



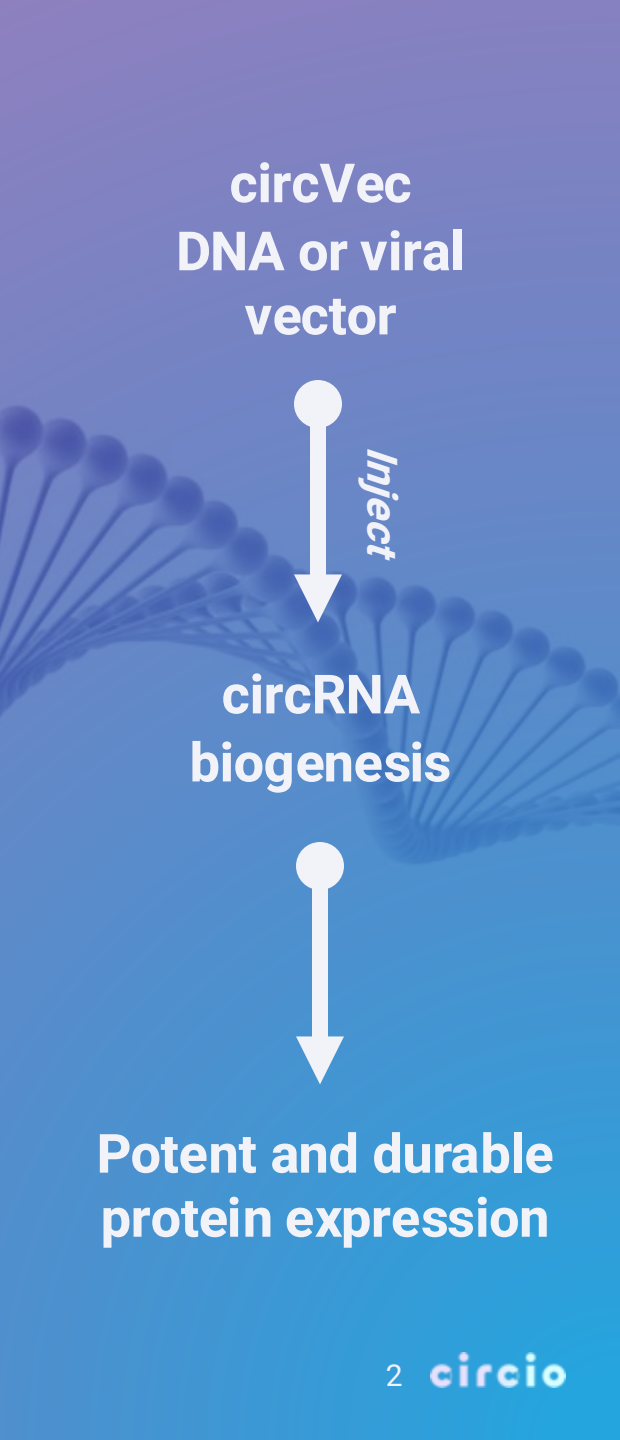
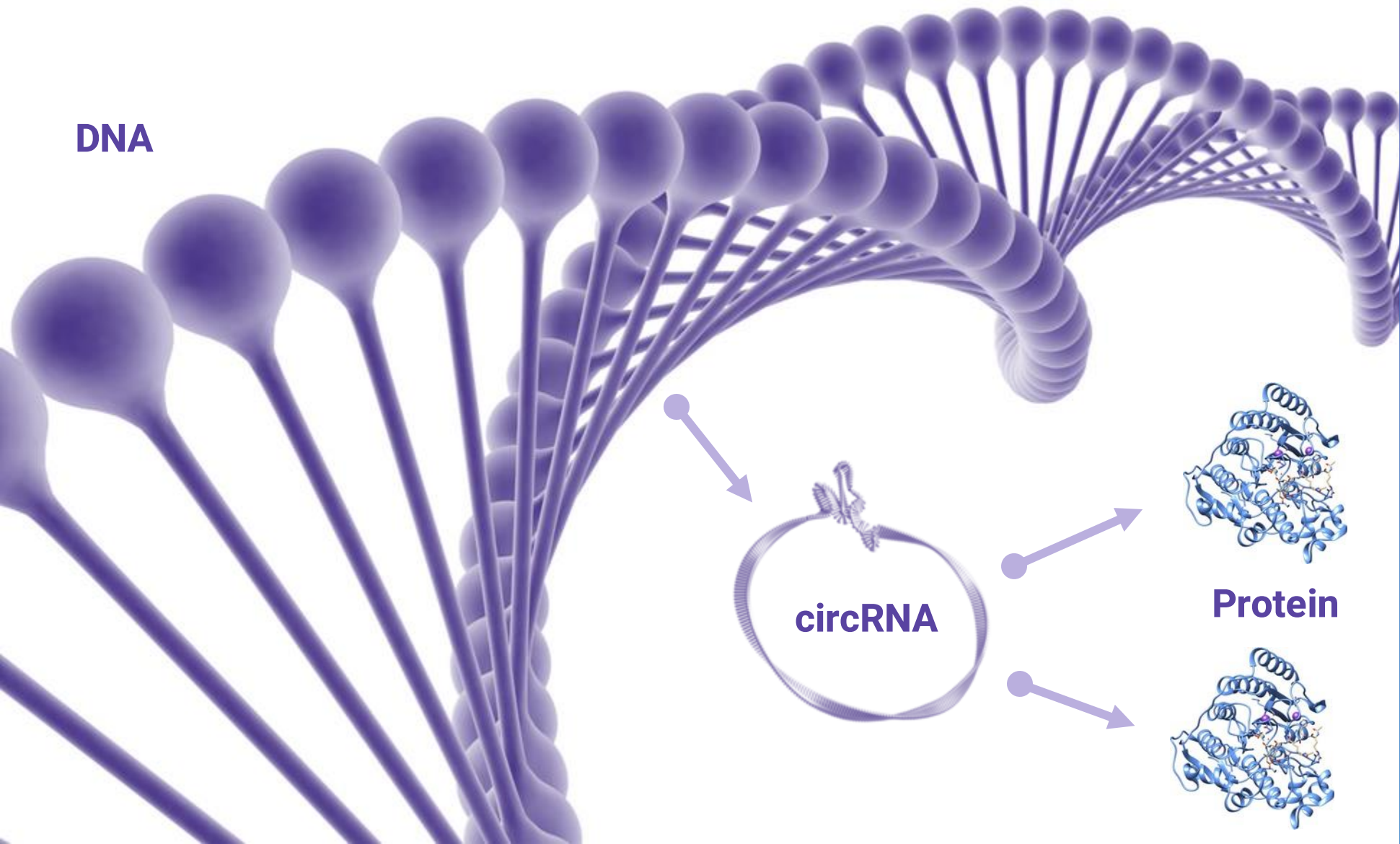
circio

**In vitro and in vivo
performance of circVec**

**a vector-based circular RNA expression platform
for enhanced gene therapy**

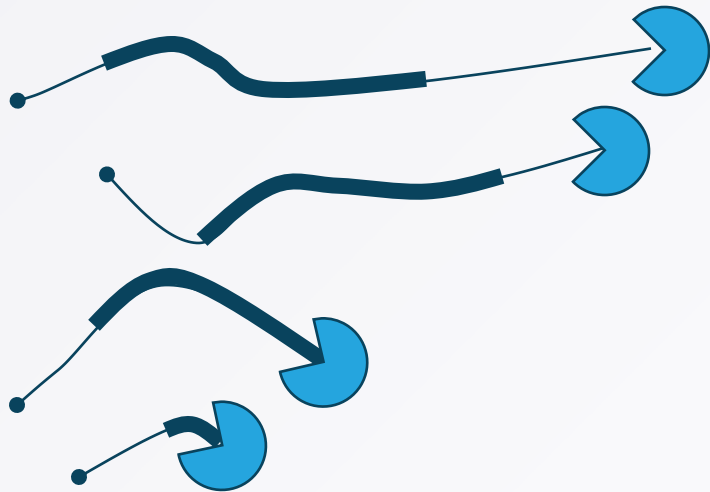
Dr. Thomas Hansen, CTO
ESGCT 2024, Rome

The unique circVec expression system: Turning the patient's cells into circRNA factories

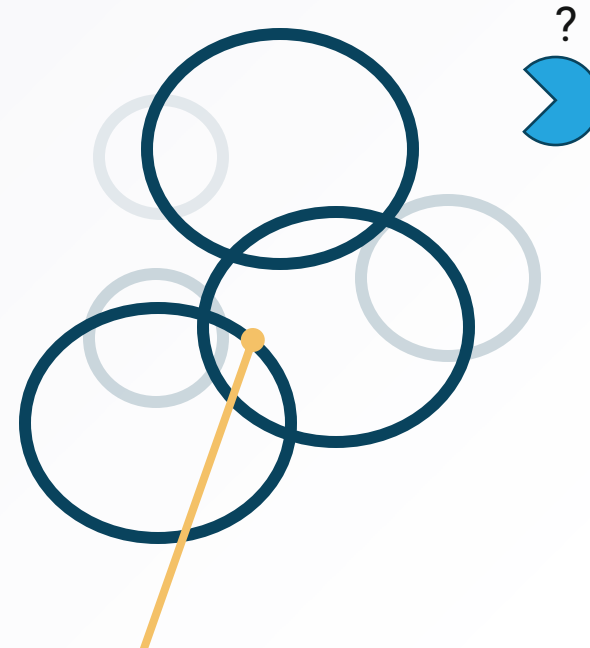


Why use circular RNA?

mRNA



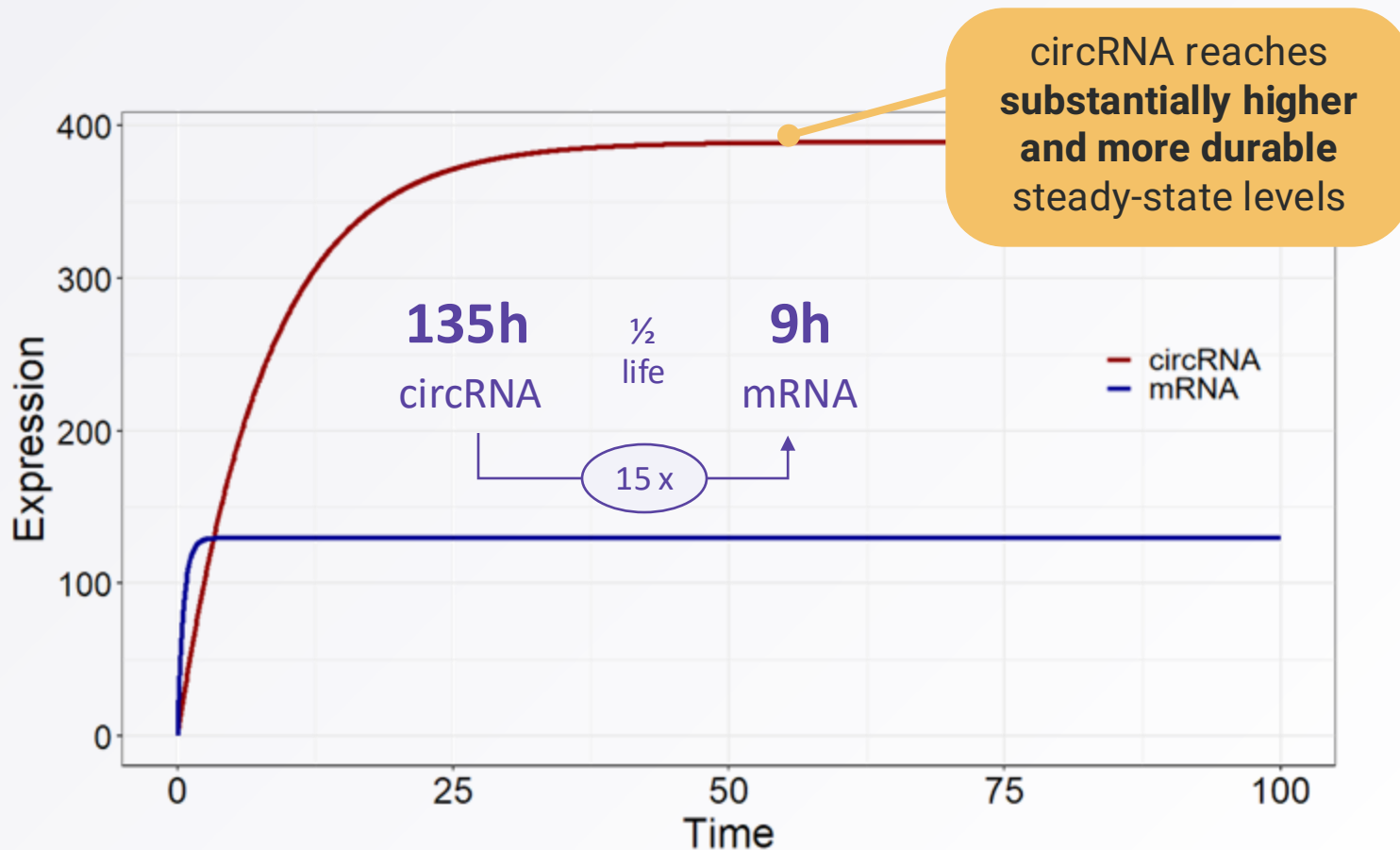
circRNA



circRNA is **resistant** to exonucleolytic decay and consequently very **stable and long-lived** within cells

Bioinformatic simulation demonstrating advantage of vector-expressed circRNA vs. mRNA

Temporal vector-based RNA expression dynamics; circRNA vs. mRNA



Input assumptions for simulation:

Non-dividing target cells

mRNA production: 10 molecules / hr

mRNA half-life: 9 hrs *

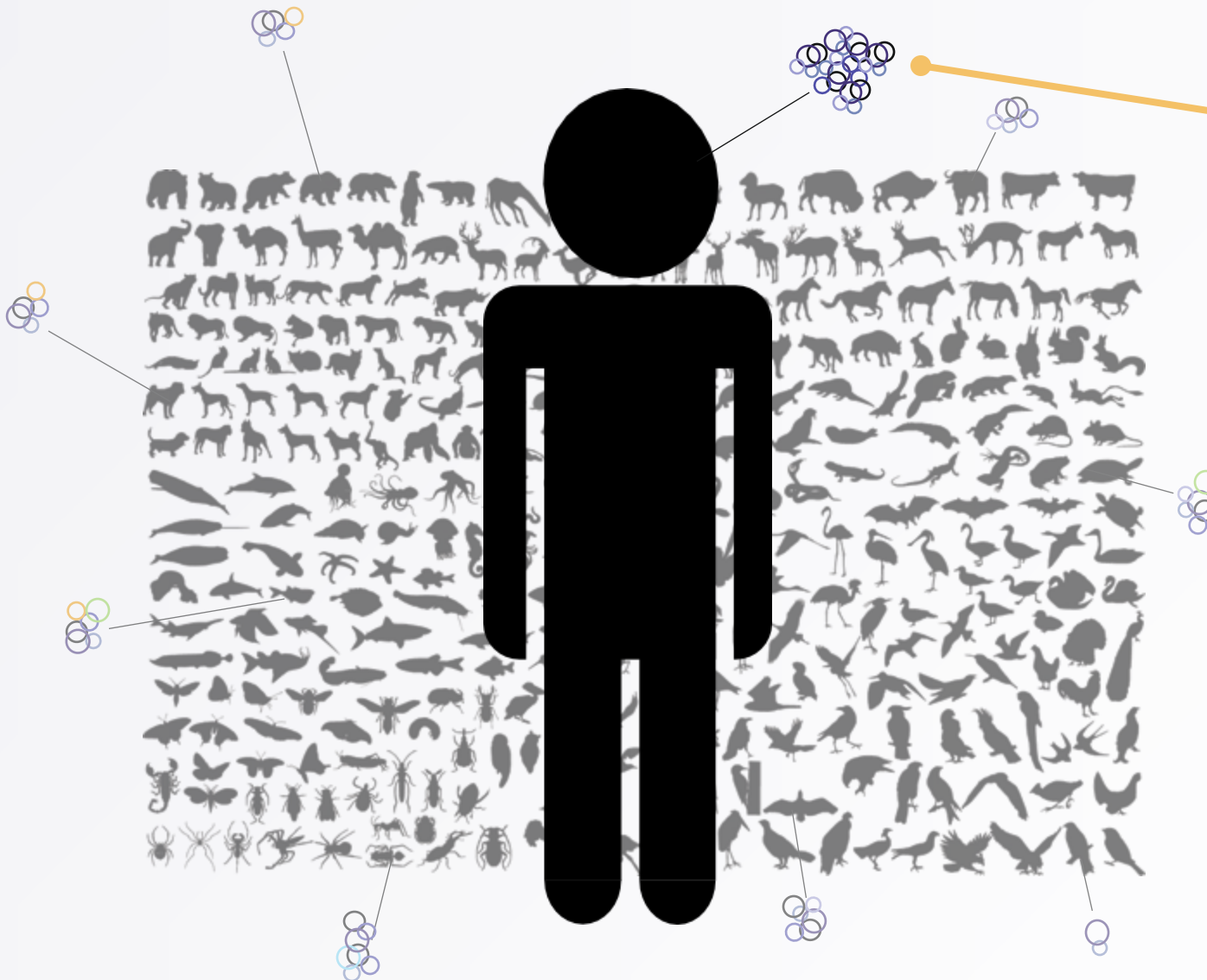
circRNA production: 2 molecules / hr
20% of mRNA rate

circRNA half-life: 135 hrs *
15x mRNA $t_{1/2}$ -life

→ circRNA translation **5x mRNA rate*** gives **>25x peak protein expression**

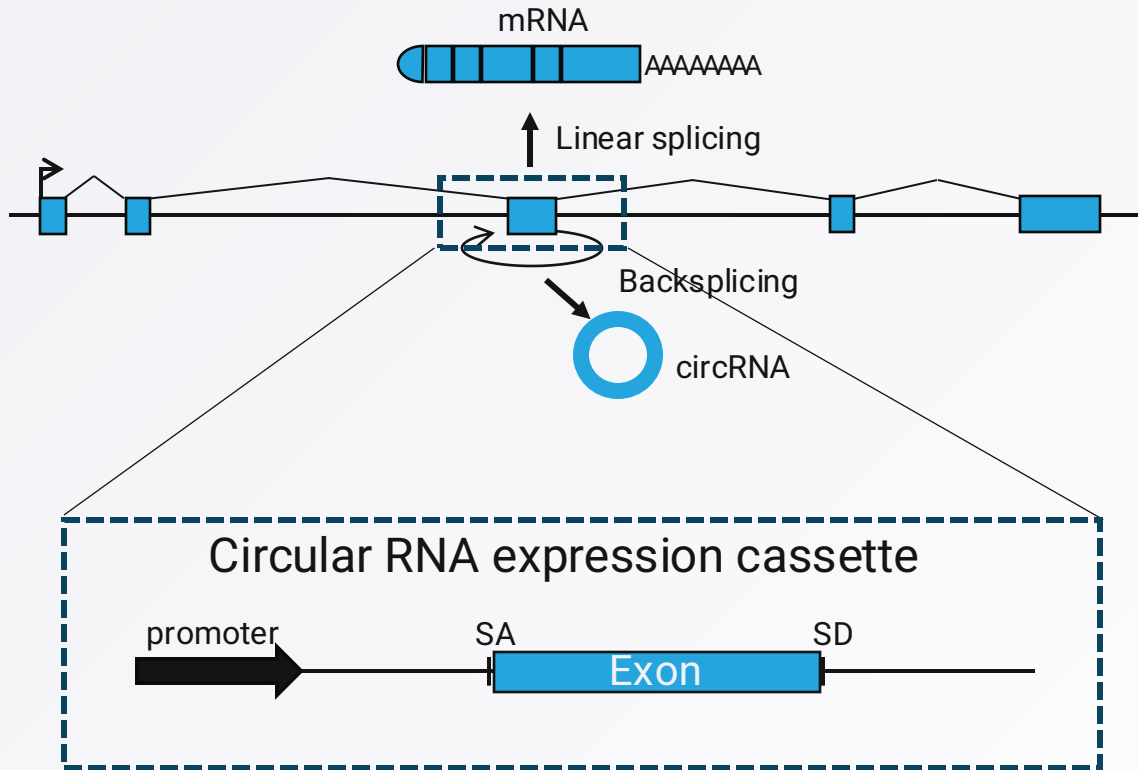
* Based on circVec experimental data

Circular RNA – a natural design

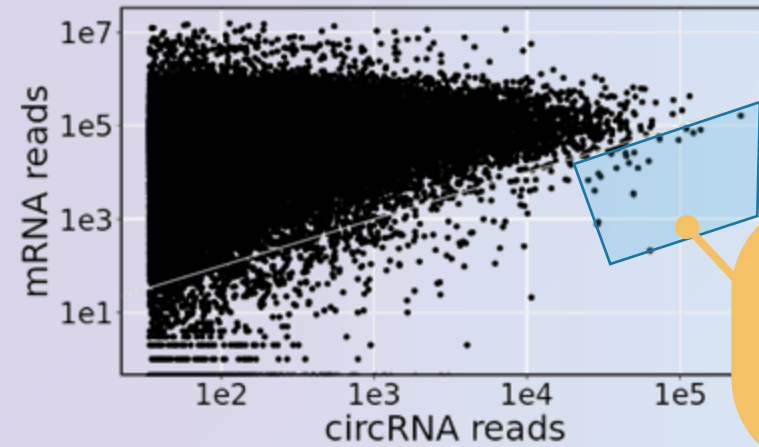


Widespread natural prevalence of circRNA in all eukaryotes, particularly in humans

circVec is based on nature's best design



Expression of human endogenous circRNA NGS analysis of 300+ RNAseq datasets

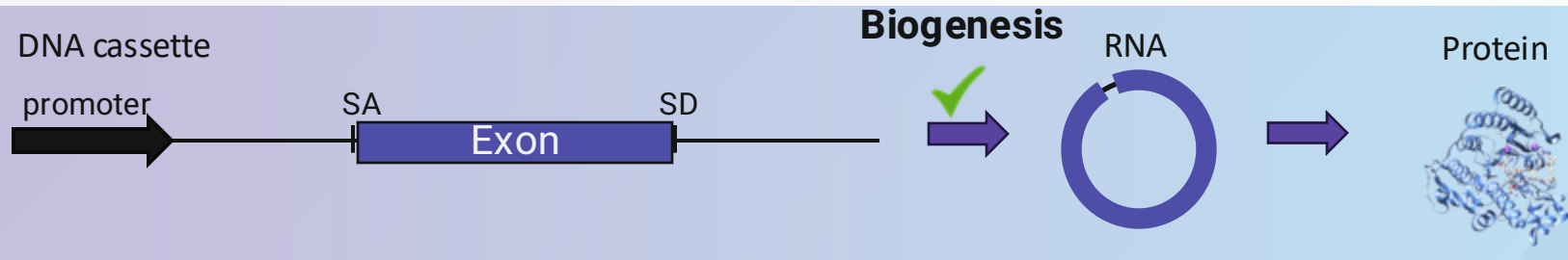


**Maximum
biogenesis-rate
of endogenous
circRNA**

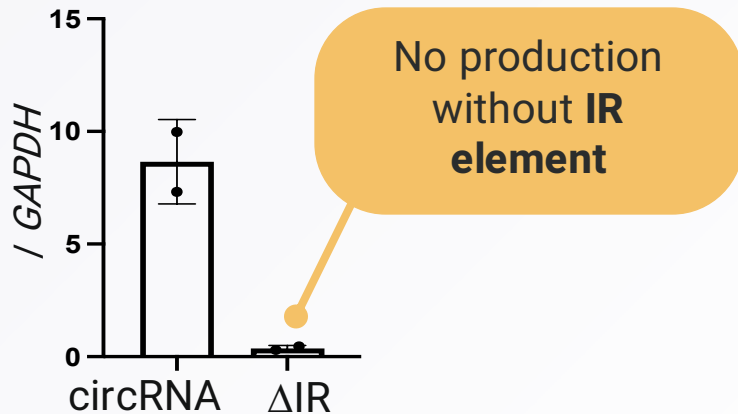
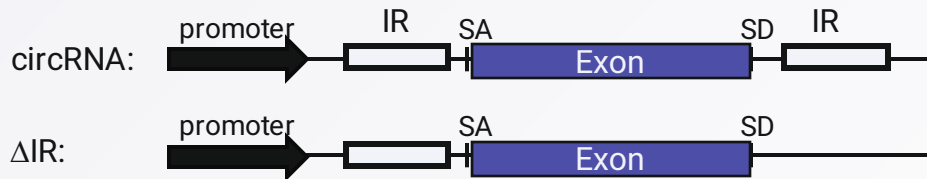
Screen and optimize the most effective loci in the human genome.

Establishing circRNA 1.0 expression

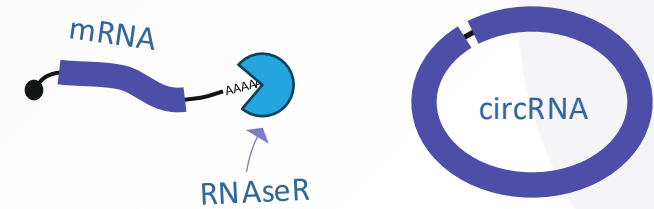
1 – Establish circVec



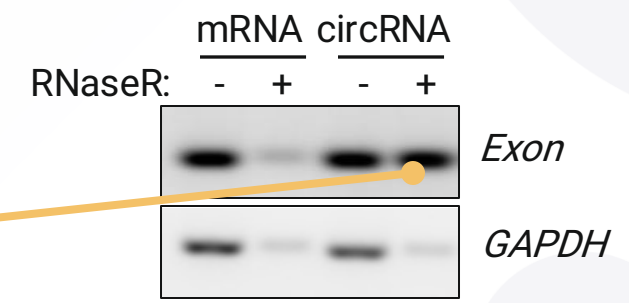
IR-dependent signal



RNaseR resistant

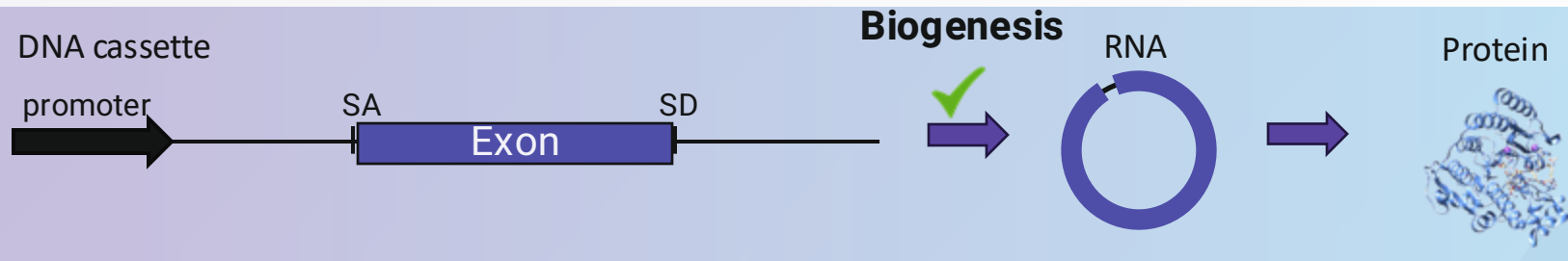


Resistance towards exonucleolytic degradation

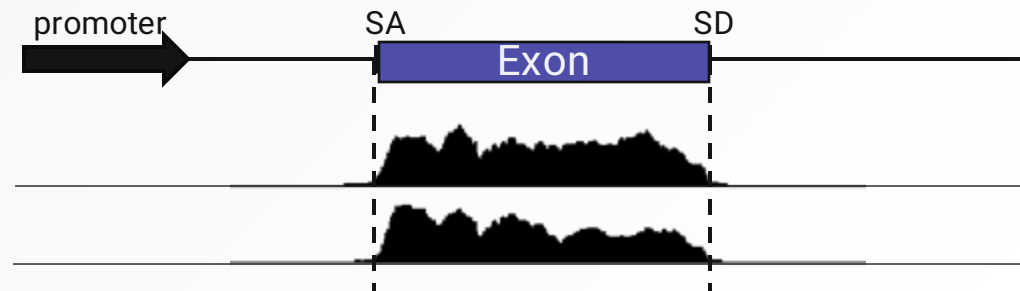


Establishing circRNA 1.0 expression

1 – Establish circVec

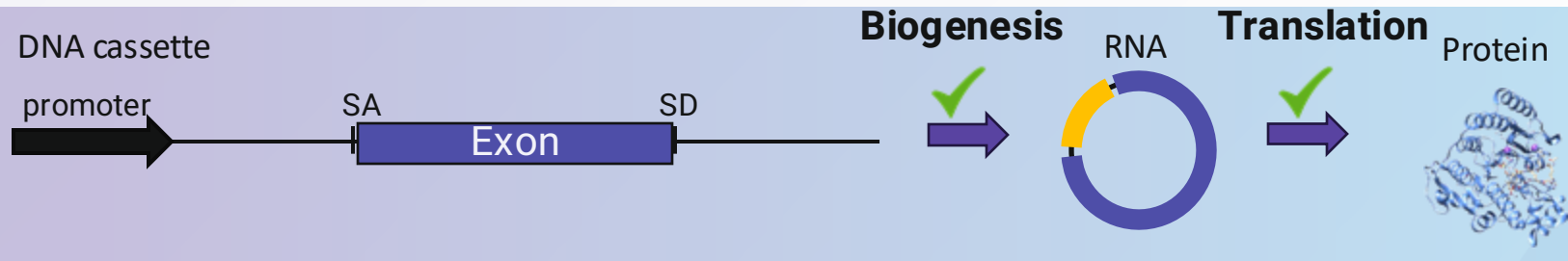


Clean expression, RNAseq



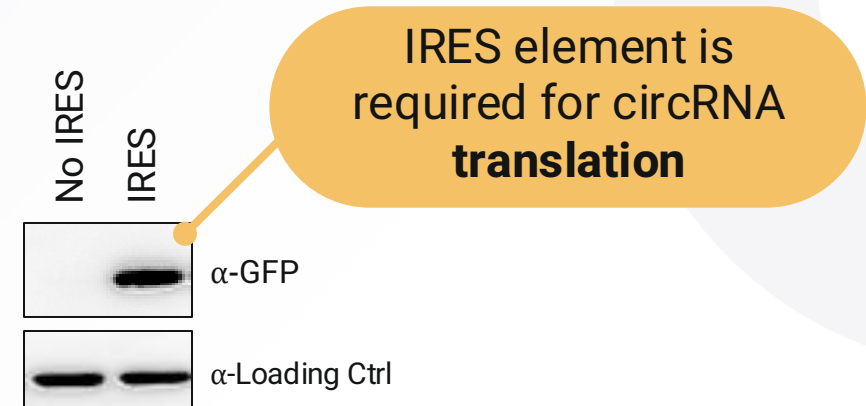
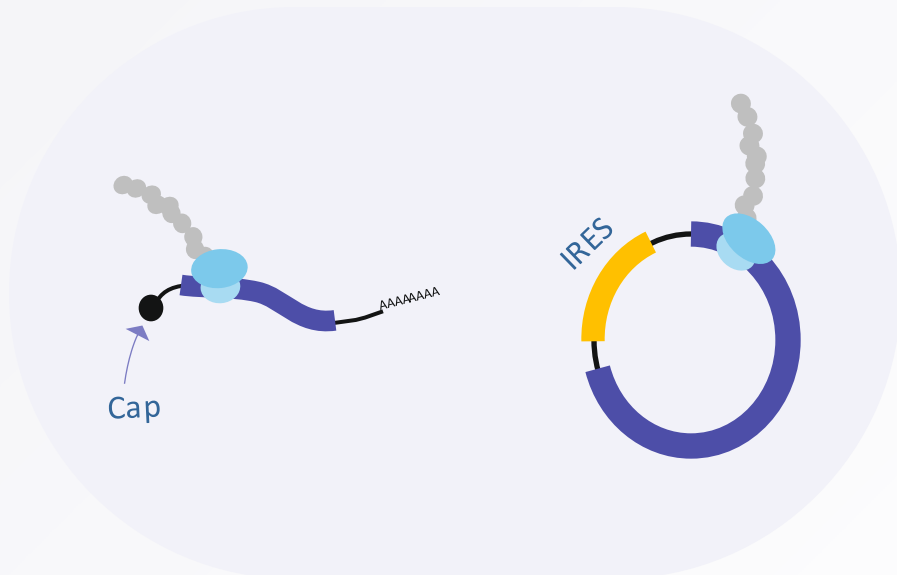
Establishing circRNA 1.0 expression

1 – Establish circVec



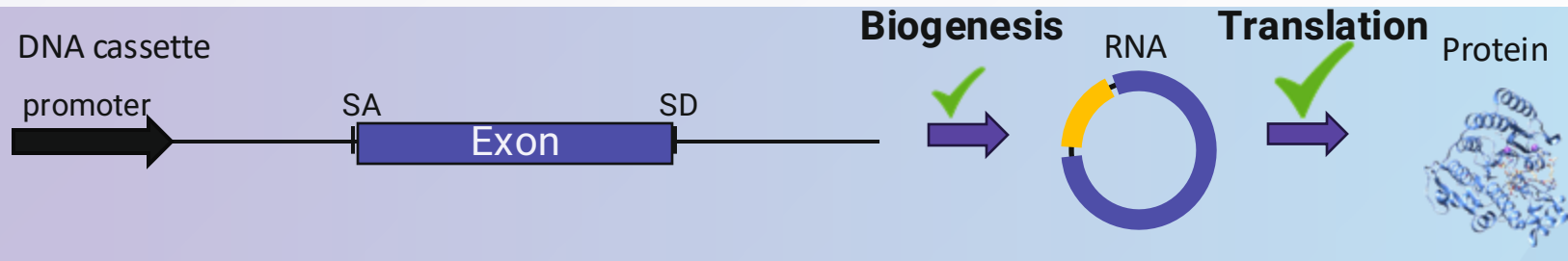
circRNAs are translated in a **cap-independent manner**

Protein expression,
Western blot

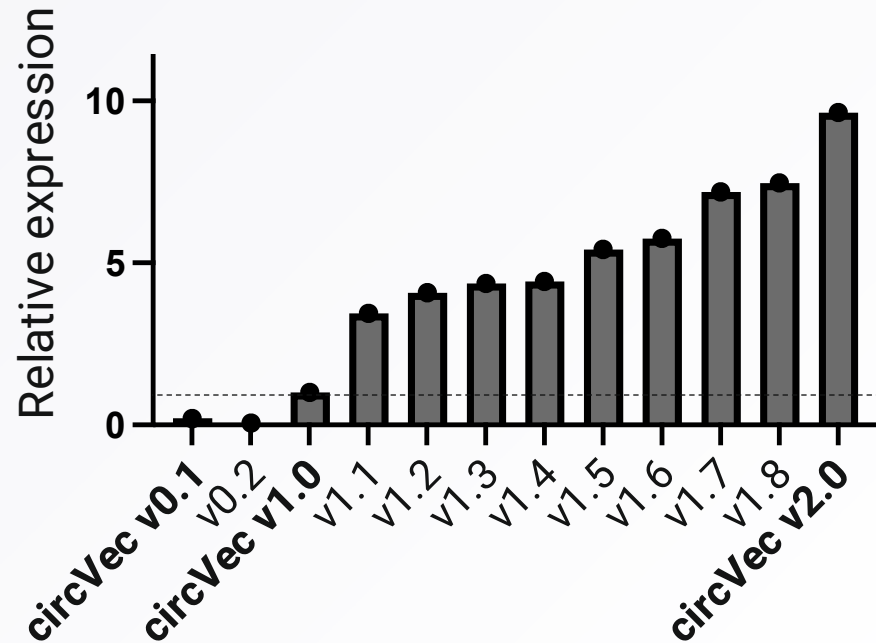


circVec 2.0: IRES optimization has resulted in >10x improvement in protein expression vs. v1.0

2- IRES selection



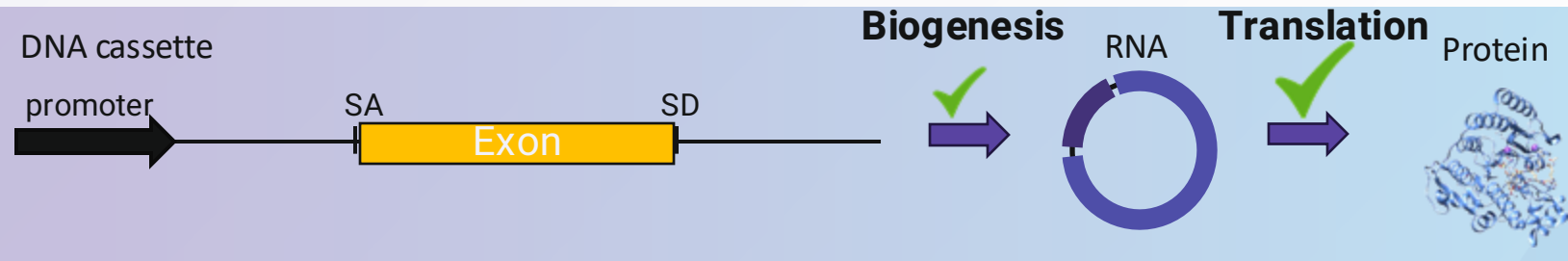
circVec IRES optimization,
protein expression level @48h post-transfection



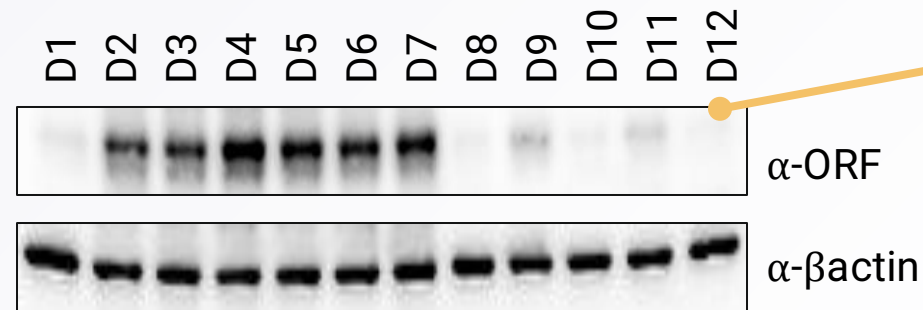
IRES screening and optimization has yielded 10x improved protein expression for circVec 2.0 vs 1.0 design

Correct cassette design is critical for circRNA-derived protein expression

3 – Cassette design



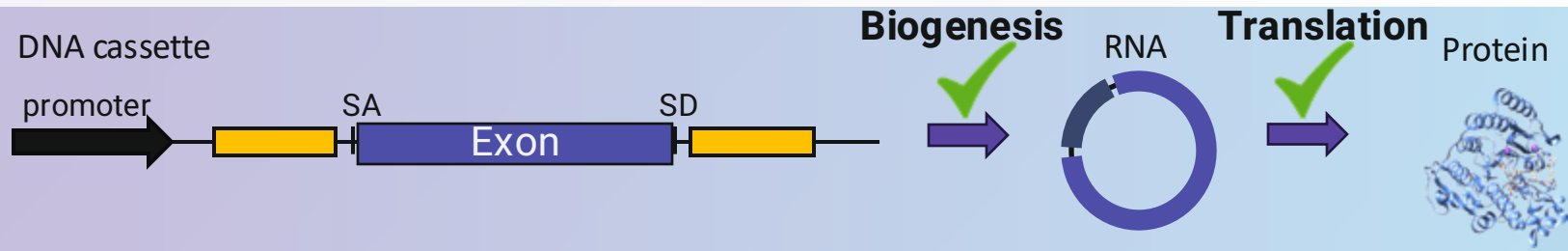
Cassette design screen, western blot



High protein yield only observed with **optimal cassette design**

... and with optimized circRNA biogenesis, circVec2.x matches mRNA expression at 48 hours post-transfection

4 – Biogenesis



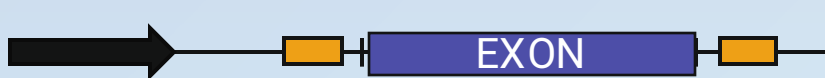
circVec1.0



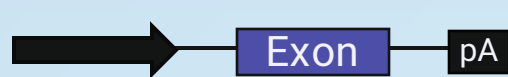
circVec2.0



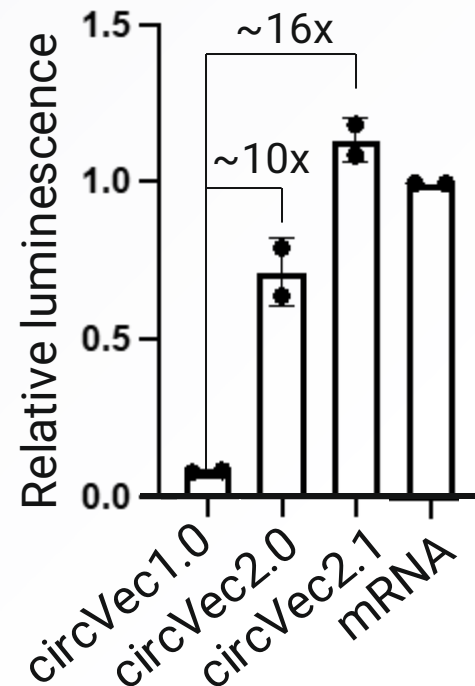
circVec2.1



mRNA



Protein expression



circVec 2.1 achieves 15x prolonged circRNA half-life and increased protein expression vs. mRNA in vitro

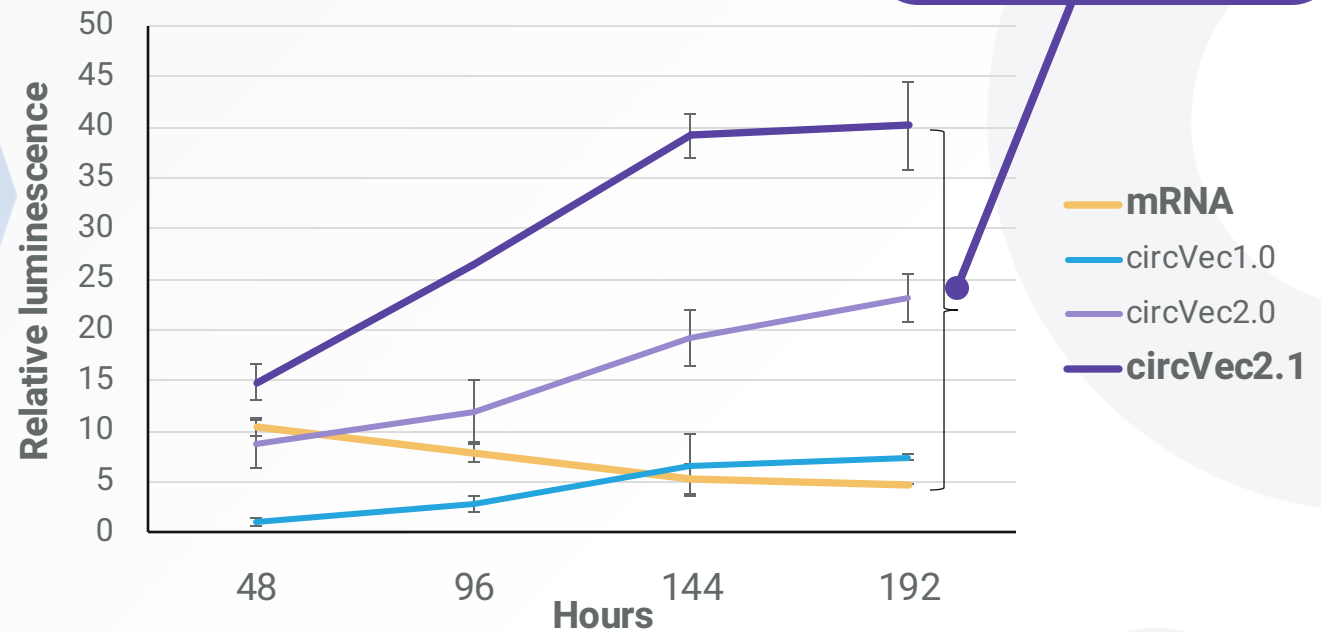
circVec RNA stability

RT-qPCR, nascent vs. total RNA

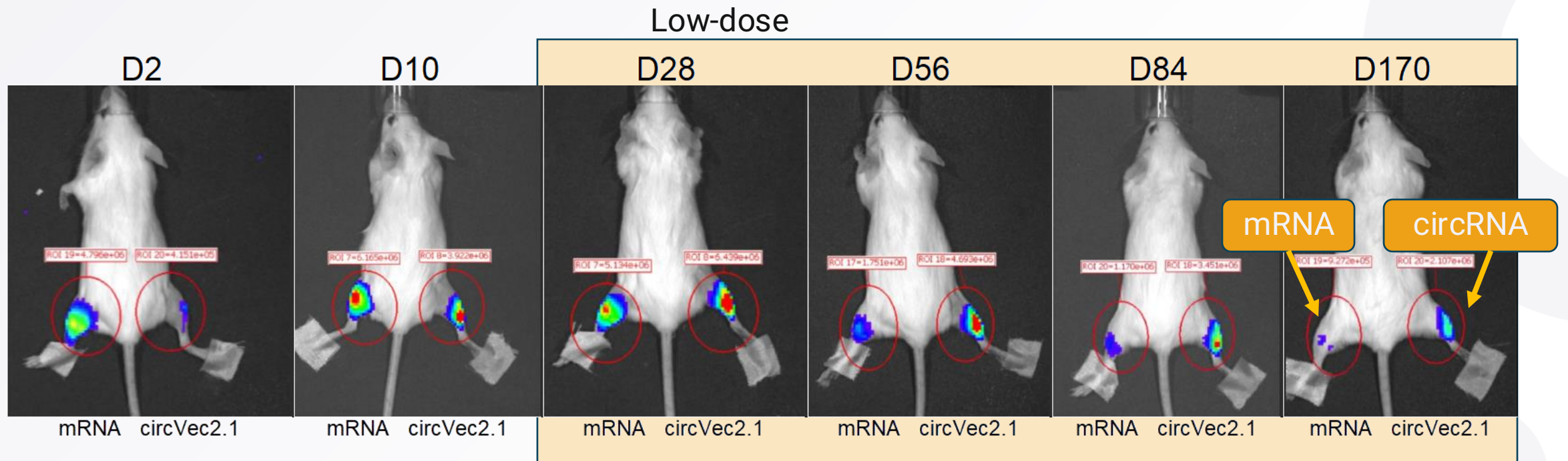
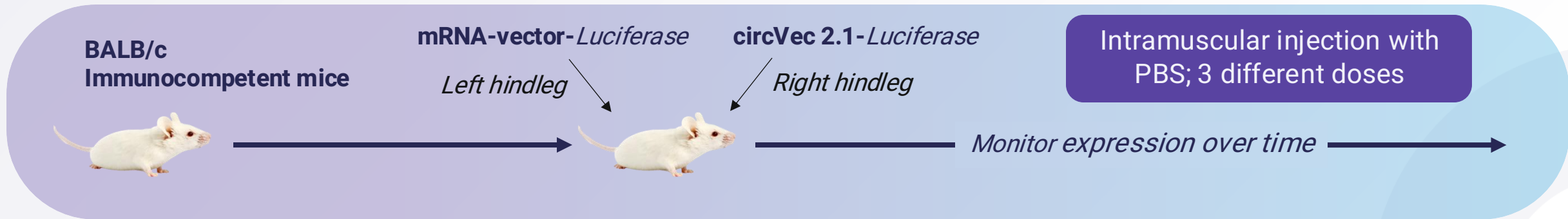
135h vs. **9h**
circRNA vs. mRNA

15x

circVec vs. mRNA luciferase reporter expression; time course

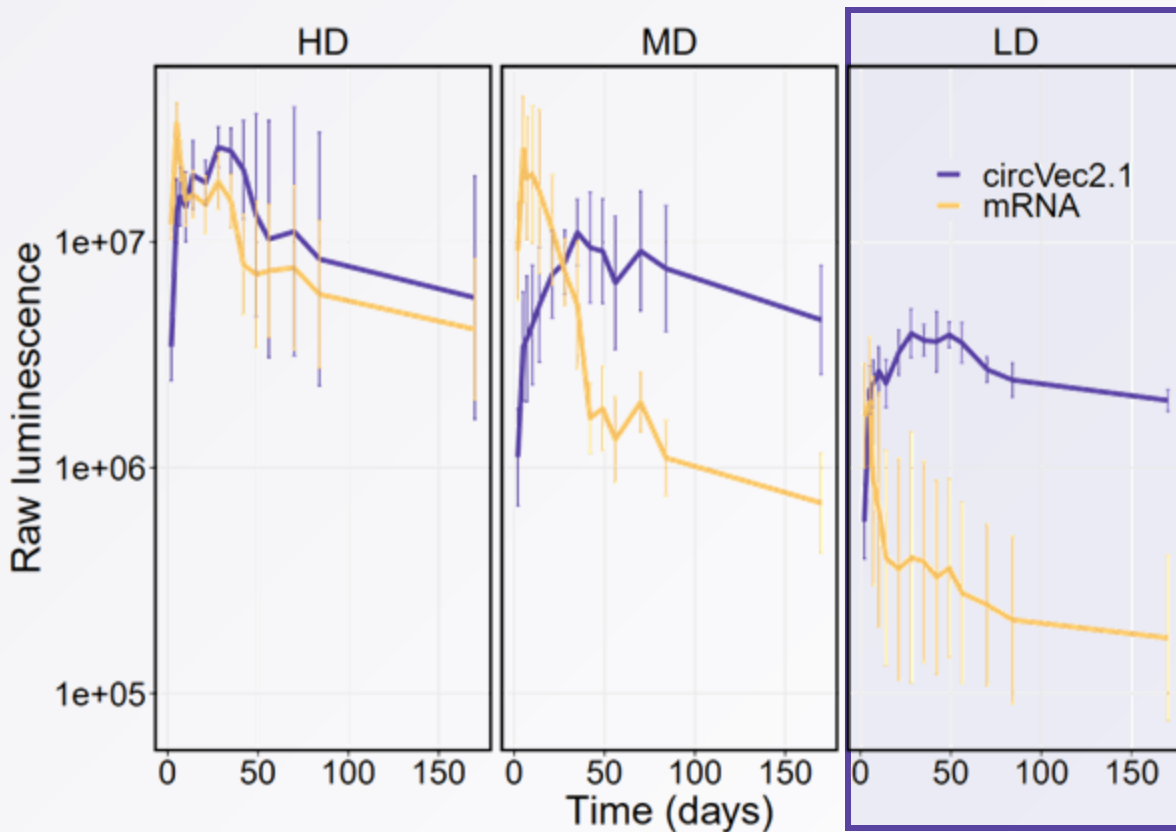


circRNA vs. mRNA expression in immunocompetent mouse muscle

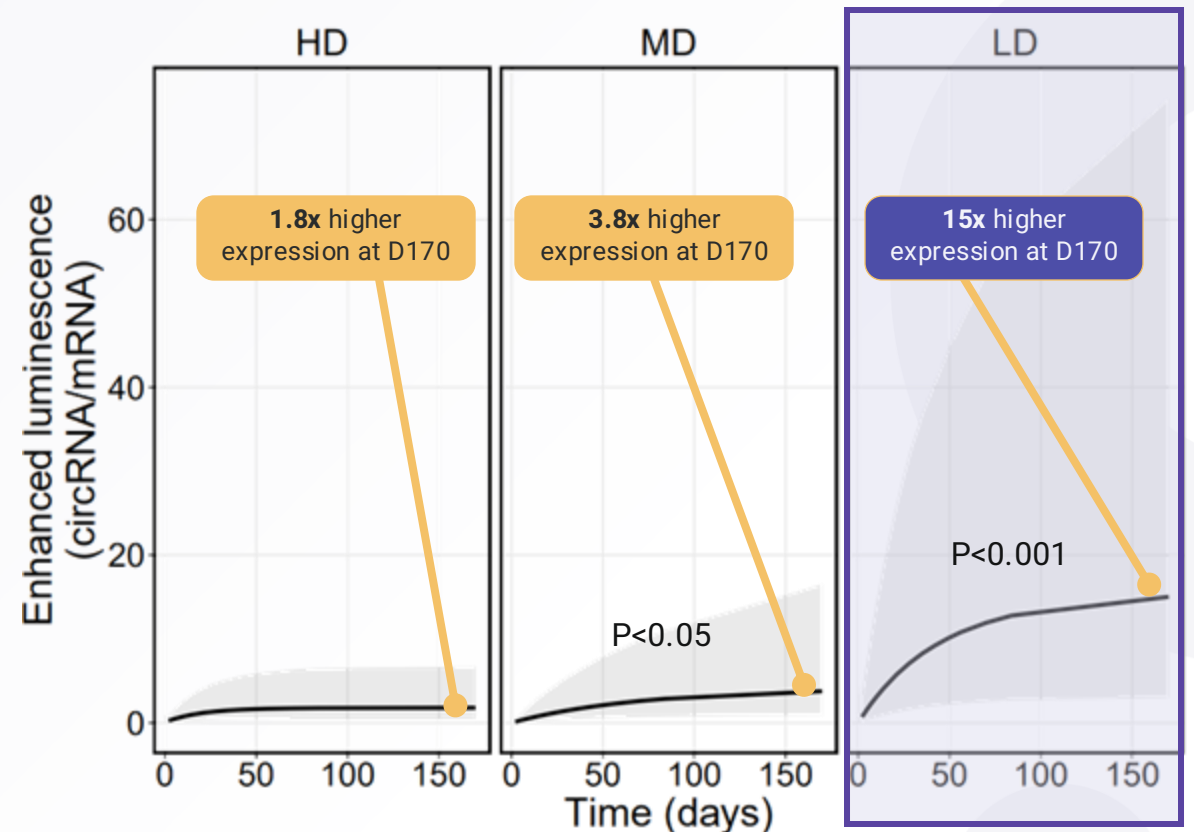


circVec in vivo advantage is enhanced at lower dose levels, up to 15x higher expression than mRNA

Absolute expression (luminescence)
circVec 2.1 vs. mRNA pDNA vector expression



Relative expression (luminescence)
-fold change circVec 2.1 vs. mRNA expression



Summary - circVec has the potential to transform how proteins are expressed in therapeutic settings

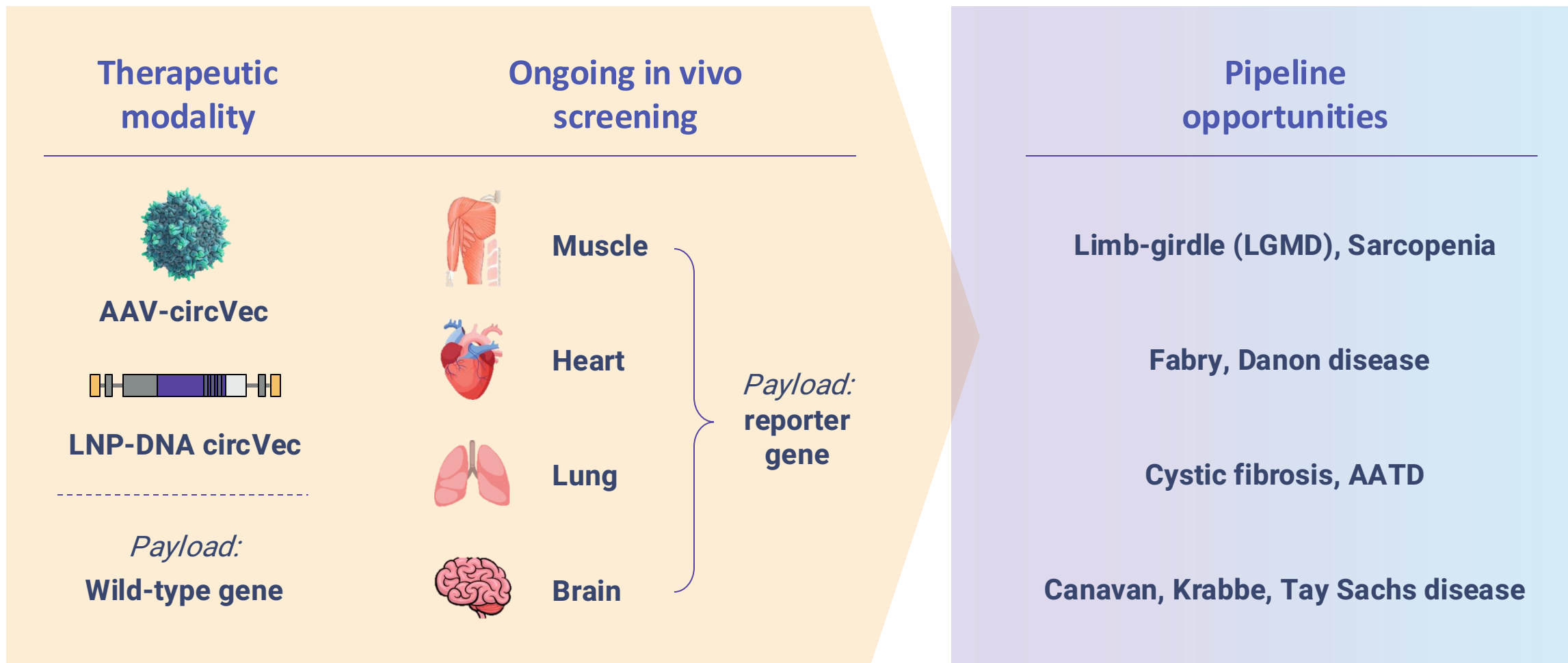
- **Superior stability** leads to accumulation of circRNA inside the cell, resulting in **higher and prolonged protein expression vs. mRNA**
- **circVec is significantly more durable and achieves up to 15x higher expression in vivo** compared to standard mRNA-based vector expression
- Choice and composition of **IR, IRES and ORF cassette design is critical for high yield expression**



Consequently, circVec may provide **higher clinical benefit** or allow for **dose-sparing**

Gene therapy development plan

Modality and disease to be selected based on experimental data



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Dr. Erik D. Wiklund

Mr. Ola Melin

Karolinska Institute:

Prof. Michael Uhlin

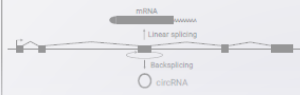
circio Optimization of in vitro and in vivo performance of circVec, a vector-based circular RNA expression platform for enhanced gene therapy

PosterID: P0947
Presented at ESCGT 2024

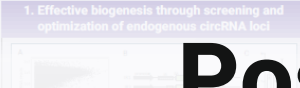
O'Leary ET^{1,2}, Zhang J^{1,2}, Hjelmqvist D^{1,2}, Vikberg S^{1,2}, Wiklund ED¹, Levitsky V¹, Hansen TD¹
1. Circio AB, Huddinge, Sweden
2. Karolinska Institutet, CLINTEC, Huddinge, Sweden

Introduction


Circular RNA (circRNA) constitutes a novel class of endogenously expressed RNA. CircRNAs are generated by a non-linear splicing event in which an upstream splice acceptor attacks a downstream donor, known as backsplicing. In contrast to mRNA, circRNAs are resistant to exonucleolytic decay which results in high intracellular stability and persistence. Here, we show our development of a circRNA expression platform, termed circVec, towards improved vector-based expression of proteins in vivo.




1. Effective biogenesis through screening and optimization of endogenous circRNA loci



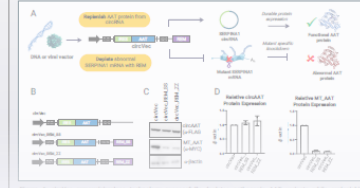
2. Superior circRNA stability confers enhanced protein expression



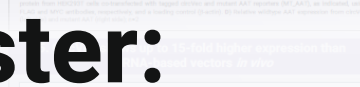
3. circRNA stability confers enhanced protein expression



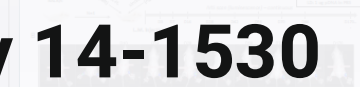
4. Bimodal circVec successfully depletes pathogenic transcripts while expressing function proteins



5. Superior circRNA stability facilitates circRNA accumulation and prolonged protein expression




6. Further improvement of circVec-derived yield by proprietary codon optimization approach



Conclusions

- Superior circRNA stability facilitates accumulation of circRNA and prolonged protein expression.
- CircVec shows enhanced and accumulating luminescence *in vivo* compared to vector-based mRNA expression.
- Choice and composition of IR and IRES/ORF is crucial for high yield expression.
- Bimodal design effectively depletes pathogenic RNA and rescues expression of functional protein
- Codon optimization outperformed available algorithms and further enhances protein yield from circVec.

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**Poster:
Thursday 14-1530
#P0947**