Arraystar eccDNA-seq

Profiling the extrachromosomal circular DNAs in cancer and diseases

Highlights

- Broad coverage: Simultaneous profiling of eccDNA(<10 kb) and ecDNA(>=10 kb).
- Maximal eccDNA enrichment: Chromosomal and mitochondrial DNA removal with Rolling Circle Amplification of eccDNA to dramatically increase eccDNA reads.
- High reliability: Well established and optimized procedures to produce best possible results.
- Easy-to-use analyses: Provided with rich annotation, genome browser track visualization, publication-ready graphics for biologists and clinicians.

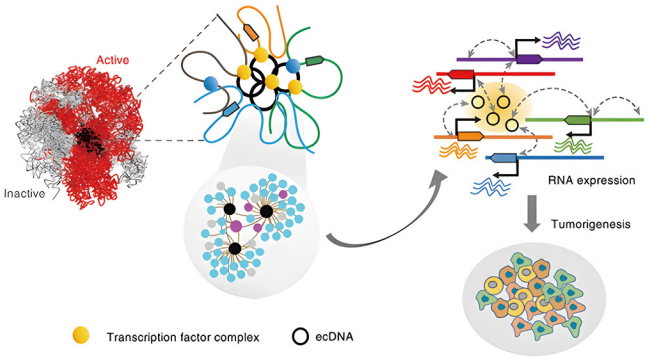


Fig 1. *ecDNA acts as a mobile super-enhancer to regulate gene transcription [2].*

Introduction

Extrachromosomal circular DNA (eccDNA) is a class of double-stranded circular DNA molecules present outside of regular chromosomal DNA. eccDNA is widely found in human normal and cancer tissues, body fluids, and other eukaryotic cells. According to the lengths, eccDNA can be classified into two main categories : eccDNA (<10 kb) and ecDNA (>=10 kb)[1]. eccDNAs may arise from any regions of the genome in normal tissues or as a byproduct of programmed cell death. These entities are typically devoid of genes and are not subjected to either amplification or growth selection within the cells. The term extrachromosomal DNA (ecDNA) is more commonly used to refer specifically to the very large eccDNAs, i.e. large clonal circular DNA molecules (10 kb to several Mb) that are clonally inherited, self-replicating, amplifying and selected only in cancer cells. These ecDNA molecules frequently harbor oncogenes, drug resistance genes, or mobile super-enhancers, which provide an evolutionary selective advantage for cancers [2](Fig. 1). Functionally, eccDNAs and ecDNAs regulate diverse molecular and physiological processes and are closely associated with a variety of diseases [3](Fig. 2). Due to the circular form, eccDNA can be excellent diagnostic and prognostic markers (Table 1). In recent years, eccDNAs have become one of the major breakthroughs and research hotspots in cancer research.

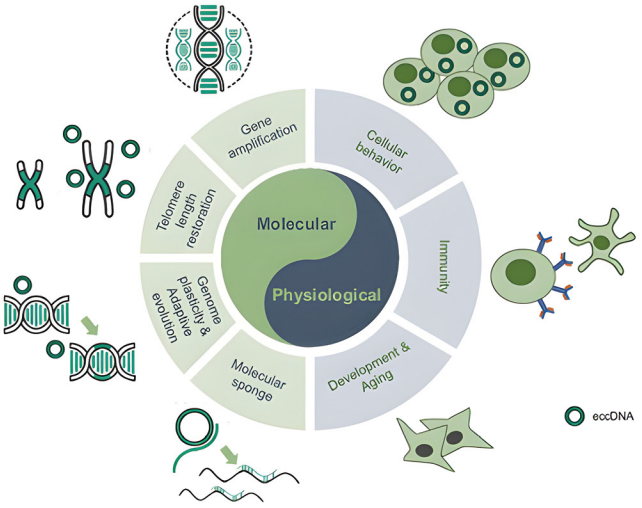


Fig 2. *eccDNAs regulate diverse molecular and physiological processes in, e.g., gene amplification, telomere length restoration, genomic plasticity and molecular sponges, cellular behavior, immunity and aging [3].*

Table 1. eccDNA as biomarker

Molecule	Form	RNase resistance	Stability	Length	Pre-Amp
cfDNA	Linear	Weak	Unstable	Short	PCR
eccDNA	Circular	Strong	Stable	Long	RCA

eccDNA Profiling

In eccDNA sequencing, the overwhelming amounts of linear chromosomal DNA and circular mitochondrial DNA (mtDNA) in the DNA sample must be removed as they can significantly reduce the eccDNA reads. Arraystar eccDNA-seq linearizes the circular mtDNAs by restriction endonuclease cleavage and then digests the linearized mtDNA and linear chromosomal DNA by a linear DNA-specific exonuclease, leaving eccDNAs intact and enriched in the sample. The eccDNAs are amplified by Rolling Circle Amplification (RCA), greatly improving the eccDNA signals and data quality (Fig. 3). The eccDNA-seq data are well annotated and analyzed for ready use (Fig. 4).

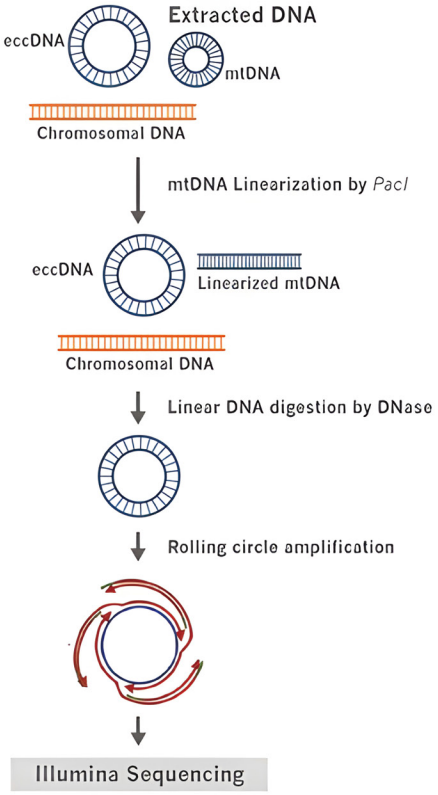


Fig 3. *eccDNA-seq workflow*

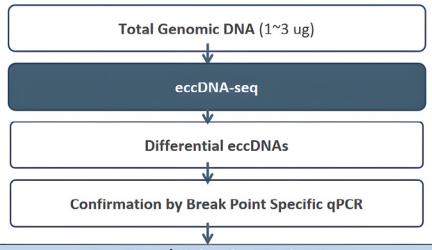
Type	Length	Soft clipped reads	Discordant pairs	Circle score	Chrom	Start	End
ecDNA	97,562,003	74	54	2733	Chr2	68799831	166361834
ecDNA	50,181,084	245	180	8117.43	ChrX	29724616	79905700
ecDNA	45,636,984	155	78	5603	Chr2	68533526	114170510
ecDNA	28,348,253	410	608	14514.49	ChrX	79870366	108218619
eccDNA	9,863	413	396	15689	Chr6	106157513	106167376
eccDNA	9,636	48	174	851.06	Chr4	169181379	169191015
eccDNA	9,403	179	124	7004	Chr13	86180618	86190021

Type	Gene ID	Overlap size	Gene ratio	Gene symbol	CancerDriverGene DB	Gene biotype	Gene locus
ecDNA	ENSG00000058085	58889	100%	LAMC2	TAG	Protein_coding	chr1:183155373-183214262:+
ecDNA	ENSG00000163041	10168	100%	H3-3A	NCGv7;AC	Protein_coding	chr1:226249552-226259720:+
ecDNA	ENSG00000158715	22658	100%	SLC45A3	IntOGen-DriverGenes;AC	Protein_coding	chr1:205626979-205649637:-
ecDNA	ENSG00000143184	5464	100%	XCL1	DriverDBv3;AC	Protein_coding	chr1:168545843-168551307:+

Fig 4. *eccDNA-seq annotation and analysis.*

eccDNA Research Roadmap

With the eccDNA-seq data, the differentially expressed eccDNAs are often confirmed by qPCR based on their characteristic break point (i.e. circular junction) sites. eccDNA functions can be predicted by their cargo genes. ChIP-seq can be used to study eccDNA chromatin modifications, epigenomic states, or transcription factor binding. The eccDNAs and their target gene expression can be correlated by lncRNA&mRNA microarray or RNA-seq. eccDNA super-enhancer activities along with all transcription factors and oncogenes can be studied by using Super-enhancer Microarrays. R-loops are involved in eccDNA formation and genomic instability, which can be profiled by DRIPc-seq. To study eccDNA interacting chromosomal DNA, proteins and other eccDNAs, HyPro enzyme can label eccDNA binding partners in close spatial proximity as used in HyPro-seq or HyPro-mass spectrometry methods.



Functions/Molecular Mechanisms	
Cargo genes	Effector functions, by qPCR
ChIP-seq	eccDNA chromatin epigenomic modification or TF/binding factors
lncRNA microarray/RNA-seq	Correlate eccDNA and target gene expression
Super-enhancer lncRNA microarray	Correlate eccDNA super-enhancer, TF, and target oncogene activities
DRIPc-seq	Correlate eccDNA and R-loop, genomic instability
HyPro-seq/MS	Find eccDNA associated genes, proteins, or other eccDNAs in close spatial proximity

References

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