

ChipShot™ Systems

Features

- **Conserve Sample:** Optimized for 5µg of total RNA or 1.5µg poly(A)+ mRNA per labeling reaction.
- **Minimize Background:** Background reduction step decreases array background autofluorescence.
- **Get Reproducible Results:** Interslide CVs below 10%.
- **Simplify Your Process:** Easy-to-follow protocols.
- **Count On Technical Support:** Outstanding technical support for the researcher from Promega.

ChipShot™ Direct System

- **Increase Productivity:** Go from RNA to purified, labeled cDNA in approximately 3 hours.
- **Get Reliable Results:** Separate protocols for CyDye™3 and CyDye™5 labeling, so dye swap experiments are not necessary.

ChipShot™ Indirect System

- **Unbiased Dye Incorporation:** CyDye™3 and CyDye™5 incorporate into cDNA at a comparable rate.
- **Get Higher Yields:** Efficient incorporation of aminoallyl dNTP by reverse transcriptase yields more cDNA for labeling.

Achieve Better Yield with the ChipShot™ Membrane Clean-Up System

We synthesized 16 Cy[®]3-labeled cDNAs using the ChipShot™ Direct Labeling System. Following RNase treatment, we pooled the reactions and then redivided them into sixteen 40µl aliquots, eliminating any sample-to-sample variation before purification. Replicate samples were purified using the ChipShot™ Membrane Clean Up System as described in Technical Manual #TM286. Post-purification yields are compared to several alternate purification systems in Figure 1. Four replicate samples were purified with each method. The ChipShot™ Membrane System delivered higher yield than the competing systems tested.

ChipShot™ Labeling and Membrane Clean-Up System Specifications

ChipShot™ Labeling and Clean-Up Systems^(a) have been tested in combination to ensure the reproducible synthesis and clean-up of cDNA for maximal sensitivity and reproducibility of DNA microarrays.

Total RNA:

- Starting from 5µg of total RNA (Cat.# C199A), 1,200–2,400ng of Cy[®]-3-labeled cDNA was obtained. The frequency of incorporation (FOI) was 20–35 Cy[®]-3-labeled nucleotides per 1,000 nucleotides of cDNA.
- Starting from 5µg of total RNA (Cat.# C199A), 900–2,400ng of Cy[®]-5-labeled cDNA was obtained. The frequency of incorporation (FOI) was 12–25 Cy[®]-5-labeled nucleotides per 1,000 nucleotides of cDNA.

mRNA:

- Starting from 1.5µg of Mouse mRNA, 350–650ng of Cy[®]-3-labeled cDNA was obtained. The frequency of incorporation was 25–45 Cy[®]-3-labeled nucleotides per 1,000 nucleotides of cDNA.
- Starting from 1.5µg of Mouse mRNA, 325–650ng of Cy[®]-5-labeled cDNA was obtained. The frequency of incorporation was 15–35 Cy[®]-5-labeled nucleotides per 1,000 nucleotides of cDNA.

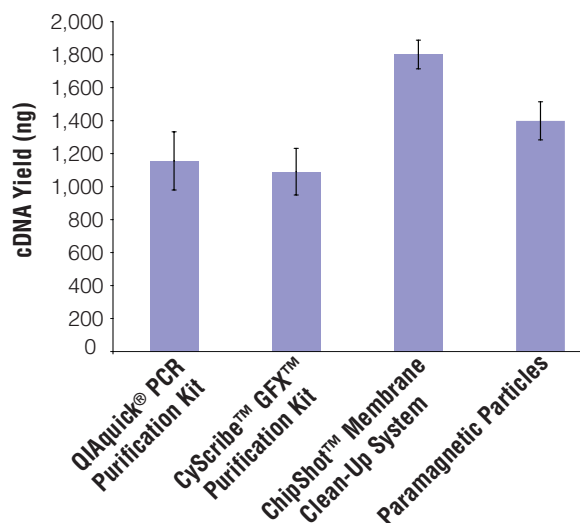


Figure 1. cDNA yield achieved using the ChipShot™ Membrane Clean-Up System compared to competing systems. Sixteen Cy[®]3-labeled cDNA reactions were synthesized using the ChipShot™ Direct Labeling System. Following RNase treatment, the reactions were pooled then redivided into sixteen 40µl aliquots, eliminating any sample-to-sample variation prior to purification. Replicate samples were purified with the ChipShot™ Membrane Cleanup System; post-purification yields are compared to several alternate purification systems (n = 4 for each method tested).

Table 1. Yield and Labeling Efficiency of Dye-Labeled cDNA Purified Using the ChipShot™ Membrane Clean-Up System.

A. Direct Labeling of cDNA from Total RNA.

| | cDNA yield (ng) | Dye Incorporated (pmol) | FOI |
|-------------------------------------|-----------------|-------------------------|--------|
| Cy³ | | | |
| ChipShot™ Membrane Clean Up (n = 8) | 2,128 ± 101 | 193 ± 10 | 29 ± 0 |
| Cy⁵ | | | |
| ChipShot™ Membrane Clean Up (n = 8) | 2,132 ± 88 | 140 ± 4 | 21 ± 0 |

B. Indirect Labeling of cDNA from Total RNA.

| | ng cDNA before dye conjugation | ng cDNA after dye conjugation | Dye Incorporated (pmol) | FOI |
|-------------------------------------|--------------------------------|-------------------------------|-------------------------|--------|
| Cy³ | | | | |
| ChipShot™ Membrane Clean Up (n = 6) | 3,633 ± 287 | 3,171 ± 213 | 174 ± 11 | 18 ± 2 |
| Cy⁵ | | | | |
| ChipShot™ Membrane Clean Up (n = 6) | 3,619 ± 204 | 3,206 ± 140 | 249 ± 14 | 25 ± 2 |

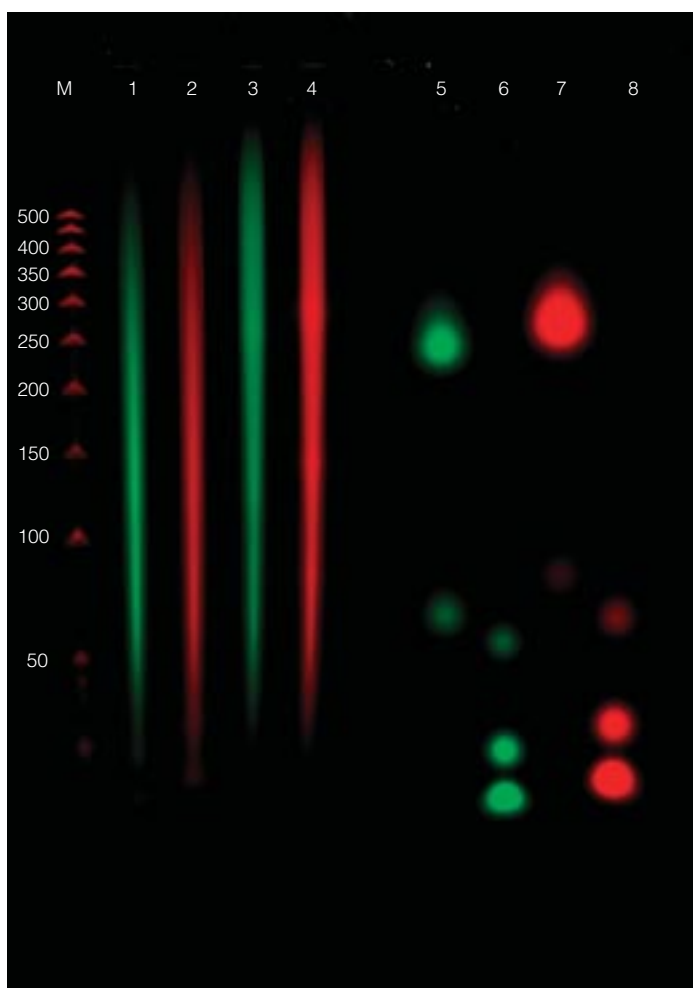


Figure 2. Highly pure cDNA produced using the ChipShot™ Systems. Fluorescently labeled cDNA was generated using the ChipShot™ Direct Labeling System (lanes 1 and 2) and the ChipShot™ Indirect Labeling System (lanes 3 and 4). The labeled cDNA samples were purified using the ChipShot™ Membrane Clean-Up System. Following purification, 1pmol of labeled cDNA was resolved on a 5% Long Ranger® denaturing acrylamide gel. Migration of 0.5pmol Cy-Dye™ NHS ester (lanes 5 and 7) and 0.5pmol Cy³-dCTP (lanes 6 and 8) are shown in comparison, demonstrating the high level of purity achieved when using the ChipShot™ Membrane Clean-Up System. Lane M, ALFexpress® Sizer™ 50-500 (Amersham Biosciences Cat.# 27-4539-01). The gel was scanned on a 9410 Typhoon® Imager (Amersham Biosciences).

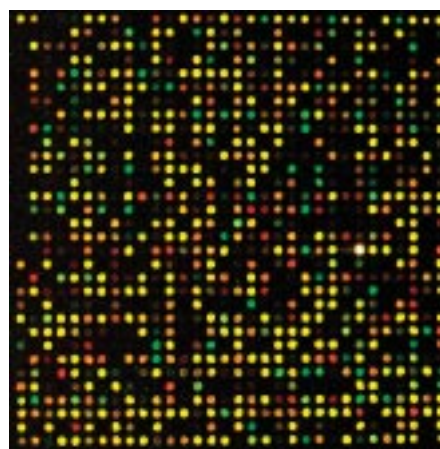


Figure 3. Array image following hybridization with Cy³-labeled cDNA purified using the ChipShot™ Membrane Clean Up System. Cy³-labeled cDNA from 293T mRNA and Cy⁵-labeled cDNA from HeLa mRNA was synthesized using the ChipShot™ Direct Labeling System and purified using the ChipShot™ Membrane Clean-Up System. The cDNA samples were hybridized to custom 4K arrays (provided by Corning, Inc.) using the Pronto!™ System (Corning, Inc.). Data were acquired using a Genepix® 4000B scanner (Axon Instruments, Inc.); a representative subgrid of the array is shown.

Ordering Information

| Product | Size | Cat.# |
|---|--------------|-------|
| ChipShot™ Indirect Labeling and Clean-Up System | 25 reactions | Z4000 |
| ChipShot™ Direct Labeling and Clean-Up System | 25 reactions | Z4100 |

Literature

| Protocol | Part.# |
|---|--------|
| ChipShot™ Indirect Labeling and Clean-Up System | TM287 |
| ChipShot™ Direct Labeling and Clean-Up System | TM286 |

Related Products

| Product | Size | Cat.# |
|--------------------------------------|---------------|-------|
| PureYield™ RNA Midiprep System | 10 preps | Z3740 |
| | 50 preps | Z3741 |
| PolyAtract® mRNA Isolation System II | 3 isolations | Z5200 |
| | 15 isolations | Z5300 |

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