ExpressArt®: The future of mRNA amplification

A "NON-Eberwine" technology with unique advantages

For the first time ever!
Specific amplification of
Bacterial mRNA

Use low amounts of input RNA,
<1 ng total RNA yields >10 µg amplified RNA
for multiple microarray hybridisations
for multiple qPCR assays
for Next Generation Sequencing

No need to remove bacterial rRNAs
specific mRNA amplification provides high sensitivity
with high signals & high signal-to-noise ratios

Simple analysis of mixed host-pathogen samples,
Use mixed bacterial & eukaryotic total RNAs
simply remove eukaryotic Poly(A) mRNAs with Oligo(dT) beads
no need to remove eukaryotic rRNAs

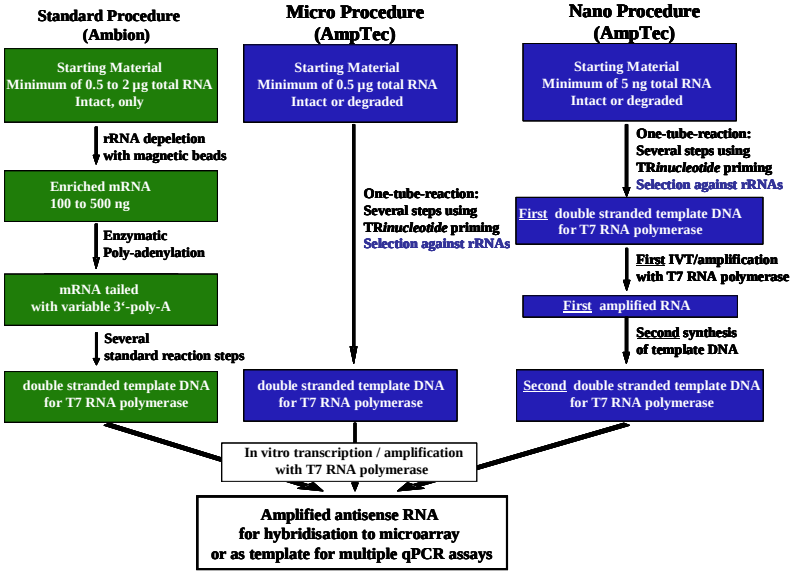
www.amp-tec.com

AmpTec
Amplification Technologies

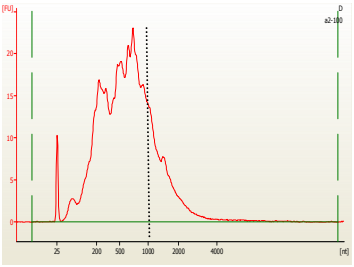
ExpressArt® Technology
Sselective amplification of bacterial mRNAs

Comparison of Flow Diagrams for Amplification of Bacterial mRNAs

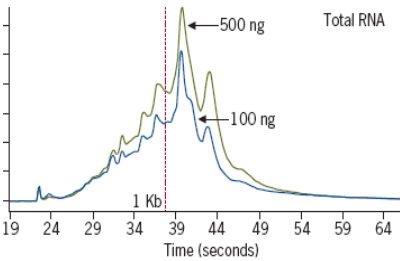
AmpTec's TR*inucleotide* priming technology results in amplification of all mRNAs, independent of a universal 3'-sequence, and in effective exclusion of rRNA sequences.
In a second step, a universal 3'-sequence is introduced in all amplified mRNAs. This permits two amplification rounds.



ExpressArt TR*inucleotide* priming with *E. coli* total RNA



Competitor (Ambion kit): Universal poly-A tailing followed by oligo(dT) priming *<u>without rRNA depletion</u>*

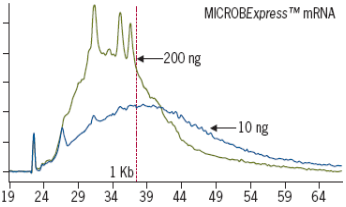


Left: Low rRNA contents in ExpressArt amplified RNA is indicated by low levels in the 16S and 23S rRNAs size range (>1.6 kb; dotted line indicates size of 1 kb). Evidently, no rRNA removal step is needed.

Right: High rRNA contents in Ambion amplified RNA is obvious by high levels in the size of 16S and 23S rRNAs.
As described in Ambion's protocol, **<u>an rRNA removal step is mandatory</u>** for optimal results (see Figure next page).

Competitor (Ambion kit):
Amplified RNAs <u>after rRNA depletion</u> with MICROBExpress kit

Only with rRNA depletion, low rRNA contents is achieved in Ambion amplified RNA. With variable, and especially with low RNA amounts (here: 10 ng of "enriched mRNA"), a high enzyme excess of Poly(A) polymerase results in extremely long poly-A tails, and a high perecentage of amplified RNA is only poly(A) sequence, this means
very low contents of mRNA sequences.



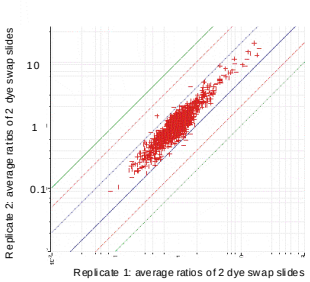
General quality of Affymetrix microarray data with ExpressArt amplified RNA *E. coli* total RNA

Data with high amounts of total RNA (0.3 µg) and one amplification round. Approximately 8 µg of biotinylated amplified RNA were hybridised with Affymetrix *E.coli* Genome 2.0 GeneChips. With *E.coli* grown at 37°C, the number of presence calls was 4804 (47.1%), signal background ratio was 98, average signals of 3378 with a Scaling Factor of 8.5. The sum of all rRNA intensities was approximately 1.2% of all detected signals. – Please note: these signals derive from hybridisation with sets of local probes, whereas with qPCR assays and different amplicon locations, up to approximately 10% rRNA sequences were estimated.

Data with low amounts of total RNA (5 ng) and two amplification rounds. Approximately 10 µg of biotinylated amplified RNA were hybridised with Affymetrix *E.coli* Genome 2.0 GeneChips. With *E.coli* grown at 37°C, the number of presence calls was 4536 (44.5%), signal background ratio was 82, average signals of 3898 with a Scaling Factor of 7.2. The sum of all rRNA intensities was approximately 1.2% of all detected signals. – Please note: these signals derive from hybridisation with sets of local probes; here we found good agreement with the estimated values of ~2% derived from qPCR assays and different amplicon locations

Reproducibility of mRNA amplification reactions

Four Ocimum *E.coli* K12 Arrays were hybridised with ExpressArt amplified cRNAs dye-swap experiments. High reproducibility was observed with a correlation coefficient (R) of 0.922.



Differential gene expression

Comparison of data obtained with conventional cDNA hybridisation (no amplification) and with ExpressArt amplified RNA

Genes induced during heat-shock in *E.coli*. Comparison of published data and results with cDNA labelling and with ExpressArt TR*inucleotide* amplification. Concordance is shaded.

Gene	**Richmond et al (1999)** **20 µg** RNA for Cy-labelled cDNAs & self-spotted microarrays	**MWG/Ocimum** **50 µg total RNA** for Cy-labelled cDNAs & Ocimum *E. coli* Arrays	**ExpressArt/MWG/Ocimum** **5 ng total RNA** for Cy-labelled amplified RNAs (2 rounds) & Ocimum *E. coli* Arrays	**ExpressArt/Affymetrix** **5 ng total RNA** for biotin-labelled amplified RNAs (2 rounds) & Affymetrix *E. coli* GeneChips
dnaj	high	medium	medium	medium
dnak	medium	medium	medium	low
grpe	medium	low	medium	medium
hslj	medium	low	low	medium
hslu	low	low	low	low
hslv	medium	low	medium	medium
htpx	medium	low	low	medium
ibpa	high	high	high	high
ipbb	high	high	high	high
lon	medium	low	medium	low

Data from a user laboratory: Prof. Chakraborty & Dr. Mraheil (Mobarak.Mraheil@mikrobio.med.uni-giessen.de) Institut für Medizinische Mikrobiologie, Giessen University, Germany.

Differential gene expression (Fold Changes) as determined with total bacterial RNAs isolated from intracellular versus extracellular *Listeria monocytogenes*

Gene	**3 µg total RNA** for Cy-labelled cDNAs No amplification	**5 ng total RNA** (~1000x lower) 2-rounds ExpressArt amplification
1	**+7**	**+13**
2	**+15**	**+13**
3	**+8**	**+6**
4	**+12**	**+8**
5	**+11**	**+17**
6	**+13**	**+22**
7	**+16**	**+44**
8	**-5**	**-4**
9	**-4**	**-4**
10	**-4**	**-3**

First publication using ExpressArt bacterial mRNA amplification kit:

Nam et al. (2009) Metatranscriptome analysis of lactic acid bacteria during kimchi fermentation with genome-probing microarrays. Int J Food Microbiol 130: 140-146.