

Novel Technology Permits Three Linear RNA Amplification Rounds and Yields Reproducible Microarray Data with Maintained Dynamics in Differential Expression Levels

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Introduction

Gene expression experiments with microarrays are a powerful tool, yielding information about thousands of mRNA levels. Without mRNA amplification, the requirement of large amounts of RNA (µg range) makes it impossible to study biopsies or low cell numbers from microdissections. Low reproducibility, loss in dynamics and limited amplification has hampered mRNA amplification with house made technologies or commercial kits.

Development of a New Technology

To overcome the common problems of mRNA amplification, we developed a novel technology which is called: *ExpressArt*TM mRNA amplification technology. The procedure is presented in Figure 1.

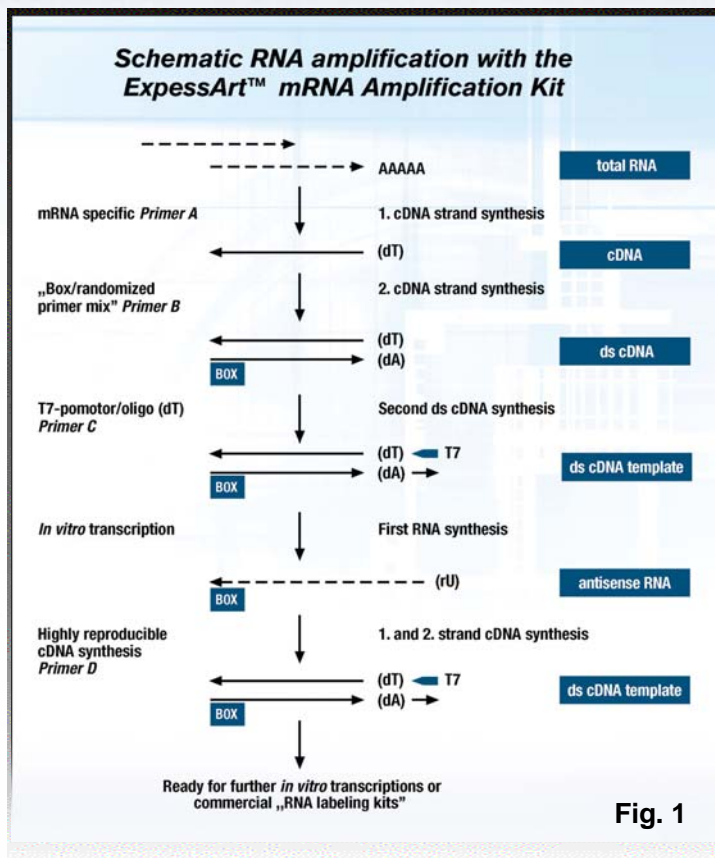


Fig. 1

*ExpressArt*TM mRNA Amplification

- *ExpressArt*TM mRNA amplification technology does not lead to any size reduction of amplified *in vitro* transcripts. Length of amplified RNA ranges from 0.2 to 3 kb (Fig. 2).
- Reproducibility after two and three rounds of amplification with the *ExpressArt*TM kit is demonstrated by data from human genome high-density microarrays (>10,000 signals on Affymetrix HG-U95A; Fig. 3).

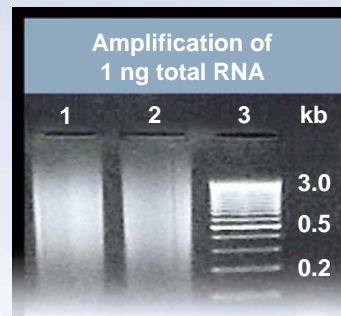


Fig. 2: Lane 1: 1 ng of total RNA amplified by 2 rounds of amplification, lane 2: 1 ng total RNA amplified by 3 rounds. RNA-content in both lanes: 1 µg. Lane 3: Ladder.

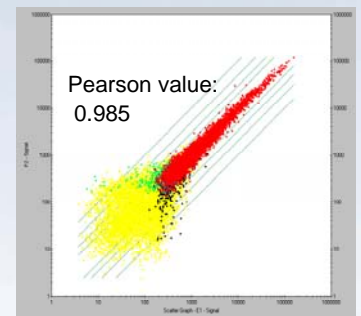


Fig. 3: High reproducibility of *ExpressArt*TM mRNA amplification. Two independent 2-round amplifications, hybridised on an Affymetrix HG-U95A chip.

- The *ExpressArt*TM mRNA amplification results in reliable identification of differentially expressed genes with faithful reproduction of quantitative differences (Fig. 4).

- This technology permits a third round of amplification, resulting in up to 100-millionfold amplification. This means, starting from 1 ng of total RNA (30-100 cells), multiple high-density microarray hybridisations can be performed.

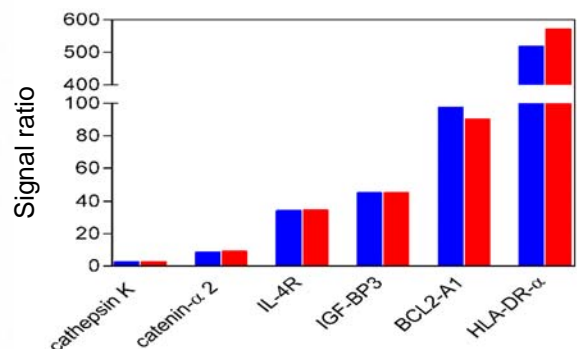


Fig. 4: High reproducibility in quantitative differences. Analysis of differential gene expression in Jurkat and H9 cell lines on Affymetrix chips. ■ Direct labeling (10 µg total RNA), ■ *ExpressArt*TM amplified mRNA (2-rounds with 100 µg total RNA).