References:

1. Van Gelder, R.N. *et al.* (1990) *Proc Natl Acad Sci USA* 87: 1663-1667
2. Cox and Singer (2004) *Biotechniques* 36 (1): 114
3. Cox, *et al.* (2004) *Analytical Biochemistry* 331(2): 243-254
4. Ståhlberg *et al.* (2004) *Clinical Chemistry* 50, No. 9, 1678-1680

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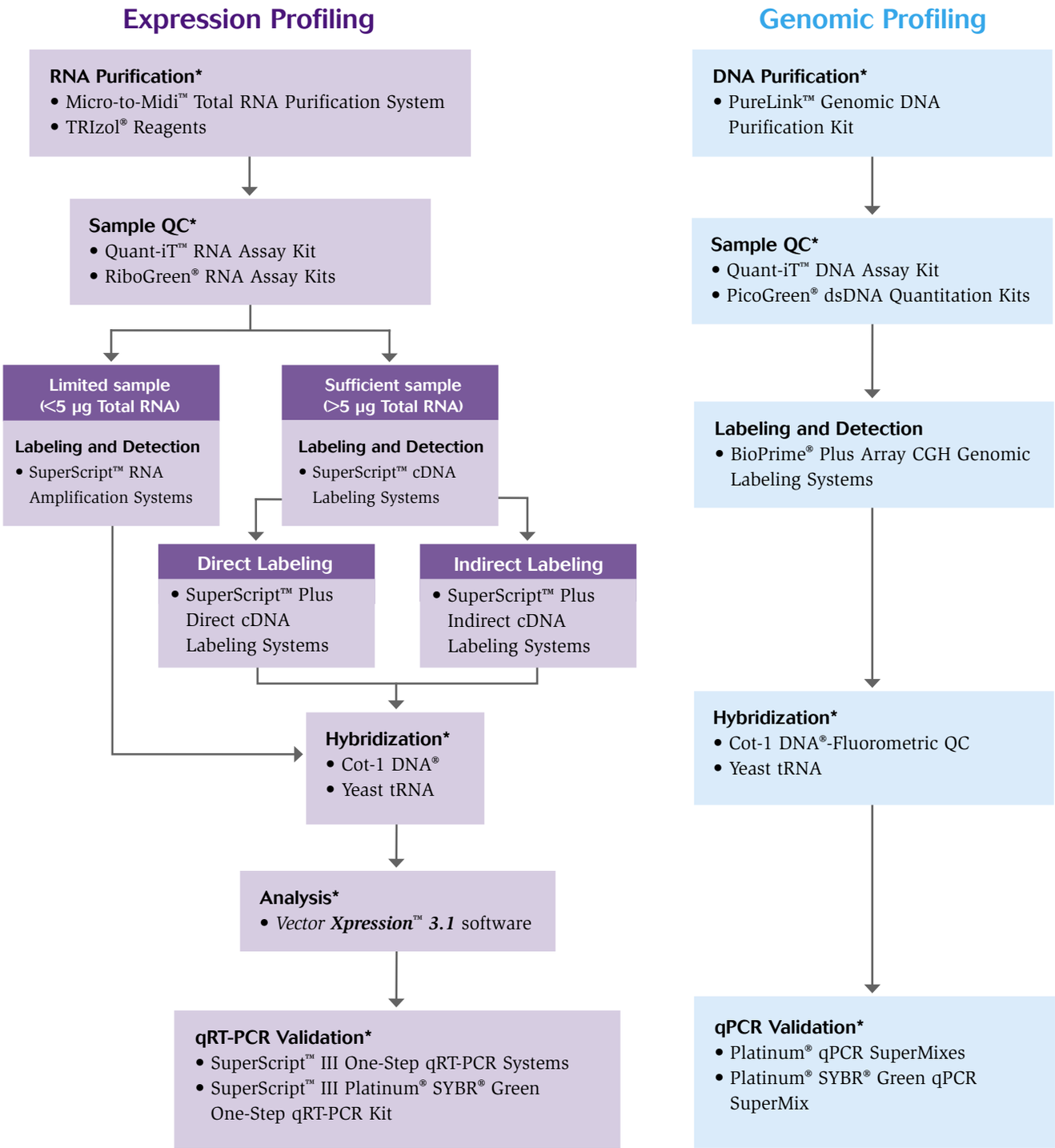
Comprehensive solutions for microarray analysis



* For more information regarding these products, please visit: www.invitrogen.com/microarray.

invitrogen™

Microarray target labeling you can trust

Whether you're performing expression or genomic profiling experiments, starting with 20 µg or 100 ng of RNA or genomic DNA, Invitrogen's robust labeling systems and detection reagents will give you superior sensitivity, flexibility, and reliability in your microarray experiments. Invitrogen array labeling systems now combine the trusted enzymatic labeling you expect from Invitrogen with improved dyes from Molecular Probes, providing a complete labeling solution.

Expression Profiling

- Improved correlation and more positive features using SuperScript™ Plus Direct and Indirect Systems with novel Alexa Fluor® Dyes
- Increased sensitivity attained with improved gene representation and consistent ≥ 1,000 fold amplification using the SuperScript™ RNA Amplification Systems

Genomic Profiling

- Improved signal-to-background and superior correlation using BioPrime® Plus Array CGH Genomic Labeling Systems with Alexa Fluor® Dyes
- Increased specificity of genomic hybridizations with Cot-1 DNA®

Table of Contents

Robust direct cDNA labeling with novel Alexa Fluor® AHA Dyes

By employing a high concentration (800 units) of SuperScript™ III RT per labeling reaction, the SuperScript™ Direct cDNA Labeling System delivers robust yields and more full-length cDNA than other direct labeling systems, providing you with sensitive and reproducible cDNA labeling.

The SuperScript™ Plus Direct cDNA Labeling System combines the performance of the SuperScript™ Direct cDNA Labeling System with the novel Alexa Fluor® AHA nucleotides, generating highly fluorescently labeled cDNA that accurately represents your initial mRNA sample.

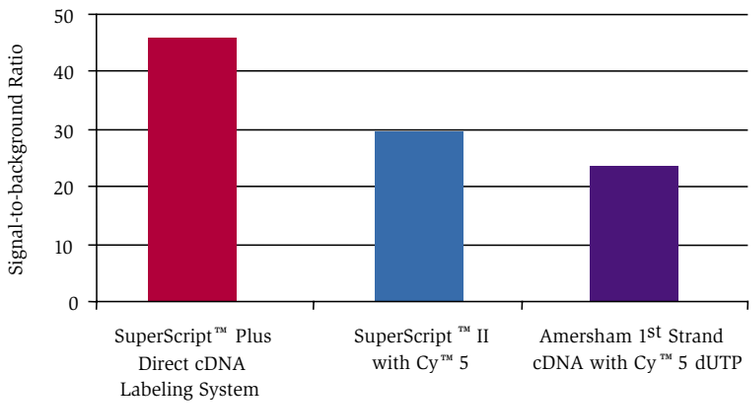
The SuperScript™ Direct cDNA Labeling Systems and the SuperScript™ Plus Direct cDNA Labeling Systems provide:

- Robust cDNA yields without an additional enzyme spike
- Higher signal-to-background ratios than protocols using SuperScript™ II and CyDyes™ or any other commercial kit (Figure 1)
- Superior correlation (R^2) than protocols using SuperScript™ II with CyDyes™ or any other commercial kit, improving data quality (Figure 2)

Superior sensitivity with the SuperScript™ Plus Direct cDNA Labeling System

Most commercially available reverse transcriptases struggle with incorporating fluorescently modified nucleotides, leading to suboptimal cDNA yields and variable signal-to-background ratios. The SuperScript™ Plus Direct cDNA Labeling System overcomes this limitation, incorporating an optimal amount of the Alexa Fluor® AHA nucleotides, resulting in higher signal-to-background ratios and ultimately more array positives (Figure 1).

Figure 1 — Improved sensitivity with higher signal-to-background ratios



Twenty micrograms total RNA was labeled with either the SuperScript™ Plus Direct cDNA Labeling System, SuperScript™ II RT with CyDyes™ or Amersham's 1st Strand cDNA Labeling Kit with Cy™ 5 dUTP (n = 3) and hybridized to MWG Human 40K arrays. The SuperScript™ Plus Direct cDNA Labeling System exhibits higher signal-to-background ratios than the competitors.

Robust direct cDNA labeling, continued

Figure 2 — Reduce false positives and improve your data quality with superior correlation

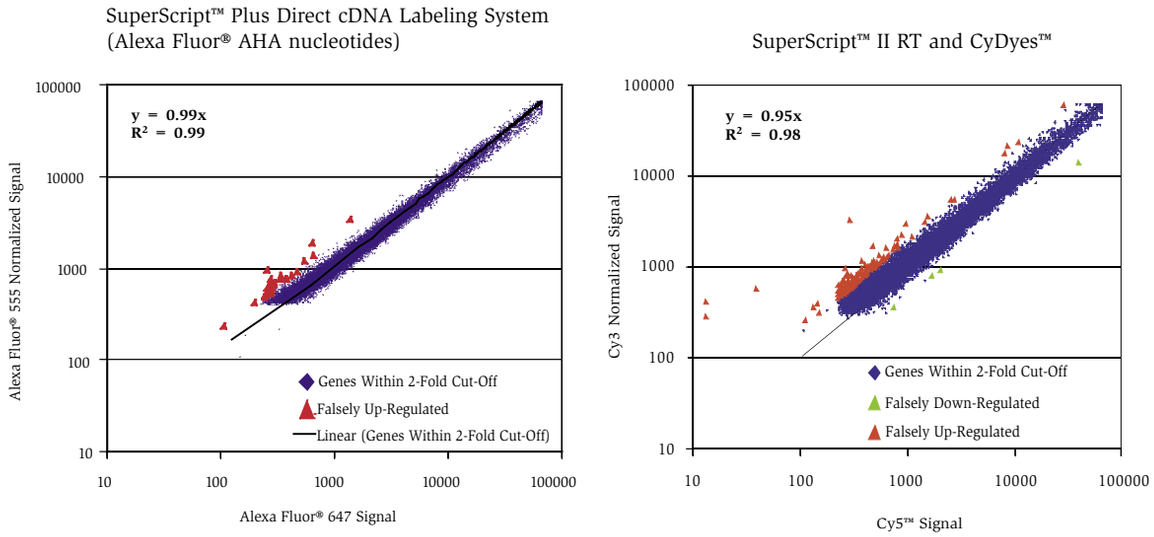


Figure 2 illustrates the improved accuracy resulting from superior correlation coefficients obtained using the SuperScript™ Plus Direct cDNA Labeling System, containing Alexa Fluor® AHA 555 and 647 modified dUTP when compared to protocols using SuperScript™ II RT and CyDyes™ together. Twenty micrograms of total RNA was used in a homotypic (self-self) hybridization to MWG Human 40K arrays.

Product	Quantity	Cat. no.
SuperScript™ Plus Direct cDNA Labeling System with Alexa Fluor® AHA dUTP	10 rxns 30 rxns	L1015-05 L1015-06
SuperScript™ Plus Direct cDNA Labeling Module with Alexa Fluor® AHA dUTP *(without purification columns)*	30 rxns	L1015-04
SuperScript™ Direct cDNA Labeling System *(without dyes)*	10 rxns 30 rxns	L1015-01 L1015-02
SuperScript™ Direct cDNA Labeling Module (*without dyes and purification columns*)	30 rxns	L1015-03

Optimized indirect cDNA labeling with Alexa Fluor® esters

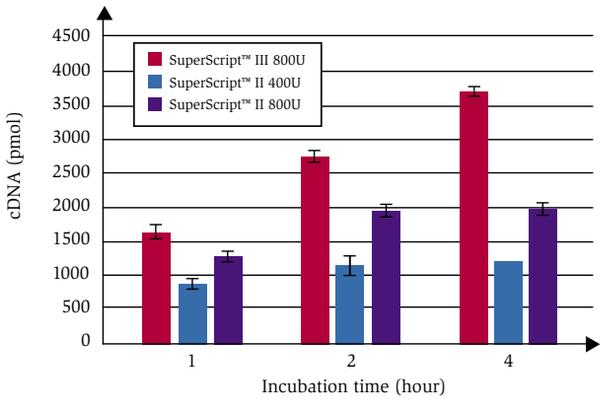
Like the SuperScript™ Direct cDNA Labeling Systems, the SuperScript™ Indirect cDNA Labeling Systems use 800 units of SuperScript™ III RT to ensure high yields of cDNA, but also contain a unique amino-allyl/ aminohexyl nucleotide mixture that increases signal-to-background ratios.

The SuperScript™ Plus Indirect cDNA Labeling Systems additionally include Alexa Fluor® 555 and 647 succinimidyl esters, providing everything needed for sensitive and convenient indirect cDNA labeling in an optimized format.

The SuperScript™ Indirect cDNA Labeling Systems and the SuperScript™ Plus Indirect cDNA Labeling Systems give you:

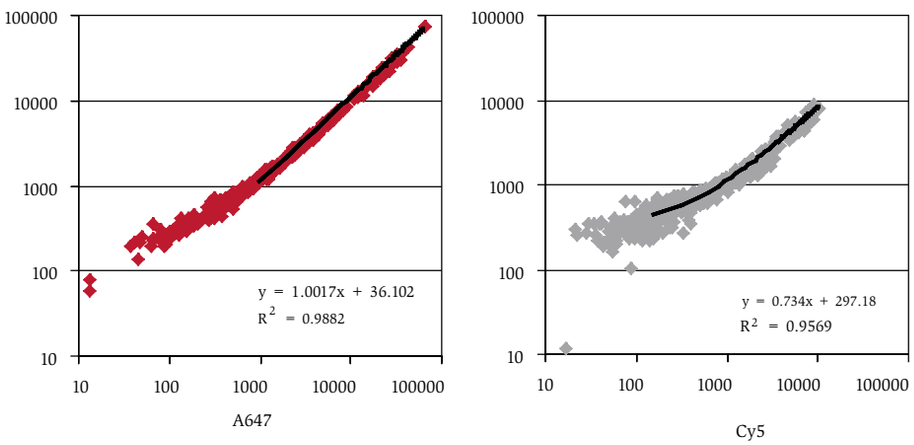
- Higher cDNA yields with SuperScript™ III RT (Figure 3)
- Increased signal-to-background ratios and more uniform labeling using a proprietary amino-allyl/aminohexyl (AA/AH) nucleotide mixture
- Superior accuracy with improved correlation coefficients (Figure 4)

Figure 3 — SuperScript™ III RT provides higher cDNA yields than SuperScript™ II RT, improving sensitivity



cDNA was synthesized with different amounts of SuperScript™ III and SuperScript™ II RTs. The reaction mixtures included 1X first-strand buffer, 5 mM DTT, 10 µg of total RNA, 2 µg oligo(dT)$_{20}$, 40 U RNaseOUT™ Recombinant Ribonuclease Inhibitor, 0.5 mM dNTP with amino-allyl and aminohexyl replacement, and 1 µCi [-^{32}P] dCTP. Five microliters of the reaction were removed and TCA-precipitated.

Figure 4 — Improve the accuracy of experimental data with superior correlation



Scatter plots display the raw data from self-self hybridizations to MWG starter arrays. Samples were prepared from 10 µg of placenta total RNA using the SuperScript™ Plus Indirect cDNA Labeling System with Alexa Fluor® NHS esters or a common protocol utilizing SuperScript™ II with Cy™ 3 & Cy™ 5. The R^2 value was calculated from the linear trend line and a section of the image is shown to illustrate the relative signal generation.

Product	Quantity	Cat. no.
SuperScript™ Plus Indirect cDNA Labeling System with Alexa Fluor® NHS esters	10 rxns	L1014-05
	30 rxns	L1014-06
SuperScript™ Plus Indirect cDNA Labeling Module with Alexa Fluor® NHS esters *(without purification columns)*	30 rxns	L1014-04
SuperScript™ Indirect cDNA Labeling System *(without dyes)*	10 rxns	L1014-01
	30 rxns	L1014-02
SuperScript™ Indirect cDNA Labeling Module (*without dyes and purification columns*)	30 rxns	L1014-03
Alexa Fluor® 555 and Alexa Fluor® 647 reactive dye decapacks	10 rxn each	A32755

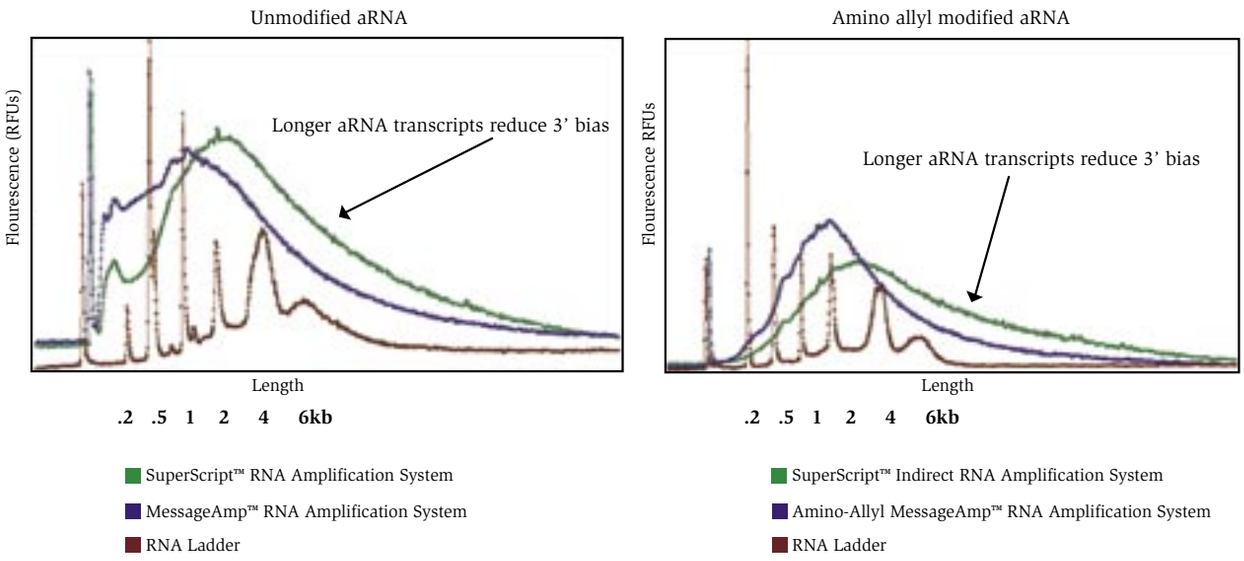
Improved RNA amplification

The SuperScript™ RNA Amplification System and the SuperScript™ Indirect RNA Amplification System are based on the proven linear RNA amplification method developed by Van Gelder *et al.* (1) that enables you to successfully perform expression profiling experiments with as little as 100 ng of starting total RNA. The SuperScript™ RNA Amplification System is an open system that can be used to produce unmodified aRNA or can be used to incorporate a modified nucleotide of your choice.

The SuperScript™ Indirect RNA Amplification System contains an amine modified *in vitro* transcription module, enabling you to generate amine modified aRNA for subsequent coupling to Alexa Fluor® NHS esters or other commercially available esters. By combining the highest quality double-stranded cDNA synthesis reagents, built around SuperScript™ III Reverse Transcriptase, with an optimized *in vitro* transcription module, the SuperScript™ RNA Amplification Systems are the best performing RNA amplification systems available. You can expect:

- Consistent, ≥1000-fold amplification of mRNA
- Improved gene representation with longer aRNA transcripts (Figure 5)
- More accurate gene expression profiling (Figure 6)

Figure 5 — SuperScript™ RNA Amplification Systems provide longer RNA transcripts, improving gene representation



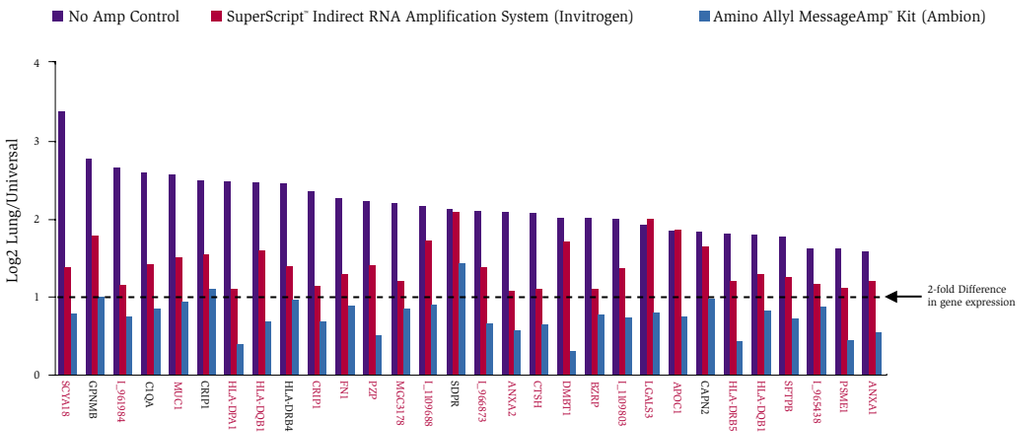
Electropherograms demonstrating the larger size for unmodified aRNA products and amino allyl-modified products generated by the SuperScript™ RNA Amplification System and SuperScript™ Indirect RNA Amplification System (Invitrogen) compared to the MessageAmp™ aRNA Kit and Amino Allyl MessageAmp™ aRNA Kit (Ambion). Samples were analyzed using the Agilent 2100 Bioanalyzer with the NanoRNA 6000 chips.

SuperScript™ III RT is key

High-quality double-stranded cDNA is vital to preserving differential gene expression ratios. SuperScript™ III RT, an improved version of SuperScript™ II RT, exhibits increased thermal stability and a longer half-life than most other commercially available reverse transcriptases. The SuperScript™ RNA Amplification Systems uses an optimal concentration (400 units) of SuperScript™ III RT that increases the length of your aRNA transcripts, improving gene representation in your microarray experiments (Figure 5). Figure 6 (page 7) illustrates how the SuperScript™ Indirect RNA Amplification Systems more accurately preserve the ratios of differential expression compared to unamplified material than does Ambion's Amino Allyl MessageAmp™ aRNA kit, improving data quality.

Improved RNA amplification, continued

Figure 6 – Detect more differentially expressed genes with the SuperScript™ Indirect RNA Amplification System



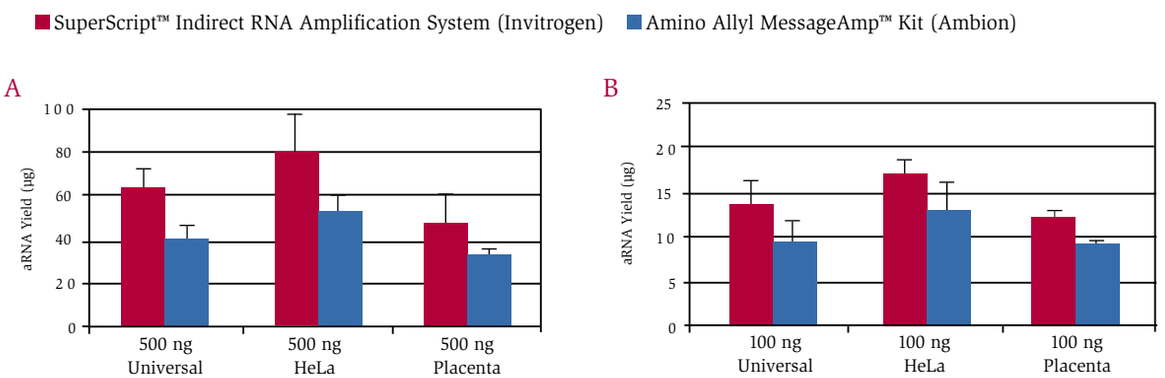
Fluorescently conjugated amino-allyl aRNA was synthesized from 500 ng of Human Lung and Universal Reference total RNA using the SuperScript™ Indirect RNA Amplification System from Invitrogen and the Amino Allyl MessageAmp™ Kit from Ambion according to manufacturer's recommendations. Labeled aRNA was hybridized to Agilent Human 1A Oligo Arrays in duplicate.

Gene names highlighted in red on the x axis represent those where a difference in gene expression would go undetected using Ambion's Amino Allyl MessageAmp™ Kit (based on 2-fold difference threshold).

Reproducible performance

Each step of the SuperScript™ RNA Amplification System and the SuperScript™ Indirect RNA Amplification System is optimized to reproducibly amplify the mRNA in your sample greater than 1000-fold (Figure 7). The systems include all the reagents necessary for first and second-strand cDNA synthesis, cDNA purification, *in vitro* transcription of amine-modified aRNA or unmodified aRNA, and aRNA purification.

Figure 7 – Improved amplification using the SuperScript™ Indirect RNA Amplification System



aRNA was generated from 500 ng (A) and 100 ng (B) of total RNA from Universal Reference RNA, HeLa, and human placenta with a single round of amplification using the SuperScript™ Indirect RNA Amplification System (Invitrogen) and the Amino Allyl MessageAmp™ Kit (Ambion). A_{260} absorbance readings were used to calculate the yield of total aRNA in micrograms and validated using quantitative RT-PCR and analysis on the Agilent 2100 Bioanalyzer (not shown).

Product	Quantity	Cat. no.
SuperScript™ RNA Amplification System	20 rxns	L1016-01
SuperScript™ Indirect RNA Amplification System	20 rxns	L1016-02
Alexa Fluor® 555 and Alexa Fluor® 647 reactive dye decapacks	10 rxn each	A32755

Efficient Genomic DNA Labeling

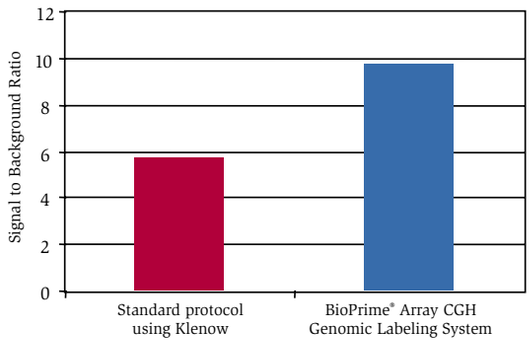
BioPrime® Array CGH Genomic Labeling Systems are fast, efficient labeling kits that allow you to generate high yields of labeled genomic samples with strong signal intensities, improving the sensitivity of genomic microarray experiments such as array-based Comparative Genomic Hybridization (aCGH).

The BioPrime® Plus Array CGH Genomic Labeling System contains all the components of the BioPrime® Array CGH Genomic Labeling System plus new Alexa Fluor® AHA modified nucleotides, providing a complete solution for your genomic labeling needs.

The BioPrime® Array CGH Genomic Labeling System and the BioPrime® Plus Array CGH Genomic Labeling System provide you:

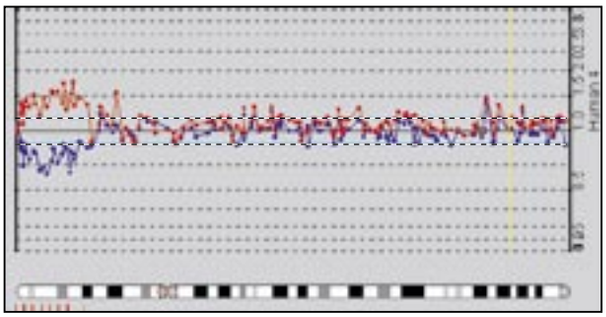
- More effective fluorescent incorporation for stronger signal intensities and greater sensitivity (Figure 8)
- Higher yields of labeled sample
- Faster results with a protocol that requires less than three hours to complete
- Improved detection of variations of gene copy number (Figure 9)

Figure 8 — Higher signal-to-background ratios with BioPrime® Array CGH Genomic Labeling System



Comparison of signal-to-background ratios of hybridizations to Spectral Genomics BAC arrays using 1 µg of genomic DNA labeled using current methods with standard Klenow compared to the BioPrime® Array CGH Genomic Labeling System.

Figure 9 — Reproducible detection of gene copy number variation using the BioPrime® Plus Array CGH Genomic Labeling System



The BioPrime® Plus Array CGH Genomic Labeling System containing the new Alexa Fluor® AHA dCTP was used to label 400 ng of genomic DNA from a Wolf-Hirschhorn and normal cell line. Gene ratios along human chromosome 4 are shown for dye-swapped arrays. A known deletion in the p arm of chromosome 4 is shown.

Product	Quantity	Cat. no.
BioPrime® Plus Array CGH Genomic Labeling System with Alexa Fluor® AHA dCTP	30 rxns	18095-013
BioPrime® Plus Array CGH Genomic Labeling Module *(without purification columns)*	30 rxns	18095-014
BioPrime® Plus Array CGH Indirect Genomic Labeling System with Alexa Fluor® NHS esters	30 rxns	18096-011
BioPrime® Plus Array CGH Indirect Genomic Labeling Module with Alexa Fluor® NHS esters *(without purification columns)*	30 rxns	18096-012
BioPrime® Array CGH Genomic Labeling System *(without dyes)*	30 rxns	18095-011
BioPrime® Array CGH Genomic Labeling Module (*without dyes and purification columns*)	30 rxns	18095-012
Human Cot-1 DNA®-Fluorometric QC	1 mg	15279-101
Mouse Cot-1 DNA®	500 µg	18440-016
Yeast tRNA	25 mg	15401-011

Flexible array labeling product summary

Expression Profiling Labeling Systems	Core Labeling Reagents	Dye Conjugated Nucleotides/Esters	Purification Columns
Direct cDNA Labeling for Microarrays			
Superscript™ Plus Direct cDNA Labeling System	•	•	•
Superscript™ Plus Direct cDNA Labeling Module	•	•	
SuperScript™ Direct cDNA Labeling System	•		•
Superscript™ Direct cDNA Labeling Module	•		
Indirect cDNA Labeling for Microarrays			
SuperScript™ Plus Indirect cDNA Labeling System	•	•	•
SuperScript™ Plus Indirect cDNA Labeling Module	•	•	
SuperScript™ Indirect cDNA Labeling System	•		•
SuperScript™ Indirect cDNA Labeling Module	•		
RNA Amplification			
SuperScript™ RNA Amplification System	•		•
SuperScript™ Indirect RNA Amplification System	•		•

Genomic Profiling Labeling Systems	Core Labeling Reagents	Dye Conjugated Nucleotides/Esters	Purification Columns
Direct gDNA Labeling for Microarrays			
BioPrime® Plus Array CGH Genomic Labeling System	•	•	•
BioPrime® Plus Array CGH Genomic Labeling Module	•	•	
BioPrime® Array CGH Genomic Labeling System	•		•
BioPrime® Array CGH Genomic Labeling Module	•		
Indirect gDNA Labeling for Microarrays			
BioPrime® Plus Array CGH Indirect Genomic Labeling System	•	•	•
BioPrime® Plus Array CGH Indirect Genomic Labeling Module	•	•	

Hybridization reagents and accessories

Product	Application	Quantity	Cat. no.
Human Cot-1 DNA®	Placental DNA predominantly 50 to 300 bp in size enriched for repetitive DNA sequences, such as the *Alu* and *Kpn* family members; commonly used to block non-specific hybridization in microarray screening.	500 µg	15279-011
Human Cot-1 DNA® - Fluorometric QC	Quantifying Cot-1 DNA® by fluorometry provides more accurate concentration data of higher molecular weight products, making it beneficial to certain genomic-based array experiments, such as array CGH using BAC arrays. By choosing fluorometric QC, you'll be sure to suppress the prevalent repetitive elements during hybridization, improving your results.	1 mg	15279-101
Mouse Cot-1 DNA®	Mouse DNA predominantly 50 to 300 bp in size enriched for repetitive DNA sequences, such as the B1, B2, and L1 family members; commonly used to block non-specific hybridization in microarray screening.	500 µg	18440-016
Yeast tRNA	Use as a carrier in nucleic acid purification and precipitation procedures; supplied in lyophilized form.	25 mg	15401-011
Oligo(dT)$_{20}$ Primer	A string of 20 deoxythymidylic acid residues that hybridizes to the poly(A) tail of mRNA; useful for first-strand cDNA synthesis at temperatures ≥ 50°C; recommended for use with SuperScript™ III Reverse Transcriptase.	15 µg (50 rxns)	18418-020
Anchored Oligo(dT)$_{20}$ Primer	A primer mixture consisting of a string of 20 deoxythymidylic acid residues followed by dV (either dG, dA, or dC) and then by dN (dA, dT, dG, or dC); due to the variable end sequence, the primer is anchored to the 5´-end of the poly A tail of mRNA and prevents priming within the poly(A) tail; recommended for use in cDNA labeling protocols and in RT-PCR.	50 µg	12577-011

Related products

Product	Quantity	Cat. no.
Nucleic Acid Purification		
Micro-to-Midi™ Total RNA Purification System	50 rxns	12183-018
TRIzol® Reagent	100 ml	15596-026
PureLink™ Genomic DNA Purification Kit	50 rxns	K181001
Sample QC		
Quant-iT™ RNA Assay Kit	1000 assays	Q33140
RiboGreen® RNA Quantitation Kit	2000 assays	R11490
Quant-iT™ DNA Assay Kit	1000 assays	Q33130
PicoGreen® dsDNA Quantitation Kit	2000 assays	P7589
Data Analysis		
Vector ***Xpression***™ 3.1 Analysis Software	visit www.invitrogen.com/bioinformatics	
qPCR/qRT-PCR Validation		
Platinum® Quantitative PCR SuperMix-UDG	100 rxns	11730-017
Platinum® SYBR® Green qPCR SuperMix-UDG	100 rxns	11733-038
SuperScript™ III Platinum® One-Step qRT-PCR Kit	100 rxns	11732-020
SuperScript™ III Platinum® SYBR® Green One-Step qRT-PCR Kit	100 rxns	11736-051
SAGE™		
I-SAGE™ Long Kit	5 libraries	T5000-03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