Arraystar Circular RNA Microarray Version 2.0

The practical choice to profile circular RNAs in gene regulation

Highlights

- The only commercially available circRNA microarrays. Specifically designed for circRNA expression profiling. CircRNAs were comprehensively curated from the landmark publications and multiple data sources with stringent collection pipelines to produce the best circular RNA array contents.
- Highly specific circular RNA signal detection. Samples are treated with RNase R to specifically remove linear RNA in the total RNA samples. Circular junction sequence specific array probes ensure the most specific, accurate and reliable circRNA profiling, even in the presence of linear counterparts.
- Detailed Annotation. In addition to standard microarray data analysis, circRNAs specific information is further annotated with the target sites of conserved miRNAs with good mirSVR scores, to unravel their functional roles as miRNA sponges.
- The preferred choice over RNA-sequencing, as RNA-seq is currently inadequate for such task due to the particular properties of circular RNA.

Introduction

Circular RNA (circRNA) is a novel type of non-coding RNA covalently circularized in a closed loop, produced by RNA back splicing process. circRNAs are not known to translate proteins. With their extensive complementarity to linear RNA counterparts, stability against nucleases, resistance to miRNA-targeted degradation, high expression levels, enrichment in cytoplasm, and large number of miRNA binding sites, circRNAs have been increasingly recognized as exceptionally effective natural miRNA sponges and competing endogenous RNAs (ceRNAs) in gene regulation. Some intronic circular RNAs (ciRNA) have been shown to enhance the host gene transcription [6]. Additionally, the tissue/developmental-stage-specific expression and long half-lives constitute an enormous advantage as a novel class of biomarkers.

To facilitate the analysis of circRNAs, Arraystar has pioneered the circRNA microarrays for human and mouse to systematically profile circRNAs under physiological and disease conditions.

circRNAs as microRNA sponges

Circular RNAs can have multiple microRNA binding sites. For example, the physical interactions of ciRS-7 with miR-7 and gene silencing complex have been demonstrated by Photoactivatable-Ribonucleoside-Enhanced Crosslinking and Immunoprecipitation (PAR-CLIP) with AGO2 in the presence of miR-7, and by biotinylated miR-7 capture (Fig 1), showing ciRS-7 biochemically as a microRNA sponge.

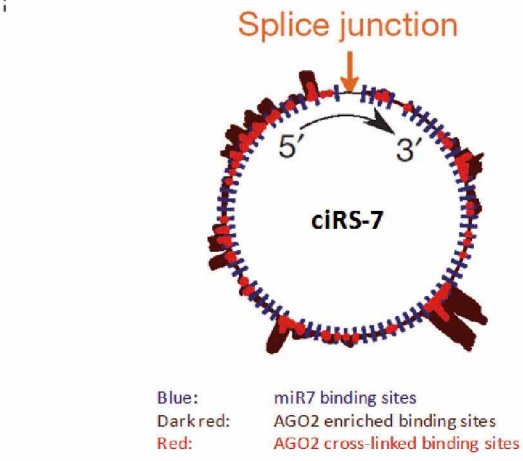


Figure 1. ciRS-7, short for "circular RNA Sponge for miR7", has more than 70 copies of predicted miR-7 binding sites. It interacts with miR-7 and the catalytic component of RNA-induced silencing complex AGO2.

circRNAs and RNA Binding Proteins

Circular RNAs may partner with very diverse RNA binding proteins (RBP) to perform wide range of molecular functions (Fig. 2). Circular RNAs may bind RBPs for subcellular delivery/localization (Fig. 3), sponge RBPs just like miRNAs, assemble different sets of RBP complexes, or act as a allosteric co-factor for enzymatic RBPs [8]. Anti-sense circular RNAs may form direct base pairing with mRNAs to regulate the activity.

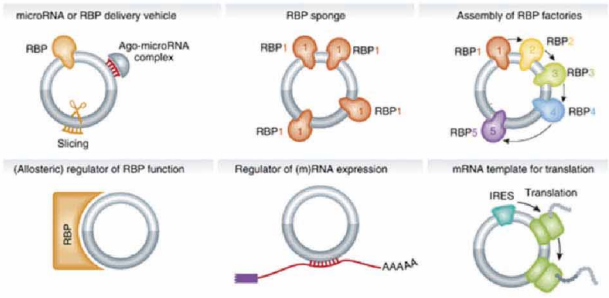


Figure 2. Interplay of circular RNA with RNA binding proteins [8].

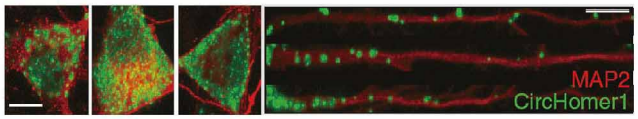


Figure 3. Dynamic subcellular localization of circular RNAs in neuronal cell body and dendrites to regulate neuronal synaptic connections [7].

circRNAs in biology and disease

MicroRNAs may regulate up to 1/3 of all gene expression. circRNA regulation of microRNA activities can lead to biological phenotypes (Fig. 4). Disproportionately large number of circRNA targets are protein kinases, which are signal transduction mediators. Aberrant circRNA expression is involved in human diseases such as cancer, Alzheimer's disease and atherosclerosis. The higher specificity and stability of circRNA in diseases are desired properties in biomarker applications.

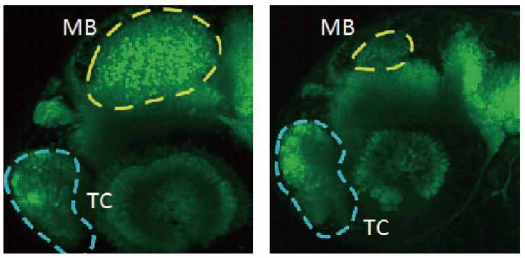


Figure 4. ciRS-7 overexpression in embryonic brain leads to profound reduction of mid brain (MB) size (right), compared to the normal control (left). Co-overexpression of miR-7 ameliorates the effect. The telencephalons (TC) are relatively unaffected.

circRNAs are stable due to resistance to exonuclease degradation

Circular RNAs lack exposed 5' and 3' termini and are resistant to exonuclease degradation. Thus, circRNAs are stable and have much longer half-lives than their linear RNA counterparts (Fig. 5). The elevated abundance contributes to functioning as microRNA sponges. It also presents a good opportunity for biomarker applications.

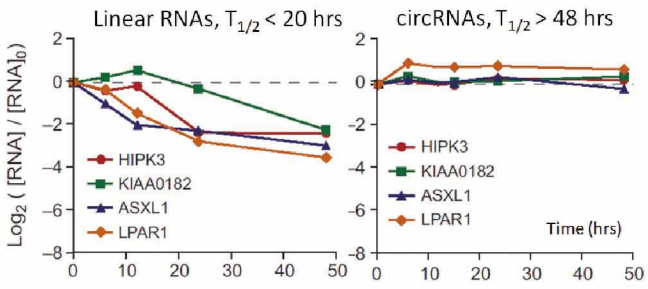


Figure 5. Circular RNAs are stable and have half-lives more than 48 hours (right), compared to their linear RNA counterparts of less than 20 hours (left).

Why use microarray over RNA-seq for circular RNA expression profiling?

Circular RNAs as a population are typically present at much lower abundance levels, at about 5~10% of linear RNAs. The cross circular junction sequences are even lower. At a typical RNA-seq depth, less than 5% of circRNAs (red circle) may be reliably quantified (Fig. 6). Even at sequencing depths of > 300 mil, the accuracy gains are only modest. In practice, generic RNA-seq are inadequate or simply una-vailable as a provided service for circular RNA profiling, as circular RNA sequencing requires very deep sequencing, paired-end chemis-try, specialized computational pipeline, complex de novo transcript assembly, and circRNA specific annotation. Novel circular RNA dis-covery, a consideration of using RNA-seq, is actually not available for the above reasons.

Circular RNA microarrays, on the other hand, use circular junction probes and enzymatic linear RNA removal to efficiently and robustly interrogate circular RNAs highly specifically at a sensitivity of one transcript per cell. Currently, it is the only practical and mature cir-cular RNA profiling technology for most research labs.

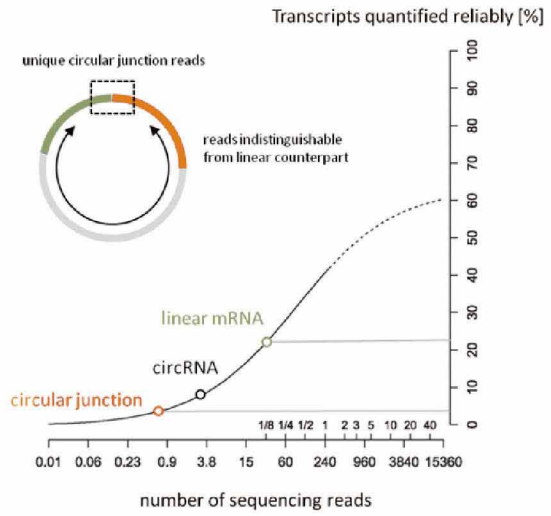
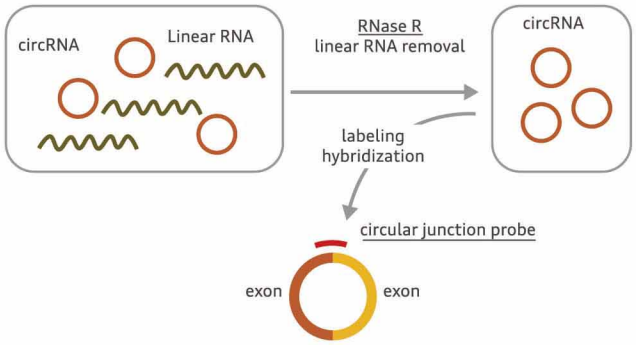


Figure 6. Quantification reliability vs read depth of RNA-seq. Typical RNA-seq has a depth of < 30 mil reads for mRNAs (blue circle), which is equivalent of < 0.5 mil for cross circular junction reads (red circle). Less than 5% circular junctions can be reliably quantified. Adopted from [9].

Arraystar circRNA Microarrays

Arraystar circRNA Microarrays were the first and are currently the only commercially available technology for sensitive and reliable circRNA expression profiling. The microarrays use RNase R linear RNA removal and circular junction probes to achieve high specificity for circRNAs, even in the predominant presence of linear RNAs (Fig. 7, top). The profiling is complete with comprehensive, systematic and detailed annotation of circRNAs, including miRNA binding sites as microRNA sponges, to gain insight into circular RNA biology (Fig. 7, bottom).



circRNA	linear RNA	miRNA	Binding Alignment	mirSVR score	PhastCons
circRNA-000482	NM_001286646	miR-340	3' uuaguCAGAGUAACGAAAUAUu 5' miR-340 uucuuGUUGUAUAACUUUAUAu circRS-9	-1.295	0.7719
		miR-125-3p	3' ccGAGGGU-UCUUGGAGUGGACa 5' miR-125a-3p caUUCAUAUAAUAGUUCACCUGg circRS-9	-1.141	0.7597
		miR-499-5p	3' uuuguaGUGACGUUCAGAAUu 5' miR-217 guugugCUUUUAAGUCUUAu circRS-9	-1.143	0.7314
		miR-217	3' agGUUAGUCAAGGACU-ACGUCAu 5' miR-217 acCAGUCUUUGAGUGAUGCAGUa circRS-9	-1.131	0.7481

Figure 7. Overview of circRNA microarray workflow (top) and detailed annotation of circRNA (bottom).

Research roadmap

The differentially expressed circRNA candidates screened by circRNA microarrays are typically confirmed by an independent method such as by qPCR (Fig. 8). The validated circRNAs are further studied for biological functions, molecular mechanisms in gene regulation and biomarker applications (Fig. 9).

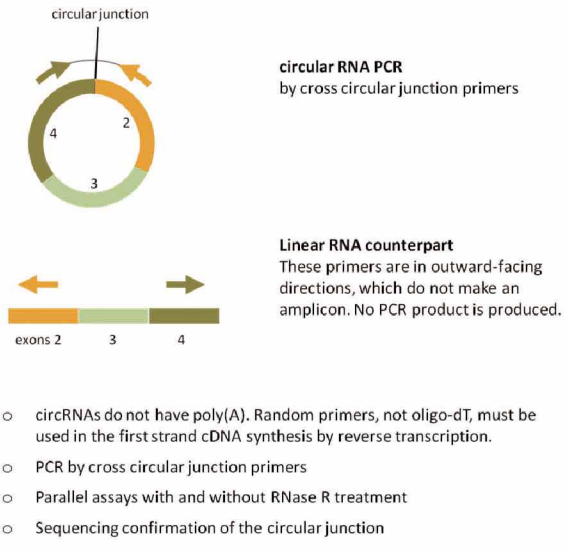


Figure 8. qPCR validation of differentially expressed circRNAs screened by circRNA microarray. The concordance between qPCR and microarray for the differentially expressed circRNA is related to the magnitude of change (FC), p-value, as well as the abundance level.

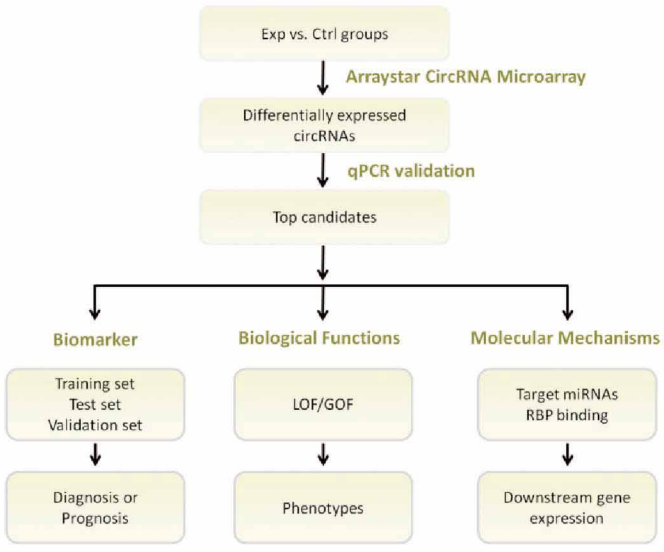


Figure 9. Roadmap of circRNA expression profiling and follow-up studies.

Arraystar CircRNA Array Contents

	Human	Mouse
Total number of unique circRNAs	13,617	14,236
Probe Length	60nt	60nt
Probe Region	circRNA junctions of circRNA	
Probe Specificity	Transcript specific	
circRNA Enrichment	RNase R treatment	
Labeling Method	Labeling by random priming	
Salzman's circRNAs (2013)	8,529	
Memczak's circRNAs (2013)	1,601	1,750
Zhang's circRNAs (2013)	93	
Zhang's circRNAs (2014)	4,980	
Jeck's circRNAs (2013)	3,769	
Guo's circRNAs (2014)	5,536	570
You's circRNAs (2015)		13,300
Array Format	8*15K	8*15K

References

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