

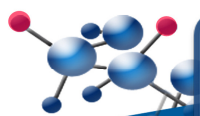
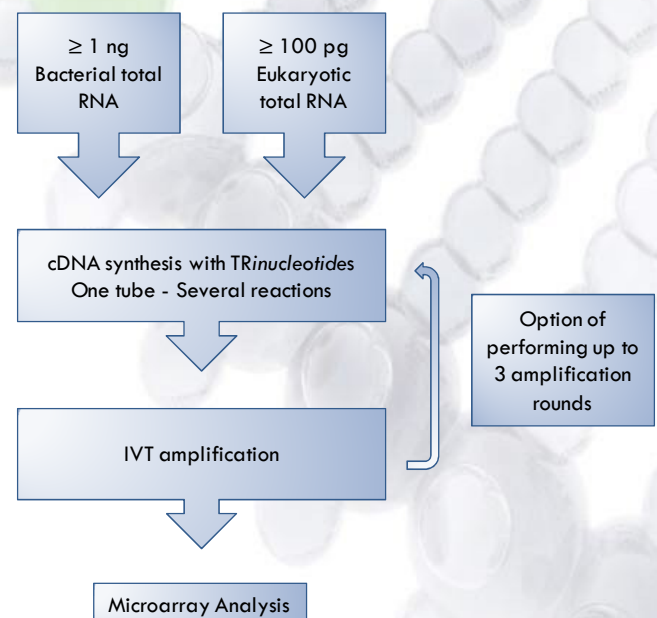
ExpressArt[®] mRNA Amplification Technology

C&E ExpressArt[®] amplification kits, add-on modules and reagents.
 ExpressArt[®] amplification technology provides simple protocols with unique advantages to overcome many of the current problems associated with other techniques for mRNA amplification.

ExpressArt[®] Features

- Amplifies high quality samples from degraded mRNA, including FFPE samples, without loss of sequence
- Amplifies from picograms of eukaryotic RNA or 1 nanogram of bacterial RNA
- Samples from two and three rounds of amplification are directly comparable
- Specifically amplifies all species of bacterial mRNA from total RNA without the need to remove rRNA
- Eliminates the need for RiboMinus steps in Exon and GENE microarray amplification protocols
- Eliminates primer derived artifacts
- Amplifies mRNA without pre-determining quantities

amsbio's ExpressArt[®] kits and reagents for high quality mRNA amplification based on a unique non-Eberwine method using *TRinucleotide primers*. ExpressArt[®] eliminates input RNA limitations with a range of micro, nano and pico kits for standard and specialized mRNA amplification needs. ExpressArt[®] *TRinucleotide* technology recovers all mRNA sequences allowing the use of severely degraded RNA samples without loss of sequence. In addition, *TRinucleotide* technology allows selective amplification of mRNA from any species of bacteria without the need to use MICROBExpress, and provides unique advantages for Exon and GENE microarray mRNA amplification. Together with aminoacyl labeling modules and exclusive reagents for enhancing RNA isolations from very small and FFPE samples, ExpressArt[®] provides solutions to problems no other amplification technology has overcome.



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Limitations of Standard mRNA Amplification Technology

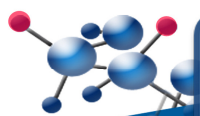
RNA amplification methods are commonly based on the Eberwine method, a linear, isothermal amplification strategy based on *in vitro* transcription with T7 RNA polymerase that has recognized technical limitations (Van Gelder *et al.* 1990; Eberwine *et al.* 1992). This technique relies on synthesizing double stranded cDNA from mRNA using a T7-promoter/oligo(dT) primer for first strand cDNA-synthesis and limited RNase H digestion for self-priming during second strand synthesis. To achieve amplification these double stranded DNAs are used as templates for *in vitro* transcription, resulting in linear amplification, maintaining the expression patterns of the original mRNAs (Poirier *et al.* 1997; Puskas *et al.* 2002).

Despite the common use of Eberwine based methods of mRNA amplification there remain a number of basic technical issues that it cannot resolve:

- Amplified RNA is 3'-biased since transcription and cDNA-synthesis with a T7-promoter/oligo(dT) primer start at the poly(A)-tail of the original mRNA.
- A second round of amplification is based on random priming, causing reduction of fragment length, which is even more pronounced when only small amounts of input RNA are available.
- The use of a T7-promoter/oligo(dT) primer in the first cDNA-synthesis can lead to large amounts of primer-derived high molecular weight artifacts that become increasingly dominant the smaller the starting amount of template (Baugh *et al.* 2001).
- Dependency on poly(A)-tail priming necessitates the use of high quality RNA samples only.

Because ExpressArt[®] is not based on the Eberwine method these technical issues can be eliminated and in addition ExpressArt[®] amplification technology offers unique advantages.

ExpressArt[®] allows amplification of high quality samples from as little as ~5 cells or 100 picograms total RNA with the option of performing up to 3 amplification rounds without compromising detection sensitivity or loss of sequence. ExpressArt[®] eliminates the need to calibrate samples and allows direct comparison of mRNA samples of different quantities and quality. ExpressArt[®] TRinucleotide technology recovers all mRNA sequences, including degraded samples, by priming selectively to the 3'-ends of mRNA independent of poly-(A) tails. Selection against rRNA eliminates the need for rRNA removal making it ideal for bacterial, exon and GENE microarray analysis.



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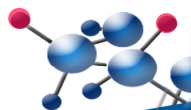
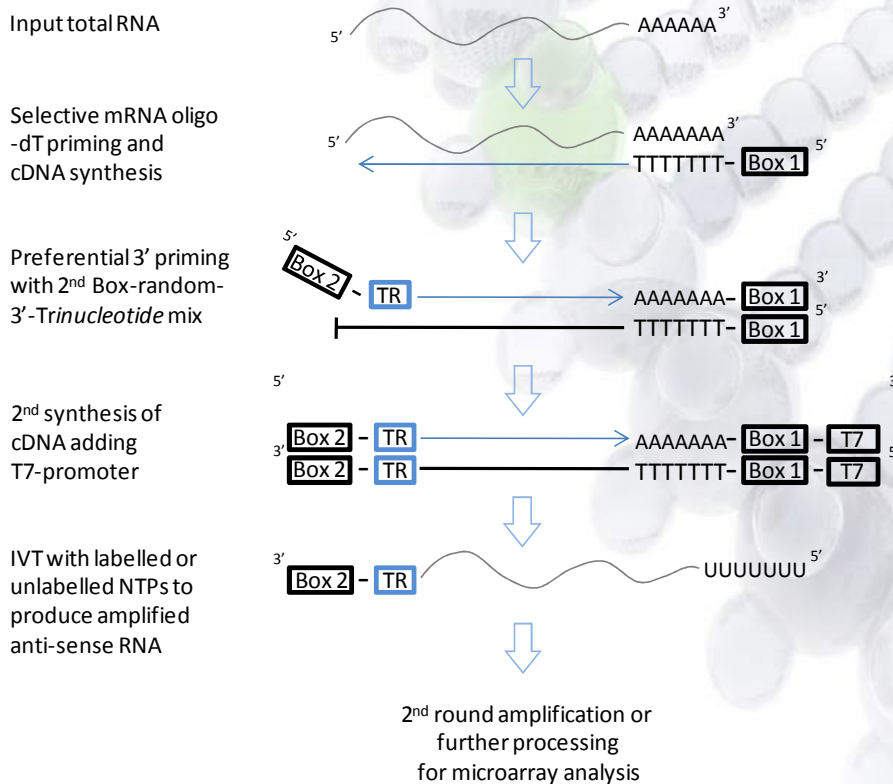
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ExpressArt[®] mRNA Amplification Technology

Using the standard ExpressArt[®] mRNA amplification kits, cDNA is synthesized from mRNA by an anchored oligo(dT)-primer, without a T7-promoter (Fig. 1). All RNAs (including mRNAs and rRNAs) are digested with a mixture of heat-labile RNases. To minimise 3'-bias, double stranded cDNA is generated using ExpressArt[®] *TRinucleotide* primer technology; a mixture of BOX-random-*TRinucleotide* primers that preferentially prime near the 3'-ends of all nucleic acid molecules. These 30-mer primers contain a unique 21-mer BOX sequence, followed by six random nucleotides, and a *TRinucleotide* sequence mix at the 3'-end. The 3' selectivity results in the generation of almost full-length double stranded cDNAs (Fig. 2 - 3). After denaturation, the second cDNA strand is primed in reverse orientation using a T7-promoter/oligo(dT) primer. This leads to double stranded cDNA with a functional T7-promotor at one end and the BOX sequence tag at the other end. This dsDNA product is used as template for *in vitro* transcription, generating amplified, antisense oriented cRNA with defined sequences at both ends. This is a major advantage for second and especially for third round amplifications, where size reductions of cRNAs are avoided and allows the comparison of samples that contain very divergent amounts of input RNA. For second and third amplification rounds, full-length cDNAs are obtained using this 21-mer BOX sequence as a primer, negating the need to use random primers that generate short mRNA fragments.



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Fig. 1 Standard ExpressArt[®] mRNA amplification technology

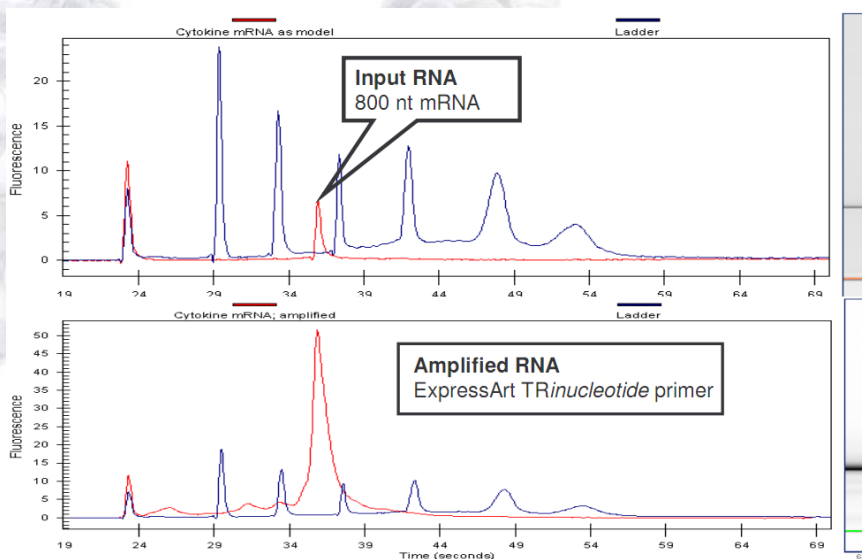


Fig. 2 Agilent Bioanalyzer profile demonstrating the preservation of sequence length using the standard ExpressArt[®] mRNA amplification technology on a single cytokine 800nt mRNA

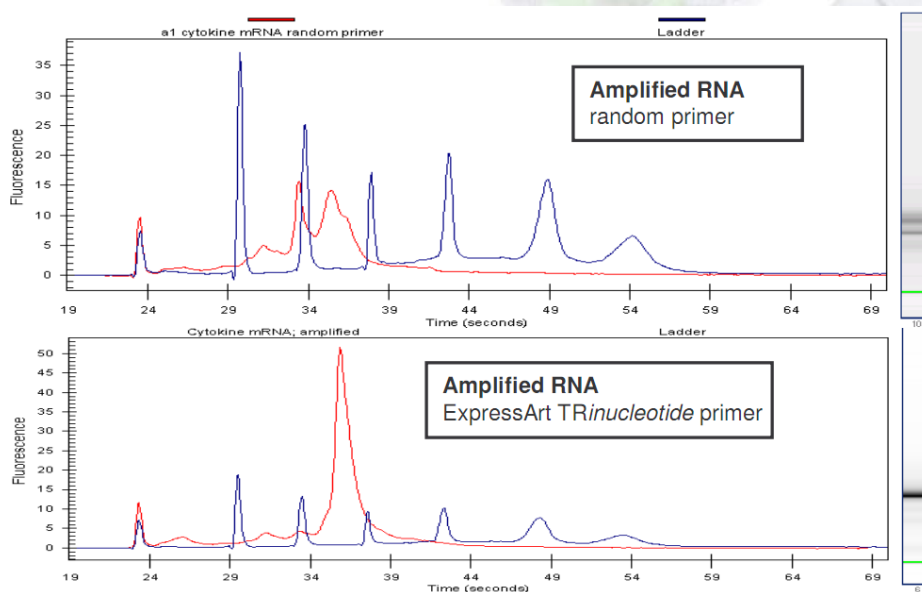
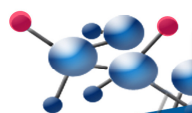


Fig. 3 Comparative Agilent Bioanalyzer profile comparing random priming with the efficiency of standard ExpressArt[®] mRNA amplification technology in preserving the sequence length of a single cytokine mRNA



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Maintain all mRNA Sequences and Eliminate Derived Artifacts Primer

Standard mRNA amplification technologies frequently produce large amounts of template-independent high molecular weight amplification artifacts that are a severe limitation in the amplification of very low amounts of input RNA. With ExpressArt® no-template-controls are free of any amplified background, even after two and three rounds of amplification. This enables the amplification of sub-nanogram amounts of input total RNA. In addition ExpressArt® eliminates continuous shortening of mRNA sequences using *TRinucleotide* priming rather than random primers (Fig. 4 and 5).

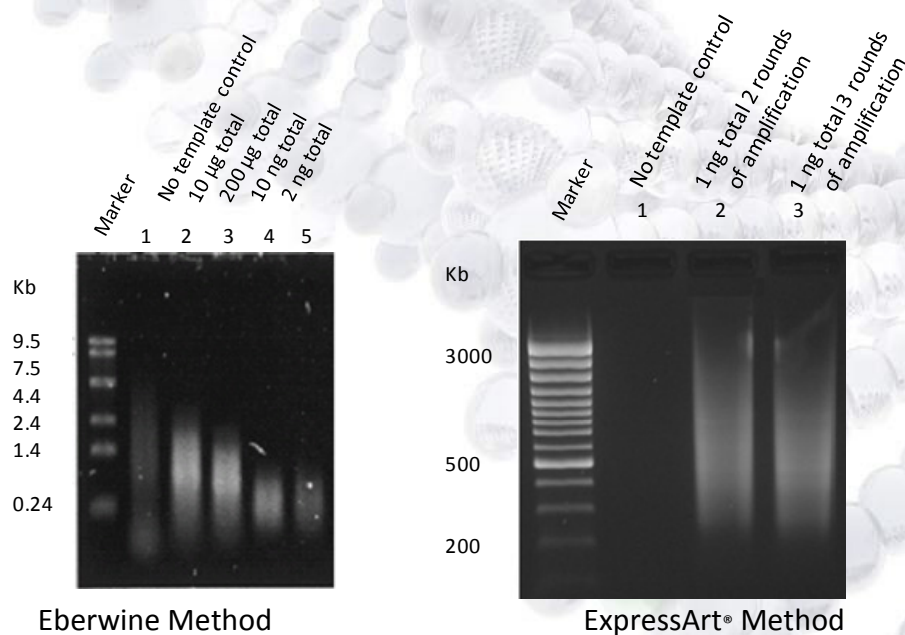


Fig. 4 ExpressArt® allows two and three rounds of amplification with minimal loss of sequence and no primer derived artifacts

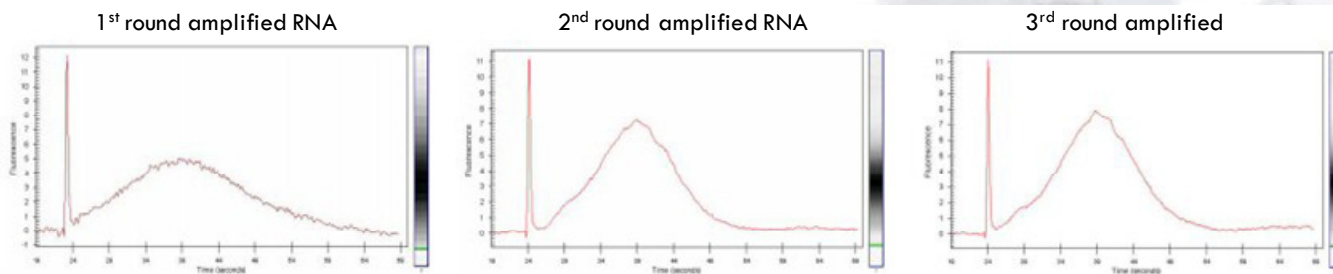


Fig. 5 Agilent Bioanalyzer profiles demonstrating conservation of mRNA sequence length through 1st, 2nd, and 3rd round amplification using ExpressArt®



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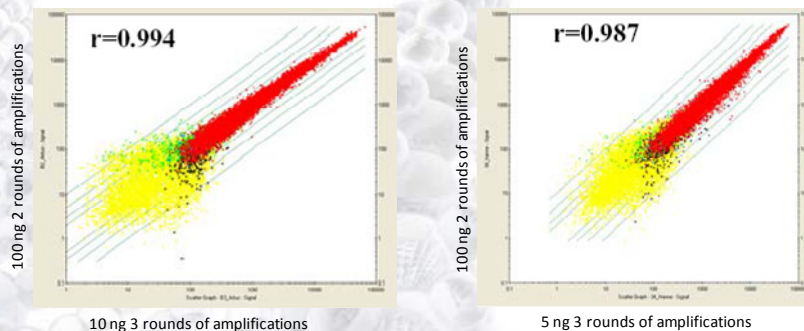


Fig. 6 Log-Log plots demonstrating the concordance of microarray data with two and three amplification rounds

ExpressArt[®] mRNA Amplification of Degraded mRNA

ExpressArt[®] eliminates restricting your research due to low quality RNA, all mRNA sequences can be recovered from large and small samples using samples previously unsuitable for standard RNA amplification and subsequent microarray analysis. Fig. 8-9 demonstrate that mRNA chemically degraded to an RNA Integrity Number (RIN) of 2.2 can still be used to a high quality cRNA suitable for microarrays analysis.

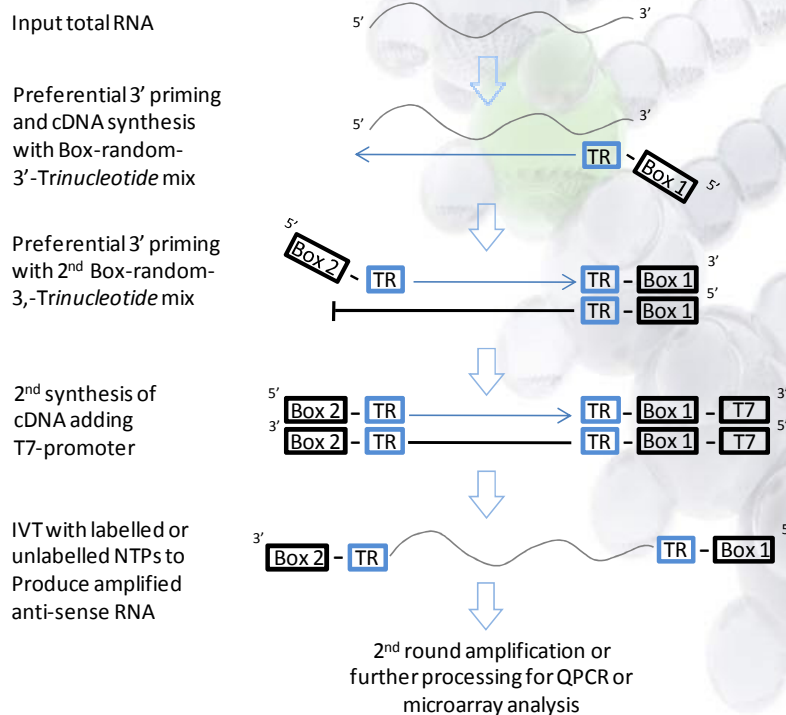
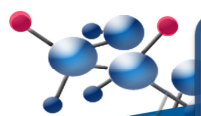


Fig. 7 ExpressArt[®] mRNA amplification technology for degraded and FFPE samples and for Exon as well as GENE microarray samples



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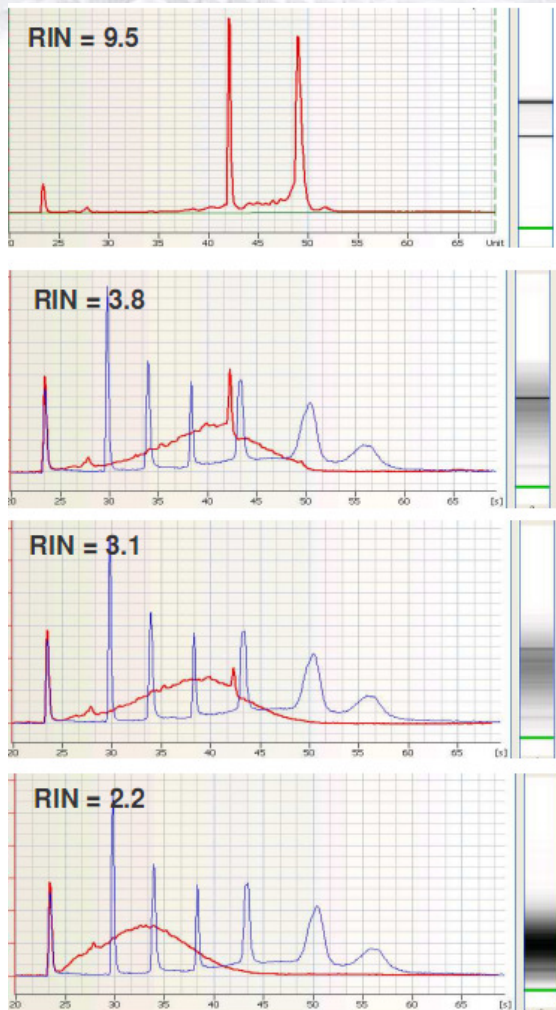
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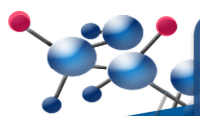
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Results of hybridization to the Affymetrix GeneChip HG-U133A

Presence Call	3' – 5' ratios		rRNA%
	GAP-DH	β-actin	
9,794 (:=100%)	3.2	1.6	1.8%
98%	2.8	1.1	2%
98%	2.0	0.9	1.9%
96%	2.5	0.9	1.8%

Fig. 8 Agilent Bioanalyser profile of high quality and degraded rRNA and their presence calls, 3'-5' ratios and rRNA %

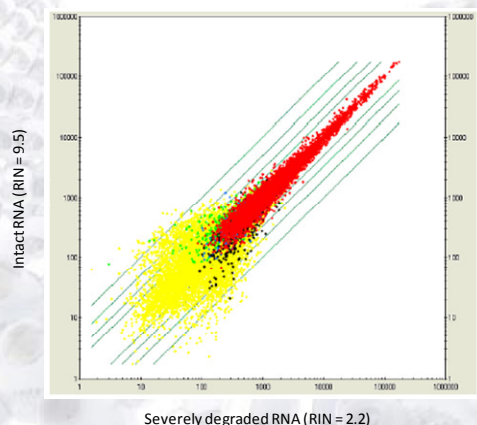


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Pearson value, $r = 0.993$

96% Relative Presence Calls

3'-5'-Ratios:
GAP-DH = 2.1
 β -Actin = 0.9

Less than 2% rRNAs in amplified

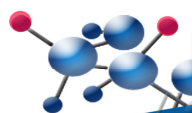
Fig. 9 Comparative hybridisation of intact and severely degraded mRNA on the Affymetrix Genechip HG-U133A

ExpressArt® Features for Amplifying mRNA for Exon and GENE Microarray Analysis

- Eliminates the need for RiboMinus steps in Exon and GENE microarray amplification protocols
- Allows the use of degraded RNA and maintains uniform representation of all mRNA sequences
- Amplifies sufficient cRNA for microarrays from as little as 1 nanogram total RNA
- Samples from two and three rounds of amplification are directly comparable
- Greatly reduced rRNA background and the elimination of primer derived artifacts

Method of cRNA synthesis	Starting Material Quantity and Quality	cRNA yields (μ g)	Sensitivity (% P)	Mean Signal vs Background	Replicates (Pearson values)
Standard Affymetrix	2.0 μ g/intact RIN = 9.8	21 \pm 5	51 \pm 1	280 vs 310 (0.9)	0.98
ExpressArt® TRinucleotide	50 ng/intact RIN = 9.8	2 rounds* 61 \pm 10	64 \pm 2	360 vs 21 (1.7)	0.98
ExpressArt® TRinucleotide	50 ng/degraded RIN = 3	2 rounds* 58 \pm 10	53 \pm 2	280 vs 200 (1.5)	0.96
ExpressArt® TRinucleotide	50 ng/severely degraded RIN = 2.3	2 rounds* 52 \pm 10	47 \pm 3	265 vs 250 (1.1)	0.95

Proof of Principle – John Arrand Cancer Research Institute, Birmingham, UK * Aliquots of ~500ng were used in 2nd round amplification



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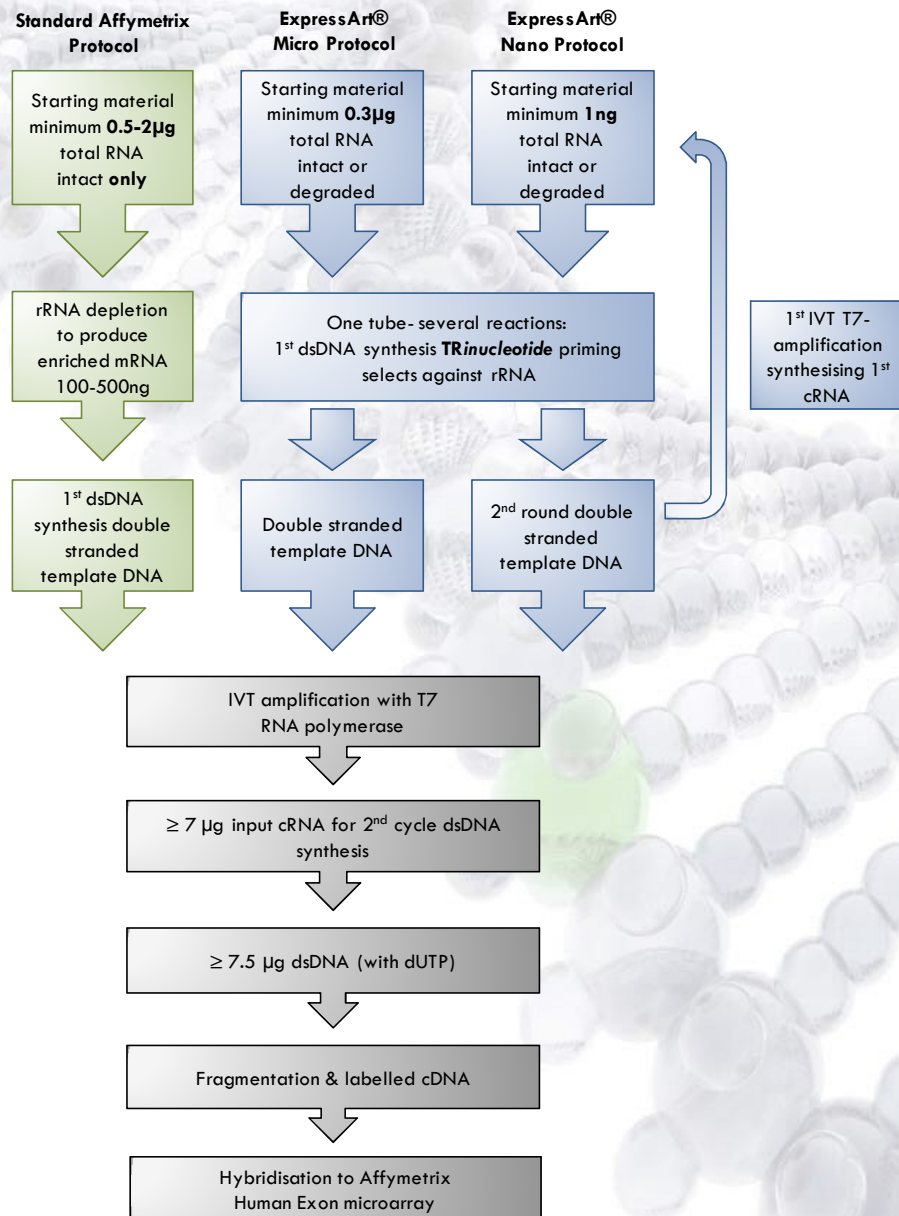


Fig. 10 Comparison of standard Affymetrix and ExpressArt® Exon microarray mRNA amplification protocols

ExpressArt® Amplification of mRNA from FFPE Samples for Microarray Analysis

Challenges of performing microarray analysis with FFPE samples

- Handling, storage and RNA isolation results in degraded total RNA not suitable for microarray analysis
- Cross-Linking of RNA-protein and RNA-RNA inhibits reverse transcription
- Chemical modification such as methyl-adenines and dimerized adenines limit 3'-poly(A) binding

ExpressArt® features for the new FFPE mRNA amplification kits and reagents

- Allows the use of degraded RNA and maintains uniform representation of all mRNA sequences
- Amplify sufficient aRNA for microarray analysis from as little as 1 nanogram of total RNA
- Provides DeCrossLinker reagents, universal nuclease and RNase inhibitor for improved RNA quality
- Produce aRNA from two and three rounds of amplification that are directly comparable
- Eliminates primer derived artifacts

ExpressArt® Bacterial mRNA Amplification Kits

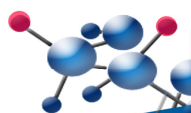
Challenges of Amplifying Bacterial mRNA

ExpressArt® bacterial mRNA amplification kits offer unrivaled advantages for mRNA amplification where other kits suffer numerous technical problems including:

- Bacterial total RNA consist of >90% rRNAs
- Limited numbers of bacterial species from which mRNA can be amplified
- Reliance on time consuming and costly rRNA removal steps
- Prerequisite for high quality mRNA samples from logarithmic cultures
- rRNA removal is not 100% effective causing high background levels in microarray analysis

ExpressArt® Features for Amplifying Bacterial mRNA

- Allows the use of degraded RNA and maintains uniform representation of all mRNA sequences
- Amplifies sufficient aRNA for microarrays from as little as 1 nanogram of total RNA
- Specifically amplifies mRNA from all species of bacteria without the need to remove rRNA
- Amplify mRNA from bacteria in any physiological condition
- No enzymatic poly-adenylation steps



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Conversion of bacterial mRNA sequences to cDNA is achieved using *TRinucleotide* primers, that preferentially prime to 3'-ends and select against reverse transcription of rRNAs. Synthesis of double stranded DNAs is performed with a second *TRinucleotide* primer that produces cRNA contain a 5'-Box-1 and a 3'-BOX-2 sequence (Fig. 7) that provides a priming sequence for second and third amplification rounds of full length sequences.

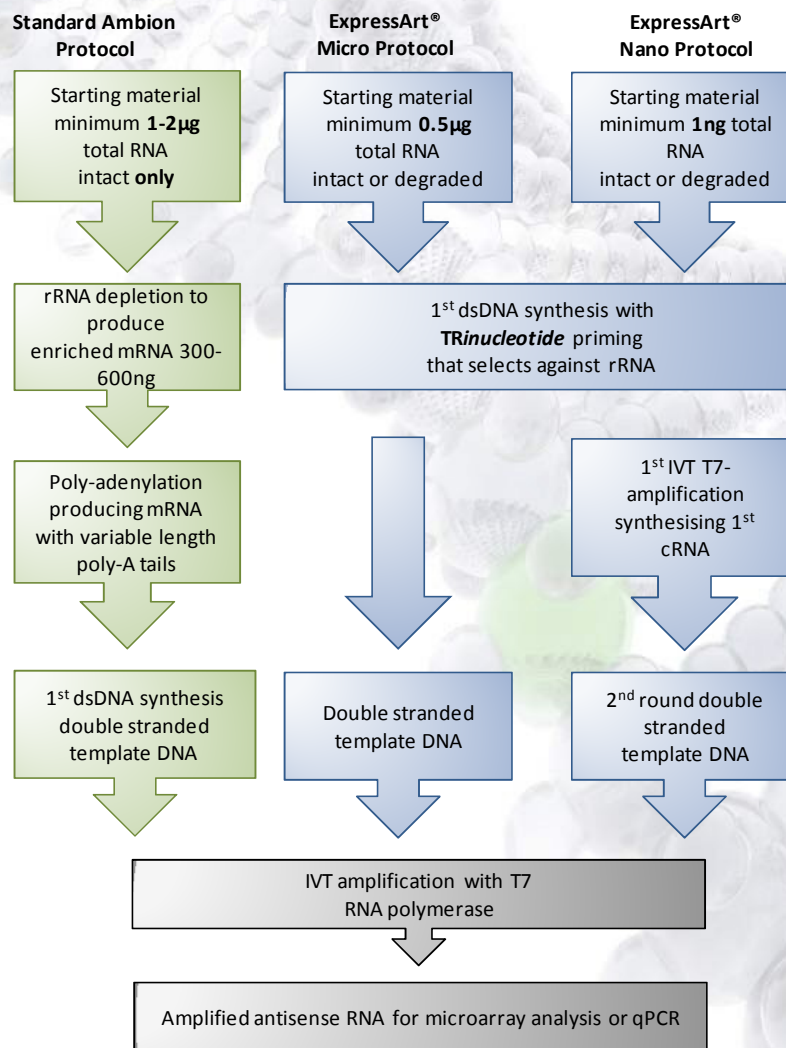
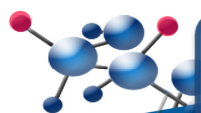


Fig. 11 Comparison of standard Ambion and ExpressArt[®] bacterial mRNA amplification protocols



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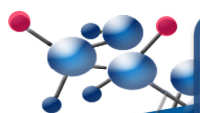
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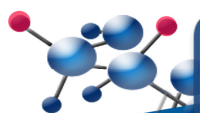
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ExpressArt® Product Line

Standard ExpressArt® C&E mRNA amplification kits for high quality eukaryotic mRNA

C&E MICRO kit for 30x 1-round (0.3-3 µg)

C&E NANO kit for 15x 2-rounds (1-700 ng)

C&E PICO kit for 15x 3-rounds (100-1000 pg)

Qty

Cat. No.

30 reactions

7199-A30

30 reactions

7299-A15

45 reactions

7399-A15

ExpressArt® TRinucleotide mRNA amplification kits for degraded mRNA, FFPE, Exon & GENE microarrays

C&E TR MICRO kit for 30x 1-round (0.3-3 µg)

C&E TR NANO kit for 15x 2-rounds (1-700 ng)

C&E TR PICO kit for 15x 3-rounds (100-1000 pg)

30 reactions

6199-A30

30 reactions

6299-A15

45 reactions

6399-A15

ExpressArt® bacterial mRNA amplification kits

C&E Bacterial MICRO kit for 30x 1-round (0.3-3 µg)

C&E Bacterial NANO kit for 15x 2-rounds (1-700 ng)

30 reactions

5199-A30

30 reactions

5299-A15

ExpressArt® RNA Isolation Kits

FFPE RNAready

LCM RNAready

RNAready

RNA Clean RNAready

RNA Clean RNAready

100 samples

9000-A100

100 samples

9001-A100

100 samples

9002-A100

100 samples

9003-A100

100 samples

9004-A100

ExpressArt® Add-On Modules

AminoAllyl Add-On Module - excludes NHS-activated dyes

AminoAllyl Add-On Module - excludes NHS-activated dyes

Archival Template Add-On Module

Archival Template Add-On Module

15 samples

2000-A15

30 samples

2000-A30

15 samples

2010-A15

30 samples

2010-A30

ExpressArt® Reagents

Pico RNA Care- Carrier compounds for very small samples

NucleoGuard (NG) - Universal nuclease and RNase inhibitor

FFPE RNA Enhance- DeCrossLinker and NG

100 samples

8999-A100

50 ml lysate

8998-M50

50 ml lysate

8990-M50

ExpressArt® cDNA Synthesis Kits

ExpressArt TR cDNA synthesis kit

Eukaryotic H-TR cDNA synthesis kit

Bacterial H-TR cDNA synthesis kit

30 samples

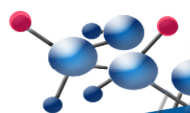
8994-A30

30 samples

8114-A30

30 samples

8004-A30



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