USER GUIDE

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BioPrime® Total Genomic Labeling System

Catalog Numbers 18097-010, 18097-011, and 18097-012

Document Part Number 25-1006
Publication Number MAN0000596
Revision 3.0

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Kit Contents and Storage

Kit Sizes and Modules

This manual supports the following BioPrime® Total kit configurations:

Catalog No.	# of Reactions	Modules
18097-010	10	Labeling and Purification
18097-011	30	Labeling and Purification
18097-012	30	Labeling only

Shipping and Storage

The BioPrime® Total Labeling Module is shipped on dry ice and should be stored at –80°C. The 2X Reaction Mixes may be stored at 4°C for up to 4 weeks (store at –80°C long-term).

The BioPrime® Purification Module is shipped and should be stored at room temperature.

Labeling Module

The following components should be stored at –80°C. The 2X Reaction Mixes may be stored at 4°C for up to 4 weeks (store at –80°C long-term).

Component	10-reaction kit	30-reaction kits
Alexa Fluor® 3 2X Reaction Mix	125 µL	125 µL × 3
Alexa Fluor® 5 2X Reaction Mix	125 µL	125 µL × 3
Exo- Klenow Fragment, 40 U/µL	30 µL	30 µL × 3
5 mM EDTA (pH 8.0)	1.2 mL	1.2 mL
TE Buffer (10/1, pH 8.0)	660 µL	660 µL
Control DNA (Salmon Sperm), (10 mg/mL)	10 µL	10 µL

Important

The fluorescently labeled nucleotides in the Alexa Fluor® 2X Reaction Mixes are sensitive to photobleaching. Store the mixes protected from light.

Kit Contents and Storage, Continued

Purification Module

The following components should be stored at room temperature.

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

Overview

Introduction

Array comparative genomic hybridization (aCGH) is a microarray-based method for analyzing genomic DNA to detect variations in gene copy number between samples (Pollack et al., 1999; Pollack et al., 2002). In aCGH, two genomic DNA samples are labeled with different fluorophores. The samples are hybridized to a microarray and the ratio of the fluorescent intensities of the fluorophores is measured for each feature on the array (Beheshti et al., 2003; Cai et al., 2002; Snijders et al., 2001). This ratio provides a relative measure of the difference in gene copy number between the samples.

The BioPrime® Total Genomic Labeling System uses a mutant form of the Klenow fragment of DNA Polymerase I (Exo- Klenow) and nucleotides labeled with two novel, application-specific dyes (Alexa Fluor® 3 and 5) to differentially label genomic DNA samples for analysis by aCGH. The kit provides the dye-labeled nucleotides in an optimized master-mix formulation that includes unlabeled nucleotides and random primers, for ease of reaction setup.

Labeled DNA generated using this system can detect differences in gene copy number from as little as 50 ng of genomic DNA, depending on the sample and array type.

Advantages of the System

- Amplified products labeled with novel Alexa Fluor® 3 and 5 dyes have greater yields and higher signal intensities on the array.
- Exo- Klenow polymerase incorporates fluorescently modified nucleotides more effectively and provides higher yields than standard Klenow, for greater reproducibility of results.
- PureLink® purification columns, included with Cat. no. 18097-010 and 18097-011, are designed to effectively remove all unincorporated nucleotides for the most accurate quantitation of labeled product and reduced background on the array.
- Dye-specific 2X Reaction Mixes include random primers and both labeled and unlabeled nucleotides for simplified reaction setup and workflow.
- Provides a complete solution for fluorescent labeling of genomic DNA.

Overview, Continued

Workflow Overview

Add Alexa Fluor® 3 2X Reaction Mix and Alexa Fluor® 5 2X Reaction Mix to separate reaction tubes, then add genomic DNA samples.

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

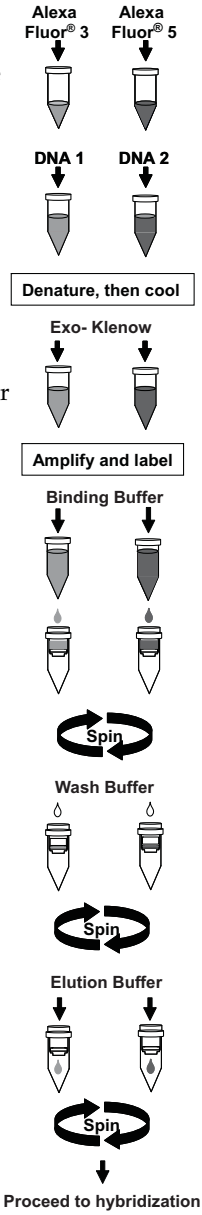
Add Exo- Klenow, then incubate at 37ºC for 2 hours to amplify and label the DNA.

Add Binding Buffer B1 to each labeled sample, transfer the mixture to PureLink® spin columns in collection tubes, and centrifuge.

Add Wash Buffer W1 to each column and centrifuge.

Transfer the spin columns to Amber Recovery Tubes, add Elution Buffer E1 to each column and centrifuge.

Proceed to hybridization.



Overview, Continued

Alexa Fluor® 3 and Alexa Fluor® 5 Dyes

The novel, application-specific Alexa Fluor® 3 and Alexa Fluor® 5 dyes used in the kit are compatible with commonly used microarray scanners, and provide greater signal correlation (R^2) values than the spectrally similar Cy®3 and Cy®5 dye pair, improving the resolution of two-color aCGH.

The table lists the excitation/emission maxima and color of each dye:

Dye	Excitation/Emission	Color
Alexa Fluor® 3	555/565 nm	Pink
Alexa Fluor® 5	650/670 nm	Light blue

Control DNA

Control DNA (Salmon Sperm DNA) is included in the kit to help you determine the efficiency of the labeling procedure. Equations for calculating the efficiency of the labeling procedure using the Control DNA are provided on page 15.

Materials Supplied by the User

In addition to the kit components, you should have the following items on hand before using the BioPrime® Total Genomic Labeling System.

- 50 ng–3 µg genomic DNA (amount is array-dependent)
- Vortex mixer
- Microcentrifuge
- Heat block, air incubator, or thermocycler with a heated lid
- Ice
- 1.7-mL DNase-free capped tubes or thin-walled PCR tubes
- Aerosol-resistant pipette tips
- 100% isopropanol and 100% ethanol (for preparing the purification buffers; see page 5)
- Optional (if quenching the labeling reaction): 0.5 M EDTA

Methods

Before Starting

Amount of Starting Material	This kit has been designed to generate ≈8 µg of labeled DNA from as little as 50 ng to 3 µg of input genomic DNA. In general, larger amounts of starting material result in better fold changes on the array. 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 Treatment	The 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 aCGH.
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 10. Otherwise, store the isolated genomic DNA at 4°C. Note that storage in TE Buffer is recommended for greater stability.

Labeling

Before Starting

In addition to the components of the Labeling Module, the following materials are supplied by the user:

- 50 ng–3 µg genomic DNA (amount is array-dependent)
- Vortex mixer
- Microcentrifuge
- Heat block, incubator, or thermocycler with a heated lid
- Ice
- 1.7-mL capped tubes or thin-walled PCR tubes
- Optional (if quenching the labeling reaction): 0.5 M EDTA

Preparing DNA Samples in TE

If the genomic DNA is in TE Buffer, add the TE Buffer provided in the kit to a final volume of 22 µL. We recommend using the TE in the kit (10 mM Tris-HCl pH 8.0, 1 mM EDTA); not all laboratory TE is provided at this ratio.

If the sample is in water, see **Preparing DNA Samples in Water**.

Preparing DNA Samples in Water

If the genomic DNA sample is in water, you must add 4.4 µL of 5 mM EDTA to the sample to ensure optimal labeling. (The EDTA will be at a concentration of 0.44 mM in the final 50-µL labeling reaction.) Add 5 mM EDTA to the sample and bring the final volume to 22 µL as follows:

DNA sample in water	X µL
5 mM EDTA (provided in the kit)	4.4 µL
Sterile, distilled water	to 22 µL

Important

- The addition of 5 mM EDTA to DNA samples in water, as described in the previous section, is necessary to ensure optimal labeling conditions.
- Fluorescently labeled nucleotides are sensitive to photobleaching. During all steps of the procedure, be careful to minimize exposure of the 2X Reaction Mixes and labeled DNA to light.

Labeling, Continued

Preparing the Control DNA

The Control DNA is provided at a concentration of 10 mg/mL, and should be diluted in TE Buffer prior to use. To prepare 1 µg of Control DNA for labeling:

1. Dilute the Control DNA in TE Buffer to a final concentration of 1 µg/µL:

Control DNA (10 mg/mL)	1 µL
TE Buffer (provided in the kit)	9 µL
Final volume	10 µL

2. Add 1 µL of the diluted Control DNA to 21 µL of TE Buffer, for a final volume of 22 µL.

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.

Labeling Procedure

1. If necessary, thaw the Alexa Fluor® 2X Reaction Mixes at room temperature, protected from light (mixes may be stored at 4°C for up to 4 weeks).
2. Briefly vortex each 2X Reaction Mix and centrifuge to collect the contents of the tubes. Place the tubes on ice.
3. **Genomic DNA samples should be prepared in TE or in water with EDTA in a volume of 22 µL, as described on page 10.** Add the following to separate DNase-free 1.7-mL capped tubes or thin-walled PCR tubes:

Component	Tube 1	Tube 2
Alexa Fluor® 3 2X Reaction Mix	25 µL	—
Alexa Fluor® 5 2X Reaction Mix	—	25 µL
Genomic DNA Sample 1	22 µL	—
Genomic DNA Sample 2	—	22 µL
Total volume	47 µL	47 µL

4. Gently pipet up and down to mix and incubate at 95ºC, protected from light, for 5 minutes. Immediately cool on ice for 5 minutes.
5. On ice, add 3 µL of Exo- Klenow Fragment to each tube, for a final reaction volume of 50 µL.

Labeling Procedure, continued

6. Vortex tubes briefly and centrifuge to collect the contents.
7. Incubate at 37ºC for 2 hours in a heat block, air incubator, or thermocycler with a heated lid, protected from light.
8. After incubation, if you are storing the reaction for any length of time prior to purification, add 5 µL of 0.5 M EDTA to each tube to quench the reaction. If you are proceeding directly to purification, you can skip this step.

Proceed to **Purification**, page 13. The reaction can be stored at –20ºC overnight if necessary (following the addition of EDTA).

Purification

BioPrime® Purification Module

Cat. no. 18097-010 and 18097-011 include a Purification Module developed for use with the BioPrime® Total system. Follow the procedure in this section to purify your labeled DNA using this module.

Other Methods of Purification

Cat. no. 18097-012 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.

PureLink® PCR Purification

The PureLink® PCR Purification System (K3100-01 and K3100-02) has been tested with the BioPrime™ Total system, and is recommended if you are using cat. no. 18097-012. Ordering information is provided on page 19.

Purification Procedure

Follow the steps below using the Purification Module from Cat. no. 18097-010 and 18097-011 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 8, 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, Continued

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 the Efficiency of the Labeling Procedure**, page 15.

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

Assessing the Efficiency of the Labeling Procedure

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_{555}, A_{650}, and A_{750}. Wash each cuvette thoroughly between samples.

Yield:[1]

$$\text{DNA } (\mu g) = (A_{260} - A_{320}) \times 50\ \mu g/mL \times \text{volume in mL}$$

Dye Incorporation:[2]

$$\text{Alexa Fluor® 3 (pmole)} = (A_{555} - A_{650})/0.15 \times \text{volume in } \mu L$$

$$\text{Alexa Fluor® 5 (pmole)} = (A_{650} - A_{750})/0.24 \times \text{volume in } \mu L$$

Degree of Labeling:[3]

$$\text{Alexa Fluor® 3 base/dye ratio} = ((A_{260} - A_{320}) - ((A_{555} - A_{650}) \times 0.04)) \times 150{,}000/(A_{555} - A_{650}) \times 6600$$

$$\text{Alexa Fluor® 5 base/dye ratio} = ((A_{260} - A_{320}) - ((A_{650} - A_{750}) \times 0)) \times 239{,}000/(A_{650} - A_{750}) \times 6600$$

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_{555} and A_{750} from A_{650} corrects for any fluorescent background that might artificially increase the measure of dye incorporation.

[3]Absorbance at A_{555} has a very slight effect on the A_{260} reading, and the formula $((A_{555} - A_{650}) \times 0.04))$ corrects for this. Conversely, there is no effect of A_{650} on the A_{260} reading; the multiplication by zero was added to the second formula to keep the formulas consistent.

Assessing the Efficiency of the Labeling Procedure, Continued

Control DNA

Typically, if starting with 1 µg of Control DNA as specified on page 11, you should expect the following:

Yield: ≥7 µg of amplified DNA

Dye incorporation: ≥175 pmol Alexa Fluor® 3
≥300 pmol Alexa Fluor® 5

Degree of labeling: ≥0.7 for Alexa Fluor® 3
≥1.2 for Alexa Fluor® 5

Note

The Alexa Fluor® dye-labeled nucleotides and reaction conditions of the BioPrime® Total Genomic Labeling System have been optimized for use on microarrays. Signal intensity and signal/background on microarrays does not correlate directly with dye incorporation or degree of labeling when comparing different fluorescent dyes. Labeling with the BioPrime® Total 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.
	EDTA concentration in the sample is too low	The labeling reaction has been optimized for a final EDTA concentration of 0.44 mM. If your genomic DNA sample is in water, you should add EDTA to the sample before labeling as specified on page 10.
	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.
	EDTA concentration in the sample is too low	The labeling reaction has been optimized for a final EDTA concentration of 0.44 mM. If your genomic DNA sample is in water, you should add EDTA to the sample before labeling as specified on page 10.
	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 below.

Product	Quantity	Catalog No.
PureLink® PCR Purification System	50 reactions 250 reactions	K3100-01 K3100-02
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

Notes

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1 October 2012