



Instruction Manual

BioPrime® Plus Array CGH Genomic Labeling System

**For generating fluorescently labeled genomic
DNA using Alexa Fluor®-labeled primers and
nucleotides**

Catalog nos. 18095-013 and 18095-014

Version A
15 November 2004
25-0793

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Kit Contents and Storage

Kit Sizes and Modules

Both versions of the BioPrime® Plus Array CGH Genomic Labeling System are supplied with a Core Module and a Nucleotide Module. Catalog no. 18095-013 also includes a Purification Module.

<u>Cat no.</u>	<u>Number of Reactions</u>	<u>Modules</u>
18095-013	30	Core, Nucleotide, and Purification
18095-014	30	Core and Nucleotide only

Shipping and Storage

The Core Module and Nucleotide Module are shipped on dry ice, and the Purification Module is shipped at room temperature. Upon receipt, store the components of the Core and Nucleotide Modules at -20°C, and store the components of the Purification Module at room temperature.

Core Module

The components of the Core Module should be stored at -20°C.

Item	Components/Concentration	Amount
Exo-Klenow Fragment	40 U/μl, Klenow fragment in 100 mM Potassium Phosphate (pH 7.0), 1 mM DTT, and 50% Glycerol	30 μl
2.5X Reaction Buffer (for resuspending Panomer™ 9 oligonucleotides)	125 mM Tris-HCl (pH 6.8), 12.5 mM MgCl ₂	660 μl
Control DNA (Salmon Sperm)	Salmon Sperm DNA, 10 μg/μl in RNase-free, DNase-free water	10 μl
Stop Buffer	0.5 M EDTA (pH 8.0)	500 μl
Sterile Water	—	1 ml

Nucleotide Module

The components of the Nucleotide Module should be stored at -20°C.

Item	Components/Concentration	Amount
10X Nucleotide Mix with Alexa Fluor® 555-aha-dCTP	dATP, dGTP, dTTP, dCTP, and Alexa Fluor® 555-aha-dCTP in 10 mM Tris (pH 8.0), 1 mM EDTA	3 × 25 μl
10X Nucleotide Mix with Alexa Fluor® 647-aha-dCTP	dATP, dGTP, dTTP, dCTP, and Alexa Fluor® 647-aha-dCTP in 10 mM Tris (pH 8.0), 1 mM EDTA	3 × 25 μl
Alexa Fluor® 555 Panomer™ 9	Dried down 5'-end-labeled random nanomers	3 × 31 nmole
Alexa Fluor® 647 Panomer™ 9	Dried down 5'-end-labeled random nanomers	3 × 31 nmole

Continued on next page

Kit Contents and Storage, continued

Purification Module

The components of the Purification Module should be stored at room temperature.

Component	Amount
PureLink™ Spin Columns with Collection Tubes	31 columns/tubes
Binding Buffer (B2) (must be combined with 100% isopropanol to create final buffer; see below)	9 ml
Wash Buffer (W1) (must be combined with 100% ethanol to create final buffer; see below)	10 ml
Elution Buffer (E1) (10 mM Tris-HCl, pH 8.5)	3 ml
Amber collection tubes	3 × 11 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 indicated below directly to the bottle of Binding Buffer B2 to create the final buffer plus isopropanol. Be sure to mark the appropriate checkbox on the bottle to indicate that you have added the isopropanol.

	<u>Amount</u>
Binding Buffer B2	9 ml (entire bottle)
100% Isopropanol	<u>6 ml</u>
Final Volume	15 ml

Store the Binding Buffer B2 prepared 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 indicated below directly to the bottle of Wash Buffer W1 to create the final buffer plus ethanol. Be sure to mark the appropriate checkbox on the bottle to indicate that you have added the ethanol.

	<u>Amount</u>
Wash Buffer W1	10 ml (entire bottle)
100% Ethanol	<u>40 ml</u>
Final Volume	50 ml

Store the Wash Buffer W1 prepared with ethanol at room temperature.

Product Qualification

This kit was verified in replicate labeling reactions using the protocols described in this manual, with 1 µg of genomic DNA as starting material and random primers and nucleotides labeled with Alexa Fluor® 555 and Alexa Fluor® 647. The DNA yield, picomole dye incorporation, and number of dye molecules per 100 bases were calculated as described on page 11. In addition, the product was run on an agarose gel and scanned to determine the success of the fluorescent labeling.

Accessory Products

Additional Products

Invitrogen has additional reagents that may be used to prepare labeled probes for hybridization. Ordering information is provided below.

Product	Quantity	Catalog no.
BioPrime® Plus Array CGH Indirect Genomic Labeling System	30 reactions	18096-011
	30 reactions (w/o purification module)	18096-012
BioPrime® Array CGH Genomic Labeling System	30 reactions	18095-011
	30 reactions (w/o purification module)	18095-012
PureLink™ PCR Purification System	50 reactions	K3100-01
	250 reactions	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®	500 µg	15279-011
Mouse Cot-1 DNA®	500 µg	18440-016
Yeast tRNA	25 mg	15401-011
	50 mg	15401-029

Overview

Introduction

Gene amplifications and deletions in the chromosome have been identified as critical factors in tumorigenesis. DNA copy-number variations have also been shown to correlate directly with changes in mRNA levels. 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. The samples are hybridized to a microarray and 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® Plus Array CGH Genomic Labeling System uses a mutant form of the Klenow fragment of DNA polymerase I (exo-Klenow) and fluorescently labeled random primers and nucleotides to differentially label genomic DNA samples for analysis using CGH. The kit includes optimized nucleotide mixes containing Alexa Fluor® 555-aha-dCTP and Alexa Fluor® 647-aha-dCTP, respectively, as well as random primers labeled at the 5' end with either Alexa Fluor® 555 or Alexa Fluor® 647.

Using the kit, you anneal your genomic DNA with the fluorescently labeled primers, which are extended in a polymerization reaction using a high concentration of exo-Klenow and fluorescently labeled nucleotides, resulting in high incorporation of fluorescent dye and 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.

Probes generated using this system can differentially detect differences in gene copy number from as little as 400 ng of genomic DNA.

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 and thereby increasing 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.
 - Alexa Fluor® dyes have a higher signal intensity and higher signal-to-background ratio than other labeling dyes.
 - Labeled primers and nucleotides provide for more effective fluorescent incorporation for stronger signal intensities and greater sensitivity.
 - Simplified random priming protocol takes less than three hours.
 - Provides a complete solution for fluorescent labeling of genomic DNA.
-

Starting Material

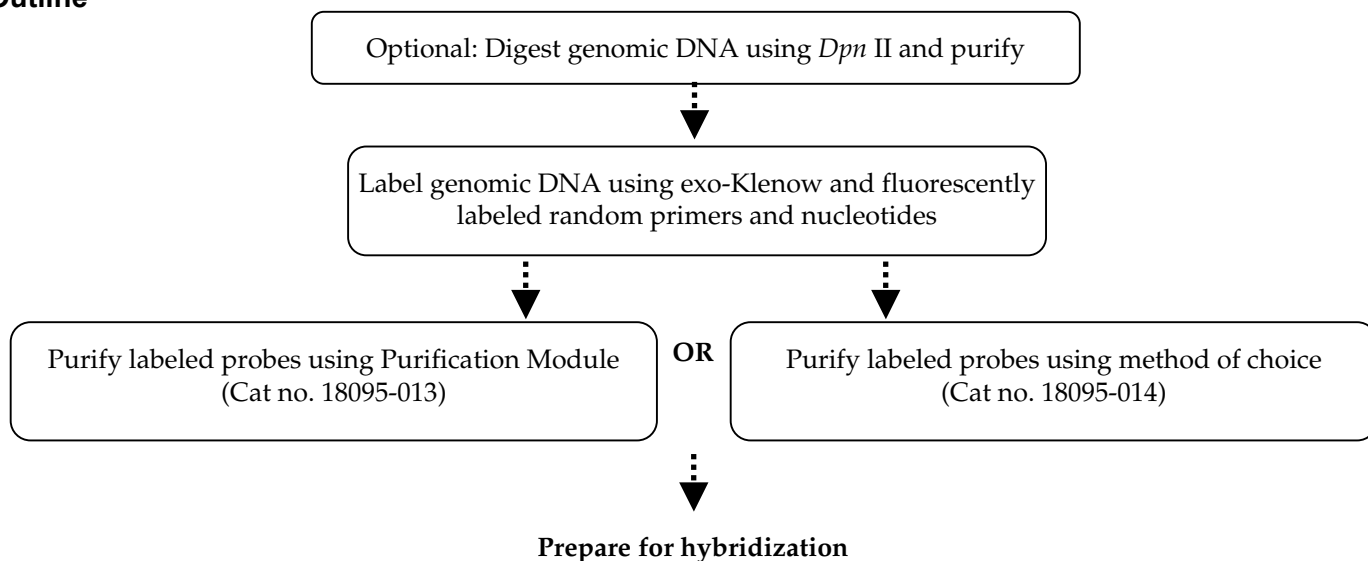
Typical amounts of starting material are 400 ng–2 µg genomic DNA (for BAC arrays) or 4 µg genomic DNA (for cDNA arrays).

Continued on next page

Overview, continued

Experimental Outline

The flow chart below outlines the experimental steps of the system:



Alexa Fluor[®] 555 and Alexa Fluor[®] 647

The Alexa Fluor[®] 555 and Alexa Fluor[®] 647 dyes used to label the nucleotides and random primers included 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 microarray gene expression assays. The exceptionally bright Alexa Fluor[®] dyes are also insensitive to pH and are highly water-soluble.

The table below shows the excitation and emission maxima and color of each dye:

<u>Dye</u>	<u>Excitation/Emission (nm)</u>	<u>Color</u>
Alexa Fluor [®] 555	555/565	Orange Fluorescent
Alexa Fluor [®] 647	650/670	Far-Red Fluorescent

Labeled Nucleotides and Primers

The labeled 5-aminohexylacrylamido-dCTP (aha-dCTP) nucleotides included in the kit are modified with a unique hexylacrylamide linker, which serves as a spacer between the nucleotide and the dye. This spacer reduces interactions between the nucleotide and the dye, resulting in brighter conjugates.

The Panomer[™] 9 random oligodeoxynucleotides are 9-base, random-sequence oligonucleotides that are covalently labeled on the 5' ends with the dye.

In the exo-Klenow reaction, the labeled primers and nucleotides are incorporated by the enzyme, ensuring efficient and unbiased labeling throughout the genomic DNA.

Continued on next page

Overview, continued

Control DNA

Control DNA (Salmon Sperm) 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 11.

Materials Supplied by the User

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

- 400 ng–2 µg genomic DNA (for BAC arrays) or 4 µg genomic DNA (for cDNA arrays)
- Vortex mixer
- Incubators
- Refrigerated microcentrifuge
- Ice
- 1.5-ml microcentrifuge tubes
- 1.5-ml amber microcentrifuge tubes
- Aerosol resistant pipette tips

The following items are optional, depending on whether you are performing the optional DNA digestion procedure (on page 5).

- *Dpn* II restriction endonuclease, including *Dpn* II buffer (NEB #R0543S)
 - Phenol:Chloroform:Isoamyl alcohol (25:24:1)
 - 3.0 M Sodium Acetate, pH 5.2
 - 100% Ethanol
 - 70% Ethanol
 - Tris EDTA (TE), pH 8.0
-

Methods

Isolating DNA

Isolating Genomic DNA

This kit is optimized for use with 400 ng–2 µg of genomic DNA (for BAC arrays) or 4 µg genomic DNA (for cDNA arrays) as starting material. Isolate genomic DNA using your method of choice.

The PureLink™ Genomic DNA Purification Kit (K1810-01) is a complete kit for the isolation of genomic DNA. Ordering information is provided on page vii.

General Handling of DNA

When handling DNA, make sure you use sterile conditions to ensure that no DNases are introduced. Make sure all equipment that comes in contact with DNA is 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

Run genomic DNA on an agarose gel to check for quantity and quality. Bufferless, precast agarose E-Gels™ (Catalog no. G6000-01) are available from Invitrogen for fast and easy electrophoresis.

Storing DNA

After preparing the DNA, we recommend that you proceed directly to **Labeling Genomic DNA** on page 7. Otherwise, store isolated genomic DNA at +4°C.

Optional: DNA Digestion and Purification

Introduction

This section provides an optional DNA digestion and purification protocol. Note that this step is not required, and you may proceed directly to **Labeling Genomic DNA** on page 7.



Note

Small amounts of starting material may be lost in the ethanol precipitation step. If this happens with your sample, skip digestion and purification and proceed directly to **Labeling Genomic DNA** on page 7.

Before Starting

The following materials are supplied by the user:

- 400 ng–2 µg genomic DNA (for BAC arrays) or 4 µg genomic DNA (for cDNA arrays)
- *Dpn* II restriction endonuclease, including *Dpn* II buffer (NEB #R0543S)
- Phenol:Chloroform:Isoamyl alcohol (25:24:1)
- 3.0 M Sodium Acetate, pH 5.2
- 100% Ethanol
- 70% Ethanol
- Tris EDTA (TE), pH 8.0
- Vortex mixer
- Incubators set at 37°C, 65°C and –80°C
- Refrigerated microcentrifuge
- Ice
- 1.5-ml microcentrifuge tubes

The following materials are supplied in the kit:

- Sterile distilled water
 - Optional: Control DNA (Salmon Sperm) (10 µg/µl)
-

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Optional: DNA Digestion and Purification, continued

Digestion and Purification Procedure

For BAC arrays, use 400 ng–2 µg of genomic DNA in the following procedure.

For cDNA arrays, use 4 µg of genomic DNA and double the amounts of all the other reagents in the following procedure. (Use the normal specified amounts of reagents for the subsequent labeling and purification procedures).

First-time users should set up a control reaction using the Control DNA supplied in the kit.

1. Prepare each reaction as follows, using a 1.5-ml microcentrifuge tube:

<u>Component</u>	<u>Amount</u>
Genomic DNA*	X µl
<i>Dpn</i> II buffer	2 µl
<i>Dpn</i> II enzyme	1 µl
Distilled water	to 20 µl

*For the control reaction, use an appropriate volume of Control DNA supplied in the kit (10 µg/µl).

2. Mix well and incubate tube at 37°C for 2 hours.
3. Inactivate the reaction at 65°C for 20 minutes.
4. Cool on ice for at least 1 min, and then add 80 µl of TE, pH 8.0, and 100 µl of Phenol:Chloroform:Isoamyl alcohol (25:24:1) to the tube. Vortex well.
5. Spin in a microcentrifuge for 5 minutes at 11,000 × g.
6. Pipette the top aqueous layer (~100 µl) from the tube into a new 1.5-ml microcentrifuge tube and add 10 µl of 3.0 M sodium acetate and 250 µl of 100% cold ethanol.
7. Incubate at –80°C for 30 minutes.
8. Centrifuge for 20 minutes at 4°C. Carefully remove and discard the supernatant without disturbing the pellet.
9. Add 500 µl of 70% ethanol to the tube and centrifuge for 5 minutes. Carefully remove and discard the supernatant without disturbing the pellet.
10. Air dry the sample for 5 minutes.
11. Completely resuspend the sample in 21 µl of sterile distilled water. The reaction can be stored at –20°C overnight, if necessary.

Proceed to **Labeling Genomic DNA** on the following page.

Labeling Genomic DNA



Important

Fluorescently labeled primers and nucleotides are sensitive to photobleaching. When preparing the reaction:

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

Before Starting

The following materials are supplied by the user:

- 400 ng–2 µg genomic DNA (for BAC arrays) or 4 µg genomic DNA (for cDNA arrays) — Optional: starting material may be first digested and purified as described on pages 5–6.
- Microcentrifuge
- Incubators or water baths set at 95°C and 37°C
- Ice
- 1.5-ml amber microcentrifuge tubes

The following materials are supplied in the kit:

- Sterile water
 - 2.5X Reaction Buffer
 - Alexa Fluor® 555 Panomer™ 9 / Alexa Fluor® 647 Panomer™ 9
 - 10X Nucleotide Mix with Alexa Fluor® 555-aha-dCTP / 10X Nucleotide Mix with Alexa Fluor® 647-aha-dCTP
 - Exo-Klenow Fragment
 - Stop Buffer
 - Optional: Control DNA (Salmon Sperm) (10 µg/µl)
-

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Labeling Genomic DNA, continued

Resuspending the Panomer™ 9 Oligonucleotides

Before proceeding with the labeling protocol, first resuspend the dried-down labeled primers you will be using in the reaction (Alexa Fluor® 555 Panomer™ 9 and/or Alexa Fluor® 647 Panomer™ 9). Resuspend each tube of primers as needed.

Add 110 µl of 2.5X Reaction Buffer directly to the amber tube of dried-down primers (31 nmole) included in the kit. Cap the tube, vortex briefly to mix, and proceed to the labeling procedure below.

Store any unused resuspended primers at -20°C protected from light.

Labeling Procedure

Follow the steps below to label genomic DNA. Each reaction will use either the Alexa Fluor® 555-labeled primers and nucleotides or the Alexa Fluor® 647-labeled primers and nucleotides.

1. If you performed the optional digestion procedure starting on page 5, use the full sample volume (21 µl) from step 11, page 6, in place of the genomic DNA in the table below. For each labeling reaction, add the following components to new **amber** 1.5-ml microcentrifuge tube:

<u>Component</u>	<u>Volume</u>
Alexa Fluor® 555 <i>or</i> Alexa Fluor® 647 Panomer™ 9 solution (resuspended as above)	20 µl
Genomic DNA	X µl
Sterile water	to 44 µl

2. Incubate at 95°C in an incubator or water bath for 10 minutes, and then immediately cool on ice, protected from light, for 5 minutes.
3. On ice, add the following to each tube:

<u>Component</u>	<u>Volume</u>
10X Nucleotide Mix with Alexa Fluor® 555-aha-dCTP <i>or</i> 10X Nucleotide Mix with Alexa Fluor® 647-aha-dCTP	5 µl
Exo-Klenow Fragment	<u>1 µl</u>
Final Volume	50 µl

4. Mix gently and briefly centrifuge to collect the contents.
5. Incubate at 37°C for 2 hours, protected from light.
6. Add 5 µl of Stop Buffer to the tube and place on ice. The reaction can be stored at -20°C overnight, if necessary.

Proceed to **Purifying the Labeled Probes** on the following page.

Purifying the Labeled Probes

Introduction

Cat no. 18095-013 includes a Purification Module developed for use with the system. Follow the procedure below to purify your labeled cDNA using this module.

Cat no. 18095-014 does not include a Purification Module. Use your preferred method of purification, and then continue to hybridization.



Note

The PureLink™ PCR Purification System (K3100-01 and K3100-02) has been tested with this kit, and is recommended if you are using catalog no. 18095-014. Ordering information is provided on page vii.

Before Starting

The following materials are supplied by the user:

- Vortex mixer
- Microcentrifuge

The following materials are supplied in the kit:

- PureLink™ Spin Columns
 - Binding Buffer B2 (prepared with isopropanol as described on page vi)
 - Wash Buffer W1 (prepared with ethanol as described on page vi)
 - Elution Buffer E1
 - Amber collection tubes
-

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Purifying the Labeled Probes, continued

Purification Procedure

Follow the steps below using the Purification Module from catalog no. 18095-013 to purify the labeled DNA probes.

1. Add 200 μ l of Binding Buffer B2 (prepared with isopropanol as described on page vi) to each tube from Step 6, page 8, and vortex to mix.
2. Load the sample from Step 1 onto the PureLink™ Spin Column. Note that the column comes preinserted in the collection tube.
3. Centrifuge at $10,000 \times g$ for 1 minute. Discard the flow-through and place the column back in the tube.
4. Add 650 μ l of Wash Buffer W1 (prepared with ethanol as described on page vi) to the column.
5. Centrifuge at $10,000 \times g$ for 1 minute. Discard the flow-through from the collection tube, and place the column back in the tube.
6. Spin at maximum speed for an additional 2–3 minutes to remove any residual wash buffer. Discard the flow through.
7. Place the Spin Column in a new, sterile **amber** collection tube (supplied in the kit).
8. Add 55 μ l of Elution Buffer E1 to the center of column and incubate at room temperature for 1 minute.
9. Centrifuge at maximum speed for 2 minutes. The flow-through contains the purified labeled DNA probes. (Discard the column after use.)

Proceed to hybridization. To determine the efficiency of the labeling reaction, proceed to **Assessing the Efficiency of the Labeling Procedure** (page 11).

Preparing for Hybridization

After you have generated fluorescently labeled genomic DNA probes, you are ready to prepare your probes for hybridization. Follow the preparation and hybridization instructions for your specific microarrays. See page vii for coprecipitants and blocking reagents available from Invitrogen.

Appendix

Assessing the Efficiency of the Labeling Procedure

Introduction

You can use UV/visible spectroscopy scanning to measure the amount of labeled DNA and dye incorporation. The expected amounts using the Control DNA provided in the kit are shown on the next page.

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 10, to a clean cuvette. Use an appropriate volume for your spectrophotometer. Blank the spectrophotometer using 10 mM Tris-HCl, pH 8.5.

2. Scan at 240–800 nm. Wash each cuvette thoroughly between samples.

Note: The labeled DNA must be purified as described on page 10 before scanning, as any unincorporated labeled nucleotides will interfere with the detection of labeled DNA.

3. Calculate the amount of DNA using the following formula:

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

4. Calculate the amount of fluorescent dye using the following formulas:

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

$$\text{Alexa Fluor}^{\text{®}} 647 \text{ (pmole)} = (A_{650} - A_{750}) / 0.24 \times \text{volume in } \mu\text{l}$$

5. Calculate the base-to-dye ratio using the following formulas:

$$\text{Base/dye ratio for Alexa Fluor}^{\text{®}} 555 =$$

$$\{(A_{260} - A_{320}) - [(A_{555} - A_{650}) \times 0.04]\} \times 150,000 / (A_{555} - A_{650}) \times 6,600$$

$$\text{Base/dye ratio for Alexa Fluor}^{\text{®}} 647 =$$

$$\{(A_{260} - A_{320}) - [(A_{650} - A_{750}) \times 0]\} \times 239,000 / (A_{650} - A_{750}) \times 6,600$$

The number of dye molecules per 100 bases is calculated using the formula:

$$100 / (\text{base/dye ratio})$$

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Assessing the Efficiency of the Labeling Procedure, continued

Expected Amounts Using Control DNA

If you prepare a control reaction using 1 µg of Control DNA (Salmon Sperm) as starting material, use the following table to calculate the DNA yield versus the number of dye molecules per 100 bases (DOL).

Squares in the table labeled P indicate an acceptable level of DNA yield versus DOL. Squares labeled F indicate a problem with the control reaction; see **Troubleshooting** on page 13.

	DOL							
yield (ug)	0.8	0.7	0.6	0.5	0.4	0.3	0.2	0.1
13	P	P	P	P	P	P	P	F
12	P	P	P	P	P	P	P	F
11	P	P	P	P	P	P	F	F
10	P	P	P	P	P	P	F	F
9	P	P	P	P	P	P	F	F
8	P	P	P	P	P	F	F	F
7	P	P	P	P	P	F	F	F
6	P	P	P	P	F	F	F	F
5	P	P	P	P	F	F	F	F
4	P	P	F	F	F	F	F	F
3	F	F	F	F	F	F	F	F
2	F	F	F	F	F	F	F	F

Troubleshooting

Problem	Cause	Solution
Yield of labeled DNA from the control reaction is low	DNA has been lost in the optional DNA digestion and purification step	For small amounts of starting material (< 500 ng), skip the digestion and purification step on pages 5–6 and proceed directly to labeling. Alternatively, run a small sample of DNA on an agarose gel to ensure recovery. Repeat the purification procedure, following all steps without modifications.
	DNA has been lost in the purification step after labeling	Verify that isopropanol and ethanol were added to the appropriate buffers as specified on page vi. 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 concentration of starting DNA. For small amounts of starting material (< 500 ng), skip the digestion step and proceed directly to labeling.
	Starting DNA has been improperly digested	After digestion, run a small sample of DNA on an agarose gel. It should produce a homogenous smear from 20 kb to approximately 600 bp.
Cannot detect labeled probes	DNA has been lost in the optional DNA digestion and purification step	Run a small sample of DNA on an agarose gel to ensure recovery. Repeat the purification procedure, following all steps without modifications.
	Wrong components used in the labeling reaction	Check all reagents added during the labeling reaction.
	DNA has been lost in the purification step after labeling	Verify that isopropanol and ethanol were added to the appropriate buffers as specified on page vi. 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 concentration of starting DNA
	Starting DNA has been improperly digested	Run a small sample of DNA on an agarose gel. It should produce a homogenous smear from 20 kb to approximately 600 bp.
	Reaction tubes have been exposed to light	Avoid direct exposure of the reaction tubes to light. Use amber-tinted reaction tubes for labeled DNA as indicated in the procedures. Repeat the labeling procedure.
	Fluorescent nucleotides have been exposed to light	Repeat 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. Verify that isopropanol and ethanol were added to the appropriate buffers as specified on page vi.

Technical Service

World Wide Web



Visit the Invitrogen Web Resource using your World Wide Web browser. At the site, you can:

- Get the scoop on our hot new products and special product offers
- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your Web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

<http://www.invitrogen.com>

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

Contact Us

For more information or technical assistance, call, write, fax, or email. Additional international offices are listed on our Web page (www.invitrogen.com).

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MSDS Requests

To request an MSDS, visit our Web site at www.invitrogen.com. On the home page, go to 'Technical Resources', select 'MSDS', and follow instructions on the page.

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Technical Service, continued

Limited Warranty

Invitrogen is committed to providing our customers with high-quality goods and services. Our goal is to ensure that every customer is 100% satisfied with our products and our service. If you should have any questions or concerns about an Invitrogen product or service, contact our Technical Service Representatives.

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