

Transcriptome Analysis

Assessing Limited Quantities of RNA

summary



Epistem's proprietary amplification technique, RNA-Amp™, allows robust gene expression information to be derived from as little as a single cell input.

RNA-Amp™

Epistem has pioneered the ability to perform highly sensitive RNA amplification from very limited quantities of RNA. Epistem's RNA-Amp™ technology enables robust and insightful transcriptomic assessments from single cell through to standard material inputs (10pg-100ng of RNA).

The amplified material is platform agnostic and can be utilised for downstream global or targeted analyses by:

- Microarray (Agilent, Affymetrix, Illumina)
- qPCR (Sybr, Taqman, Fluidigm)
- Hybridisation (nCounter)
- NGS (Illumina)

Platform Applications

Biomarker Analysis: RNA-Amp™ can be utilised to identify drug-induced changes including pharmacodynamic response, stratification of patient responders, treatment effect and target engagement.

Biomarker and Target Discovery: RNA-Amp™ is ideally suited to identify disease-induced gene expression changes and compare normal and diseased tissue to discover expression markers of disease. We have extensive experience in applying this approach together with LCM techniques in lung and liver fibrosis research.

Tissue Samples: RNA-Amp™ is compatible with a broad range of sample types where RNA is obtained. Tissue samples we have analysed include:

- Single plucked scalp hair
- Tumour biopsies
- Stem cells
- Laser capture microdissected (LCM) samples
- Skin and gastro-intestinal samples
- Lung, liver and kidney tissue
- Whole blood, fractionated PBMC populations
- Fluorescence-activated cell sorted (FACS) populations

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Sensitivity and Reproducibility

Epistem's RNA amplification methodology is highly sensitive, and reproducible with extensive transcriptome coverage at minimal input (single cell level). Figure 1 highlights RNA-Amp™ reproducibility between replicates at 25pg input level of RNA.

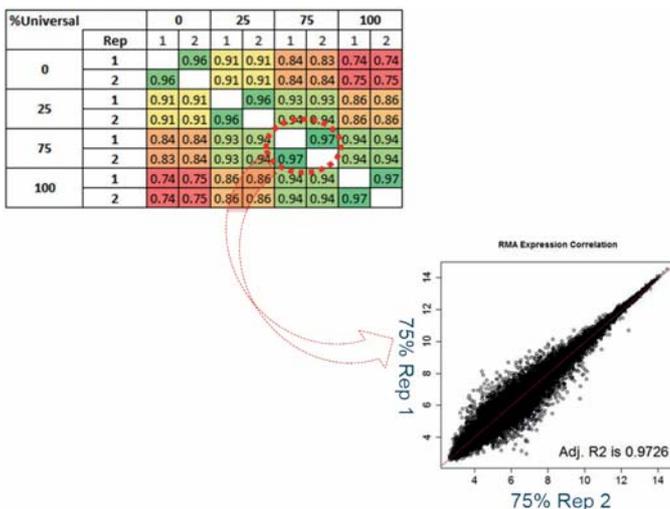


Figure 1: Replicates of MAQC brain/universal titrations achieve correlation of 0.96-0.97 (R²) - at 25pg total input RNA. At 25pg input of mixed universal/brain RNA (and Log₂>5), 34,000 probes detected (~62% of array and ~15K annotated genes). 96% reproducibility between replicates.

Linearity

Epistem's RNA amplification technology offers a high degree of linearity at minimal input levels (single cell starting materials). Figure 2 shows RNA-Amp™ is extremely correlative when comparing observed and expected gene expression levels at 25% and 75%.

%Universal	Data	Rep	25				75									
			Expected		Observed		Expected		Observed							
			1	2	3	4	Ave	1	2	3	4	Ave	1	2		
0	Expected	1	97.3	99.7	97.5	99.3	96.1	95.7	95.7	93.7	95.0	94.3	95.2	92.2	91.7	
		2	97.3		97.5	99.7	99.3	95.9	95.9	94.5	95.7	95.1	95.2	95.6	92.7	92.5
		3	99.7	97.5		97.2	99.3	96.1	95.8	95.0	94.4	95.7	93.7	95.2	92.2	91.8
		4	97.5	99.7	97.2		99.3	95.8	95.7	95.1	95.0	94.3	95.8	95.6	92.6	92.3
		Ave	99.3	99.3	99.3	99.3		96.6	96.4	95.8	95.4	95.7	95.4	96.1	93.0	92.7
	Observed	1	96.1	95.9	96.1	95.8	96.6		96.2	94.7	94.4	94.7	94.3	95.0	93.4	93.3
		2	95.7	95.9	95.8	95.7	96.4	96.2		94.6	94.5	94.8	94.3	95.0	93.6	93.8
		3	95.7	94.5	95.0	95.1	95.8	94.7	94.6		97.9	98.3	99.5	99.5	97.3	97.0
		4	93.7	95.7	94.4	95.0	95.4	94.4	94.5	97.9		99.5	98.3	99.5	97.4	97.3
		Ave	95.2	95.6	95.2	95.6	96.1	95.0	95.0	99.5	99.5	99.5	99.5	99.5	97.8	97.3
25	Expected	1	92.2	92.7	92.2	92.6	93.0	93.4	93.6	97.3	97.4	97.3	97.3	97.8	97.3	
		2	91.7	92.5	91.8	92.3	92.7	93.3	93.8	97.0	97.3	97.2	97.1	97.7	97.3	
		3	95.0	95.1	95.7	94.3	95.7	94.7	94.8	98.3	99.5		97.8	99.5	97.3	97.2
		4	94.3	95.2	93.7	95.8	95.4	94.3	94.3	99.5	98.3	97.8		99.5	97.3	97.1
		Ave	95.2	95.6	95.2	95.6	96.1	95.0	95.0	99.5	99.5	99.5	99.5	99.5	97.8	97.3
	Observed	1	92.2	92.7	92.2	92.6	93.0	93.4	93.6	97.3	97.4	97.3	97.3	97.8	97.3	
		2	91.7	92.5	91.8	92.3	92.7	93.3	93.8	97.0	97.3	97.2	97.1	97.7	97.3	
		3	95.0	95.1	95.7	94.3	95.7	94.7	94.8	98.3	99.5		97.8	99.5	97.3	97.2
		4	94.3	95.2	93.7	95.8	95.4	94.3	94.3	99.5	98.3	97.8		99.5	97.3	97.1
		Ave	95.2	95.6	95.2	95.6	96.1	95.0	95.0	99.5	99.5	99.5	99.5	99.5	97.8	97.3
75	Expected	1	92.2	92.7	92.2	92.6	93.0	93.4	93.6	97.3	97.4	97.3	97.3	97.8	97.3	
		2	91.7	92.5	91.8	92.3	92.7	93.3	93.8	97.0	97.3	97.2	97.1	97.7	97.3	
		3	95.0	95.1	95.7	94.3	95.7	94.7	94.8	98.3	99.5		97.8	99.5	97.3	97.2
		4	94.3	95.2	93.7	95.8	95.4	94.3	94.3	99.5	98.3	97.8		99.5	97.3	97.1
		Ave	95.2	95.6	95.2	95.6	96.1	95.0	95.0	99.5	99.5	99.5	99.5	99.5	97.8	97.3
	Observed	1	92.2	92.7	92.2	92.6	93.0	93.4	93.6	97.3	97.4	97.3	97.3	97.8	97.3	
		2	91.7	92.5	91.8	92.3	92.7	93.3	93.8	97.0	97.3	97.2	97.1	97.7	97.3	
		3	95.0	95.1	95.7	94.3	95.7	94.7	94.8	98.3	99.5		97.8	99.5	97.3	97.2
		4	94.3	95.2	93.7	95.8	95.4	94.3	94.3	99.5	98.3	97.8		99.5	97.3	97.1
		Ave	95.2	95.6	95.2	95.6	96.1	95.0	95.0	99.5	99.5	99.5	99.5	99.5	97.8	97.3

Figure 2: From the 0, 25%, 75% and 100% MAQC brain/universal titrations (at 25pg), utilising the expression levels noted at 0%/100% to predict *in silico* expected expression at 25% and 75%. Correlation of 0.96-0.97 (R²) of observed versus expected.

Why Choose Epistem?

Our Expertise: Epistem Pharmacogenomics provides high quality biomarker and personalised medicine information to pharmaceutical and biotechnology companies from very limited quantities of RNA. We specialise in advancing drug development programs for oncology, inflammatory and fibrotic disease indications through our innovative plucked hair analysis and laser capture microdissection techniques as well as offering GCLP accredited laboratory gene expression and DNA genotyping services. In addition to our expertise in assessing limited quantities of RNA, we also have extensive experience in developing patient stratification companion diagnostic assays using our point of care Genedrive® platform.

Outstanding Service: Epistem's high level of expertise and customer focus has led to an enviable track record and impressive repeat business ratio. Epistem has worked with over 200 pharmaceutical and biotechnology companies and has developed long term collaborative relationships with several market leaders.

Quality Management: Epistem's pharmacogenomics laboratories are GCLP accredited. Our ISO 13485 certification is for the design, development, manufacture and distribution of molecular diagnostic instruments and molecular IVD assays.