Module from Cat. no. 18095-011 to purify the labeled DNA probes.

1. Add 200 µL of Binding Buffer B2 (prepared with isopropanol as described on page 5) to each tube from Step 7, page 12, and vortex to mix.
2. Load each sample onto a PureLink® Spin Column, preinserted in a collection tube.
3. Centrifuge at 10,000 × *g* for 1 minute. Discard the flow-through and place the column back in the collection tube.
4. Add 650 µL of Wash Buffer W1 (prepared with ethanol as described on page 5) to the column.
5. Centrifuge at 10,000 × *g* for 1 minute. Discard the flow-through and place the column back in the collection tube.
6. Spin at maximum speed for an additional 2–3 minutes to remove any residual wash buffer. Discard the flow-through.

Purification Procedure, continued

7. Place the Spin Column in a new, sterile Amber Recovery Tube (supplied in the kit).
8. Add 55 µL of Elution Buffer E1 to the center of the column and incubate at room temperature for 1 minute.
9. Centrifuge at maximum speed (~20,000 × *g*) for 2 minutes. The flow-through contains the purified labeled DNA probes. (Discard the column after use.)

To determine the efficiency of the labeling reaction, proceed to **Assessing Labeling Efficiency**, page 15.

For a list of array hybridization reagents available from Life Technologies, see page 19.

Assessing Labeling Efficiency

CyDye® Wavelengths

The following table shows the absorbance and baseline wavelengths for Cy3® and Cy5® dyes:

Dye	Absorbance Wavelength	Baseline Wavelength
Cy3®	550 nm	650 nm
Cy5®	650 nm	750 nm

Calculating the Results

To calculate the amount of labeled DNA using a UV/visible spectrophotometer:

1. Transfer an appropriate volume of purified, labeled DNA from step 9, page 14, to a clean cuvette. Use an appropriate volume for your spectrophotometer. Blank the spectrophotometer using 10 mM Tris-HCl, pH 8.5.

   Important: The labeled DNA must be purified as described on page 14 before scanning, as any unincorporated labeled nucleotides will interfere with the detection of labeled DNA.

2. Measure the absorbance of the sample at A_{260}, A_{320}, A_{550}, A_{650}, and A_{750}. Wash each cuvette thoroughly between samples.

Yield:[1]

DNA (µg) = $(A_{260}–A_{320}) \times 50$ µg/mL × volume in mL

Dye Incorporation:[2]

Cy3® (pmole) = $(A_{550}–A_{650})/0.15$ × volume in µL

Cy5® (pmole) = $(A_{650}–A_{750})/0.25$ × volume in µL

Degree of Labeling:

Base/dye for Cy3® = $(A_{260} \times 150{,}000\ (cm^{-1}\ M^{-1}))/(A_{550} \times 6600)$

Base/dye for Cy5® = $(A_{260} \times 250{,}000\ (cm^{-1}\ M^{-1}))/(A_{650} \times 6600)$

Notes:

[1]Subtracting A_{320} from A_{260} corrects for any silica particles that may leak from the purification columns and artificially increase the yield calculations.

[2]Subtracting A_{650} from A_{550} and A_{750} from A_{650} corrects for any fluorescent background that might artificially increase the measure of dye incorporation.

Assessing Labeling Efficiency, Continued

Expected Results

Using the calculations on the previous page, you should expect the following:

Yield: The expected amount of labeled DNA should be ≥2.8 µg. If it is <2.8 µg, see **Troubleshooting** on page 17.

Dye incorporation: The expected level of dye incorporation should be ≥100 pmoles. If it is <100 pmoles, see Troubleshooting on page 17.

Base/dye ratio: The base-to-dye ratio should be 40–80 for both Cy3® and Cy5®.

Dye Incorporation versus Signal Intensity

Signal intensity and signal/background on microarrays do not correlate directly with dye incorporation or degree of labeling when comparing different fluorescent dyes. Labeling with the BioPrime® Array CGH Genomic Labeling System yields microarray signal intensities and signal/background ratios greater than or equal to DNA labeled with other dye-labeled nucleotides, even with lower dye incorporation and/or degree of labeling.

Troubleshooting

Problem	Cause	Solution
Yield of labeled DNA from the control reaction is low	DNA has been lost in the purification step after labeling	Make sure that isopropanol has been added to the Binding Buffer and ethanol has been added to the Wash Buffer, as specified on page 5. Measure the amount of labeled DNA in the control reaction before and after purification. Repeat the labeling and purification procedures, following all steps without modifications.
	Starting amount of DNA is too low	Increase the amount of starting DNA.
Cannot detect labeled probes	DNA has been lost in the purification step after labeling	Make sure that isopropanol has been added to the Binding Buffer and ethanol has been added to the Wash Buffer, as specified on page 5. Measure the amount of labeled DNA in the control reaction before and after purification. Repeat the labeling and purification procedures, following all steps without modifications.
Amount of incorporated labeled nucleotides is low or fluorescence is low	Starting amount of DNA is too low	Increase the amount of starting DNA.
	Reaction tubes have been exposed to light	Avoid direct exposure of the reaction tubes to light. Repeat the labeling procedure.
	Fluorescent nucleotides have been exposed to light	Repeat the labeling reaction, being careful to avoid direct exposure to light.
	Inefficient labeling due to improper purification	Follow all the purification steps as described in the procedures.

Appendix

Technical Support

Obtaining Support

For the latest services and support information for all locations, go to **www.lifetechnologies.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support (**techsupport@lifetech.com**)
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

Safety Data Sheets (SDS)

Safety Data Sheets (SDSs) are available at **www.lifetechnologies.com/support**.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to **www.lifetechnologies.com/support** and search for the Certificate of Analysis by product lot number, which is printed on the box.

Limited Product Warranty

Life Technologies and/or its affiliate(s) warrant their products as set forth in the Life Technologies General Terms and Conditions of Sale found on the Life Technologies web site at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

Additional Products

Additional Products

Life Technologies has additional reagents that may be used to prepare labeled probes for hybridization. Ordering information is provided in the following table.

Product	Quantity	Catalog No.
PureLink® PCR Purification System	50 reactions 250 reactions	K3100-01 K3100-02
PureLink® Genomic DNA Purification Kit	50 reactions	K1810-01
E-Gel® 1.2% Starter Pak	6 gels and base	G6000-01
Human Cot-1 DNA®-Fluorometric QC	1 mg	15279-101
Human Cot-1 DNA®	500 µg	15279-011
Mouse Cot-1 DNA®	500 µg	18440-016
Yeast tRNA	25 mg 50 mg	15401-011 15401-029

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Notes

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