Arraystar Downstream-of-Gene Transcript (DoG RNA) Array

One Array to Profile DoG RNAs & All Their Target RNAs Accurately

Highlights

- Designed to profile and study DoG RNAs having important biological and disease functions.
- One array to detect DoGs and all their regulatory targets: sense-overlapping lncRNA/circRNAs, DoG-derived chimera RNAs and circular RNAs, and anti-sense overlapping mRNAs/lncRNAs.
- Simple, accurate, and sensitive array probes specific to the unique splice junctions for detection and quantification, without the difficult computational and bioinformatic analyses otherwise required by sequencing
- High sensitivity at as low as 1 transcript/cell, good for DoG-derived circular RNAs and chimera RNAs often at low abundance.

What Are DoGs?

Downstream-of-gene (DoG) RNAs are RNAs transcribed beyond the polyadenylation signal (PAS) at the transcription end site (TES) and are continuous with upstream RNAs[1]. DoGs are typically 5–200 kb long and occur in about 10% of host genes[1], often overlapping with downstream transcription units called read-in genes [2, 3] (Fig. 1).

DoGs result from transcription termination failure[4]. Normal transcription termination requires coordinated slowing, pausing, and dislodging of RNA polymerase II and 3' end cleavage of the transcript. Disruption in these steps, due to stresses like osmotic shock[5], heat[6], viral infections[3, 7], or cancer mutations[2], leads to read-through transcription. DoG-producing genes often exhibit unique chromatin features that promote read-through[3], such as enriched histone marks (H3K36me3, H3K79me2) that favor elongation[8] or weaker PAS.

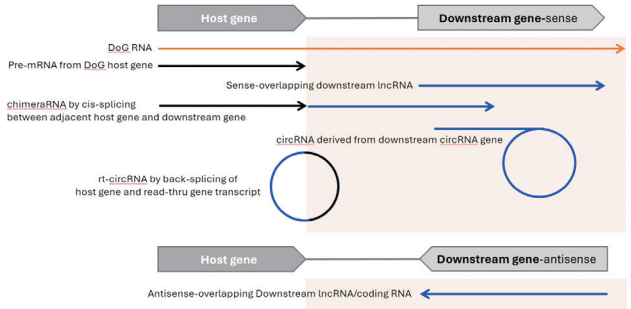


Fig 1. *DoG and its downstream genes.*

Why Study DoGs?

The functional roles of read-through transcription and DoG RNAs are currently the focus of active research, with several proposed mechanisms highlighting their involvement in gene regulation.

DoG RNAs mediate transcriptional interference in cell senescence

DoGs can act as antisense RNAs to control the gene expression of convergent protein-coding genes. In senescent cells, a family of senescence-triggered antisense read-through RNAs (START RNAs) are induced as DoGs[9]. Importantly, START RNAs repress the expression of their corresponding sense RNAs.

DoG RNA regulates genome 3D organization in viral infection

Influenza A virus nonstructural protein NS1 can induce read-through transcription of highly active host genes, causing displacement of cohesin from the host chromatin, elimination of chromatin loops, and decompaction of chromatin in the read-through regions[7].

DoG-derived chimera RNAs in cancers and diseases

~5% of the tandem gene pairs in the human genome can be transcribed into single precursor RNAs and eventually spliced into chimeric RNAs. A remarkable feature of RNA chimeras is that some are recurrently detected in different tumor samples. For example: chimeraRNA CTSC-RAB38 in 20% of the TCGA cancer samples[2], high frequency chimeraRNA CHFR-GOLGA3 in bladder cancer[10].

DoG-derived lncRNAs in cancers and diseases

Although DoG derived SLC45A3-ELK4 can encode the same ELK4 protein, SLC45A3-ELK4 enriched in the nucleus actually functions as a lncRNA not by its translated protein[11]. SLC45A3-ELK4 is expressed significantly higher in prostate cancer and controls the cancer cell proliferation/cancer progression.

DoG-derived circRNAs and rt-circRNAs with regulatory potentials

DoG read-through transcription can result in downstream circular RNA production (e.g. MATR3-PAIP2)[12](Fig. 1). Recently, a new type of circRNAs was discovered as read-through circRNAs (rt-circRNAs). They are hybrid circular RNAs that include coding exons from two adjace nt and similarly oriented genes (Fig. 1)[13, 14]. Circular RNAs can have potentials in gene and disease regulation.

Epigenetic and epitranscriptomic regulation of DoG RNAs

Dysregulation of H3K36 Tri-Methylation: In clear cell renal cell carcinoma (ccRCC), mutations in H3K36 methyl transferase SETD2 lead to extensive read-through transcription and DoG formation due to defective transcription termination[2], a mechanism common in various cancers[15, 16].

Absence of Histone H2A.Z: In senescent cells, the absence of histone variant H2A.Z triggers the generation of DoGs as antisense transcripts, regulating corresponding sense gene expression[9].

RNA Modifications: m6A modifications influence transcription termination[17, 18]. In plants, mutations in m6A-writer FIP37 trigger read-through transcription and chimeric mRNA formation, while the m6A-assisted polyadenylation pathway (m-ASP) prevents inappropriate gene expression[19].

m6A and R-loops: m6A promotes R-loop formation at gene terminators, aiding transcription termination[20]. Loss of METTL3 disrupts this, causing read-through transcription, defective termination, and DoG formation, potentially leading to DNA damage[21].

How to Study DoGs?

Arraystar Human Downstream-of-Gene Transcript (DoG RNA) Microarray is designed to profile and study human DoG RNAs. The array includes more than 13,000 probes to simultaneously detect and quantify DoG RNAs, pre-mRNAs of the host genes, and downstream overlapping transcripts as their potential regulatory targets at high accuracy and specificity.

Compared to sequencing, DoG profiling by microarray is simpler, more sensitive, and accurate. While sequencing requires complex analyses and specialized computational pipelines due to the diverse RNA types of DoGs targets, the microarrays utilize simple probe designs to target unique splice junctions (Fig. 2). The microarrays provide highly sensitive signals that detect and quantify DoGs and downstream target RNAs, including low-abundance circRNAs and rt-circRNAs. Sequencing would require costly, deep coverage that remains inadequate. For DoG profiling, the Arraystar DoG microarray offers a unique tailored solution.

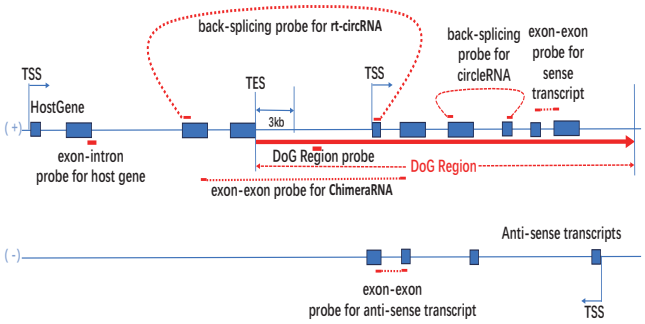
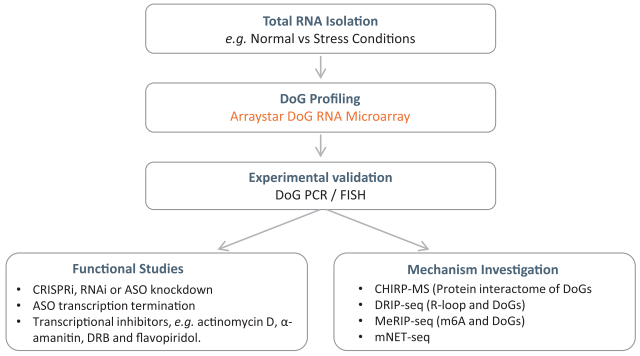


Fig 2. *Arraystar DoG RNA Microarray probe design, targeting unique sequences in splice junctions in DoG Region, Host gene, and Downstream mRNA/lncRNA/circular RNA/chimeraRNA/rt-circRNA.*

Human DoG Array Specifications

DoGs	4,460
DoG gene pre-mRNAs	4,460
Downstream lncRNAs (Sense)	480
Downstream circRNAs	1,546
chimeraRNAs	539
rt-circRNAs	356
Downstream antisense transcripts	1,866
Drosophila spike-in controls	1,000
DoG and downstream transcripts Sources	**DoGs:** Scientific publications **Host genes:** GENCODE human V44 **Downstream coding/non-coding RNAs:** GENCODE human V44 **Downstream circular RNAs:** circBase **Downstream chimeraRNAs:** FusionGDB2, GENCODE human V44,and scientific publications **Downstream rt-circRNAs:** scientific publications **Drosophila RNAs:** ENSEMBL BDGP6.46
Array Format	8 x 15K

DoG Research Roadmap



References

1. Mol Cell (2015) [PMID: 26190259]
2. Elife (2015) [PMID: 26575290]
3. Nat Commun (2015) [PMID: 25989971]
4. Trends Biochem Sci (2022) [PMID: 34489151]
5. Mol Cell (2021) [PMID: 33400923]
6. Mol Cell (2022) [PMID: 35114099]
7. Cell (2018) [PMID: 30146161]
8. Proc Natl Acad Sci (2017) [PMID: 28928151]
9. Cell Rep (2017) [PMID: 29186682]
10. Int J Biochem Cell Biol (2019) [PMID: 30818082]
11. Cancer Lett (2017) [PMID: 28716526]
12. Mol Cell (2017) [PMID: 29174924]
13. Cell (2019) [PMID: 30735636]
14. RNA Biol (2020) [PMID: 32783578]
15. Nature (2009) [PMID: 19136943]
16. Proc Natl Acad Sci (2011) [PMID: 21571633]
17. Science (2018) [PMID: 30262497]
18. Trends Biotechnol (2021) [PMID: 32620324]
19. Life Sci Alliance (2019) [PMID: 31142640]
20. Cell Res (2019) [PMID: 31606733]
21. Mol Cell (2024) [PMID: 38569554]

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