



# Platform Development of CircRNA-based Protein Expression vectors

Life Science Investor Conference, 2023

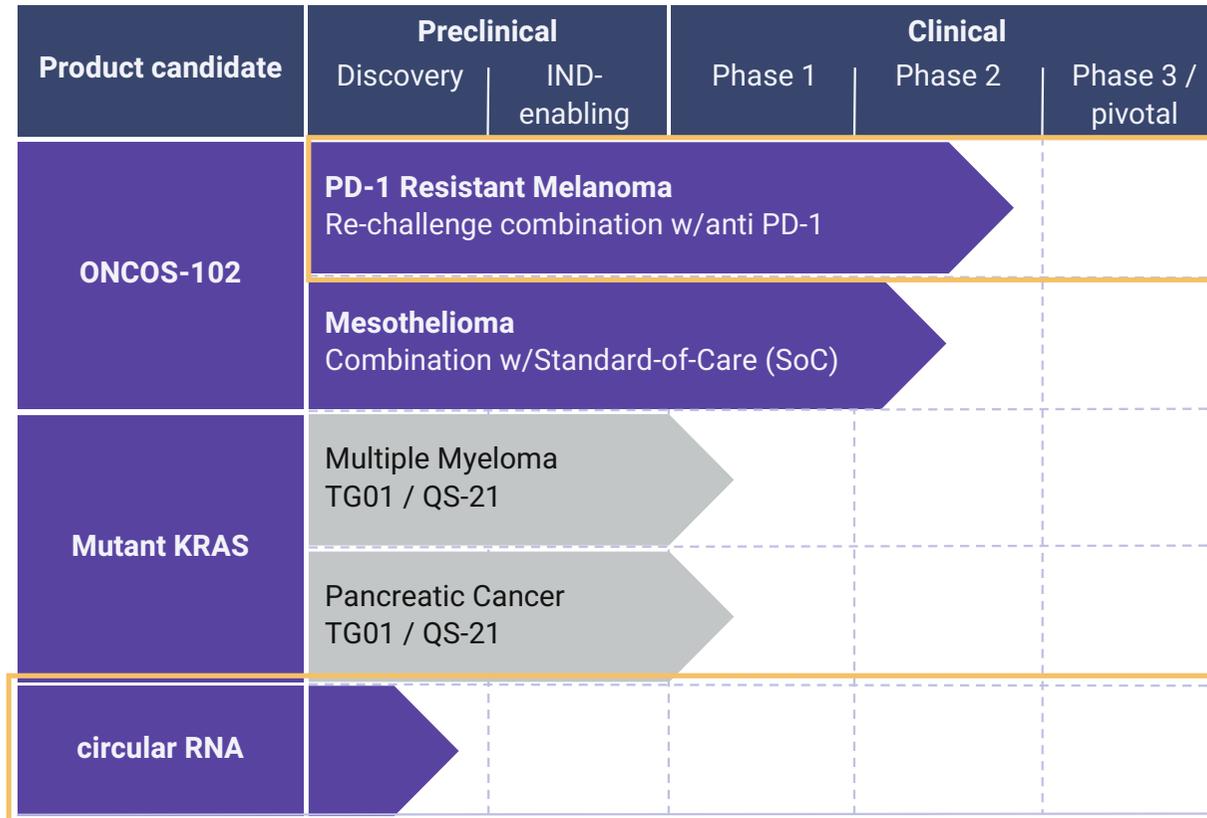
Thomas Hansen  
Head of Research

# Important notice and disclaimer

This report contains certain forward-looking statements based on uncertainty, since they relate to events and depend on circumstances that will occur in the future and which, by their nature, will have an impact on the results of operations and the financial condition of Circio and the Circio Group. Such forward-looking statements reflect the current views of Circio and are based on the information currently available to the company. Circio cannot give any assurance as to the correctness of such statements.

There are a number of factors that could cause actual results and developments to differ materially from those expressed or implied in these forward-looking statements. These factors include, among other things, risks or uncertainties associated with the success of future clinical trials; risks relating to personal injury or death in connection with clinical trials or following commercialization of the company's products, and liability in connection therewith; risks relating to the company's freedom to operate (competitors patents) in respect of the products it develops; risks of non-approval of patents not yet granted and the company's ability to adequately protect its intellectual property and know-how; risks relating to obtaining regulatory approval and other regulatory risks relating to the development and future commercialization of the company's products; risks that research and development will not yield new products that achieve commercial success; risks relating to the company's ability to successfully commercialize and gain market acceptance for Circio products; risks relating to the future development of the pricing environment and/or regulations for pharmaceutical products; risks relating to the company's ability to secure additional financing in the future, which may not be available on favorable terms or at all; risks relating to currency fluctuations; risks associated with technological development, growth management, general economic and business conditions; risks relating to the company's ability to retain key personnel; and risks relating to the impact of competition.

# Circio development pipeline



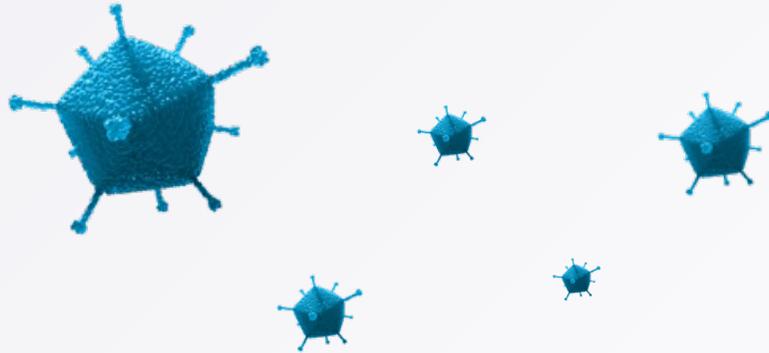
■ Trials run and financed by collaboration partners

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# ONCOS-102 in PD-1 Resistant Melanoma

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# ONCOS-102: intra-tumoral oncolytic immunotherapy

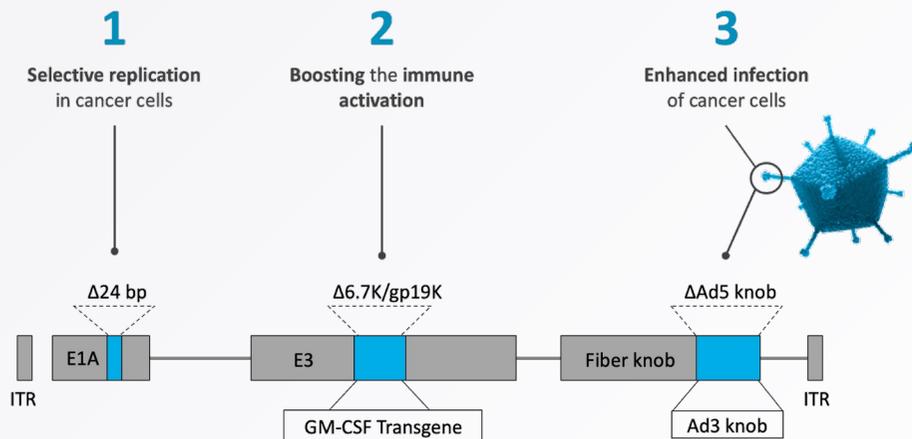


**Reverses** immuno-suppressive defence mechanisms in the tumor

**Primes** the patient's T-cells to target cancer cells

**Delivers** immune system stimulatory payloads

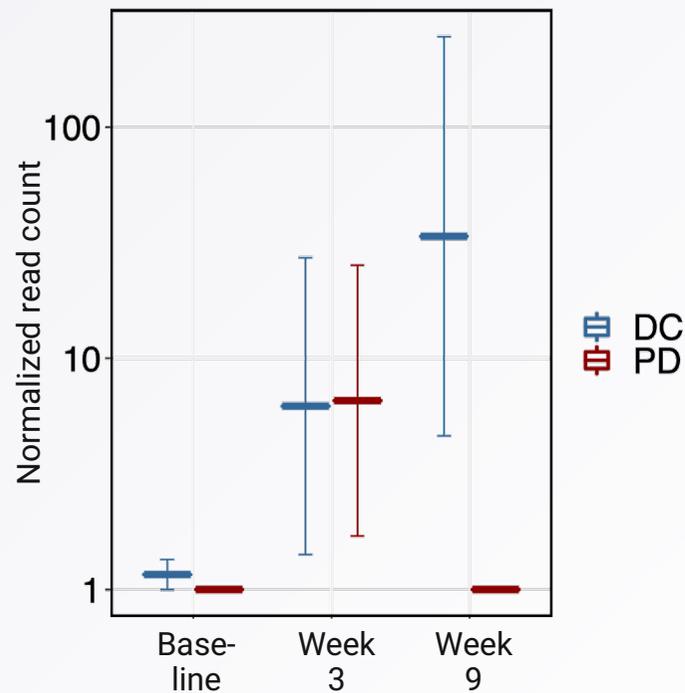
**Unblinds** the tumor to the immune system





# Persistence of payload expression correlate with outcome

**GM-CSF mRNA level in tumor biopsies,**  
Total RNA sequencing



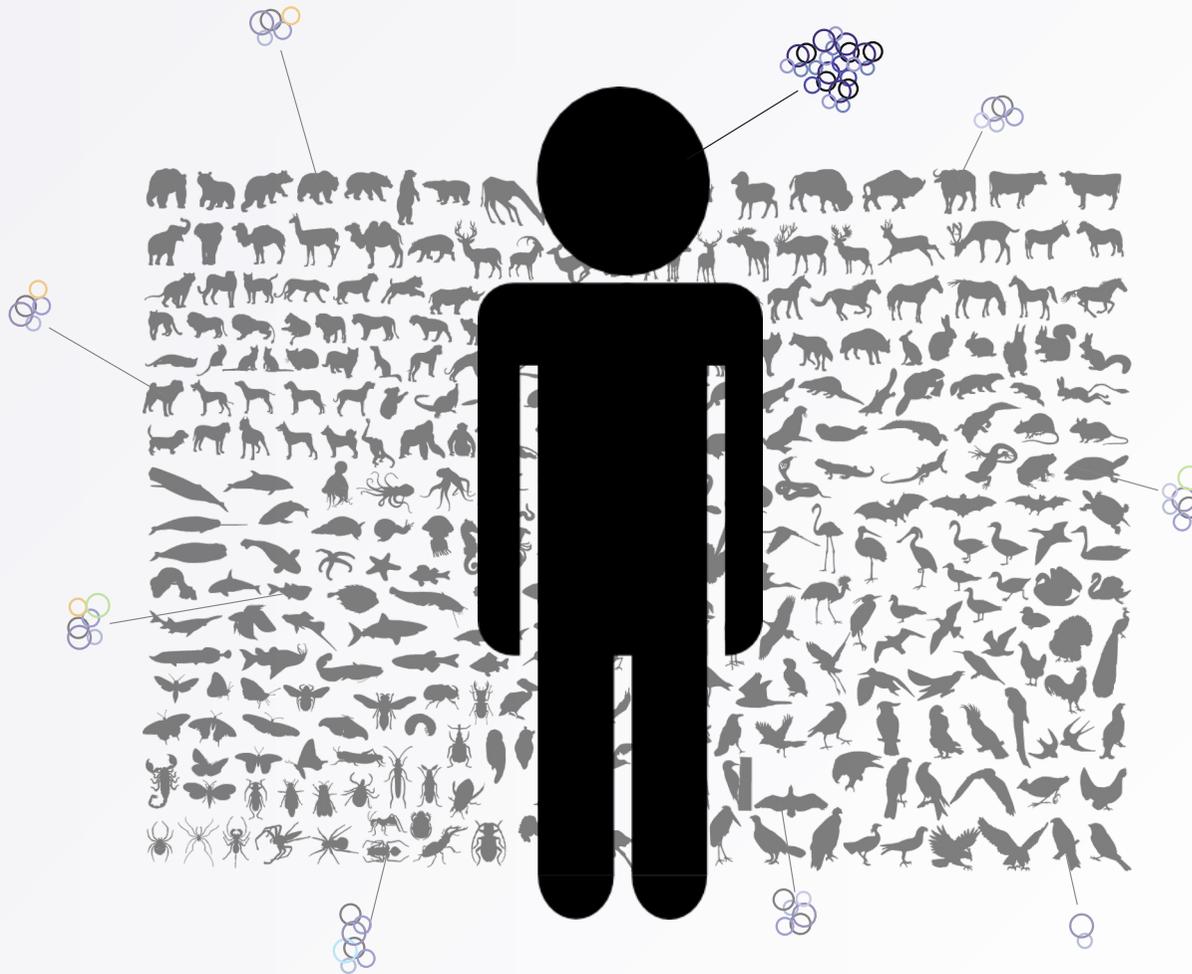
**How to prolong vector-based gene expression?**

2

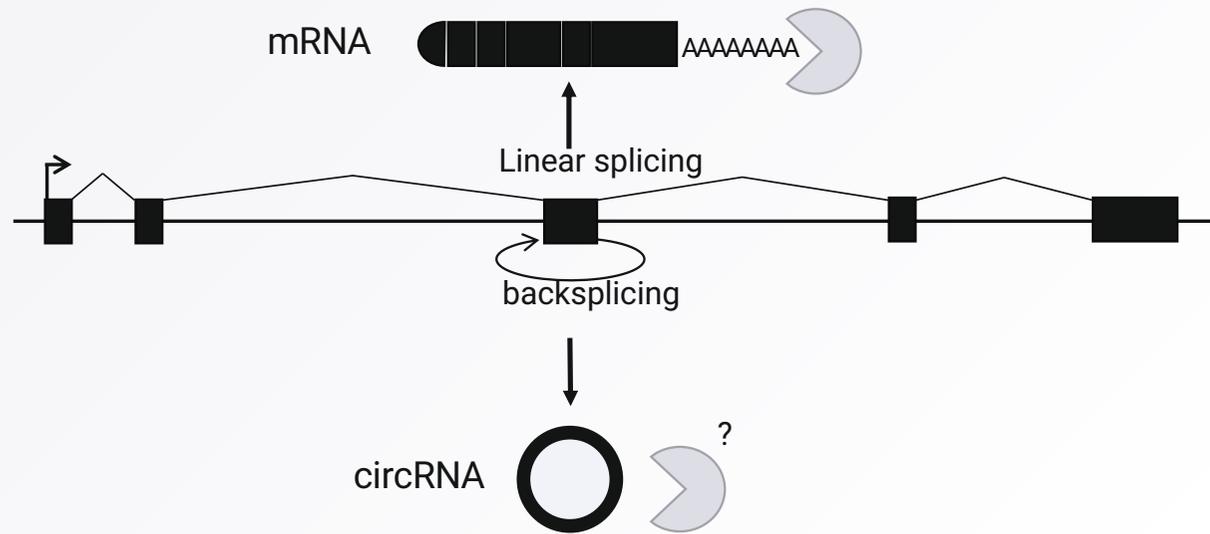
circRNA platform development

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# Circular RNA – a natural design



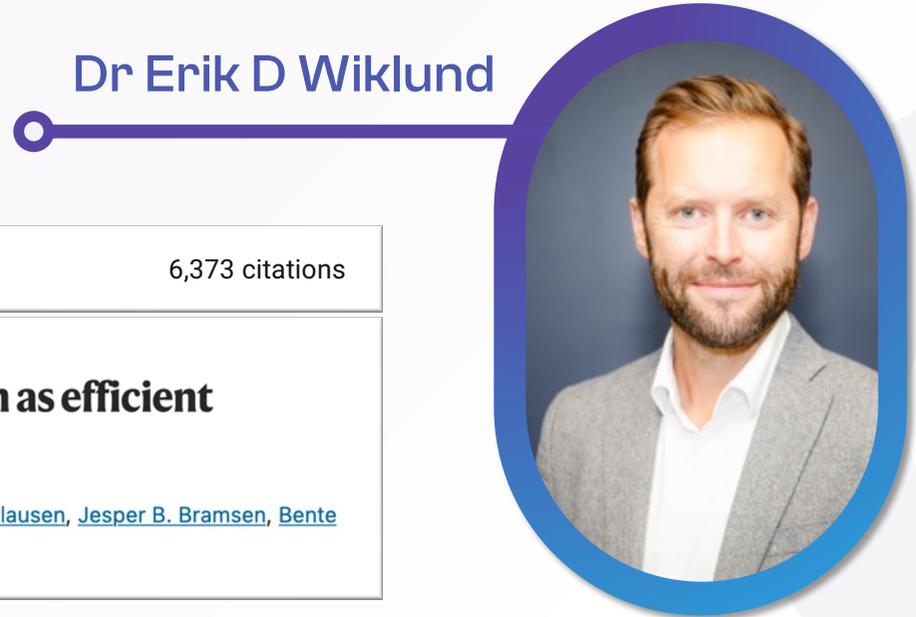
# circRNA and host genes



# The discoverers of circRNA work for Circio



Dr Thomas B Hansen



Dr Erik D Wiklund

**nature**

6,373 citations

Published: 27 February 2013

**Natural RNA circles function as efficient microRNA sponges**

[Thomas B. Hansen](#), [Trine I. Jensen](#), [Bettina H. Clausen](#), [Jesper B. Bramsen](#), [Bente Finsen](#), [Christian K. Damgaard](#) & [Jørgen Kjems](#)

THE EMBO JOURNAL | EMBOpress | 30 September 2011 | 922 citations

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**miRNA-dependent gene silencing involving Ago2-mediated cleavage of a circular antisense RNA**

[Thomas B Hansen](#), [Erik D Wiklund](#), [Jesper B Bramsen](#), [Sune B Villadsen](#), [Aaron L Statham](#), [Susan J Clark](#), [Jørgen Kjems](#)

**nature reviews genetics** | 2,291 citations

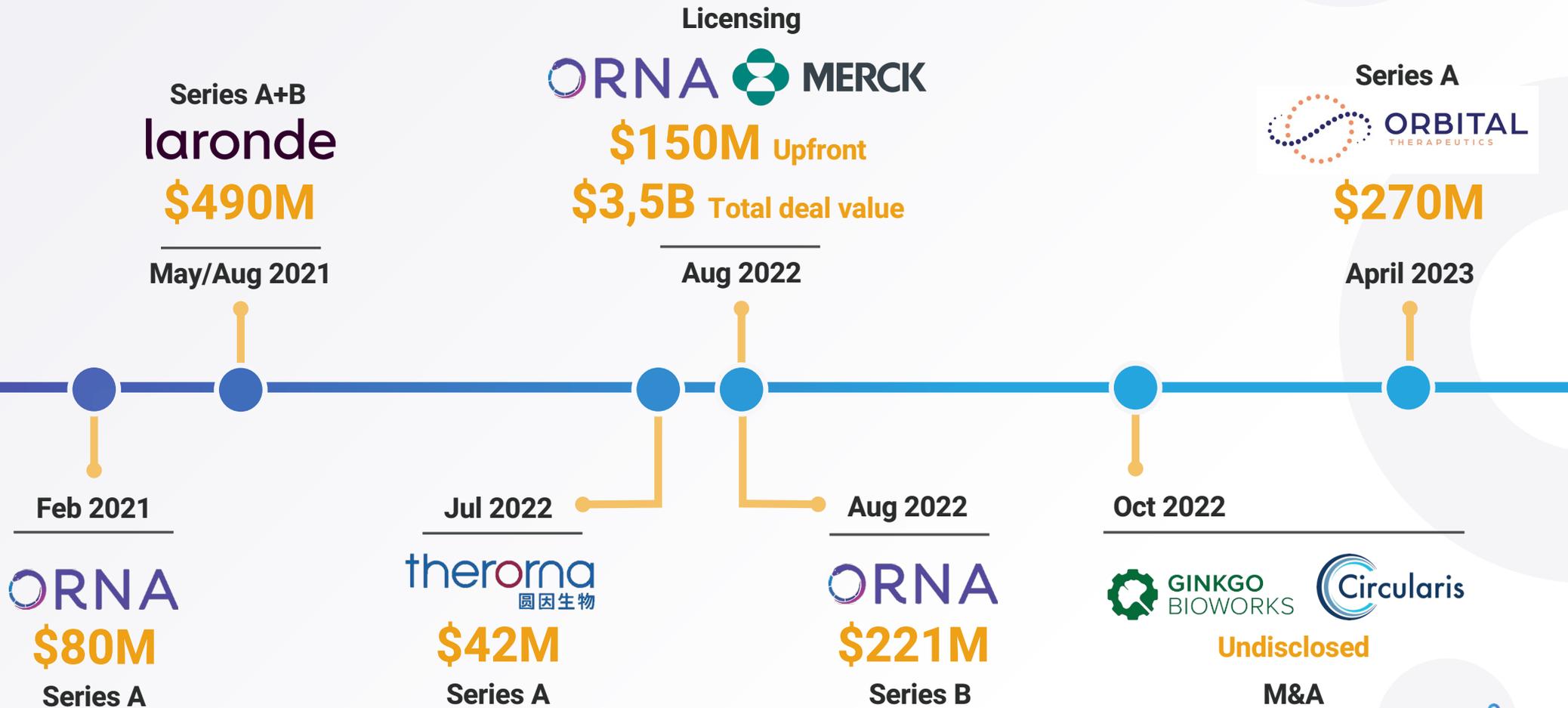
Review Article | Published: 08 August 2019

**The biogenesis, biology and characterization of circular RNAs**

[Lasse S. Kristensen](#), [Maria S. Andersen](#), [Lotte V. W. Stagsted](#), [Karoline K. Ebbesen](#), [Thomas B. Hansen](#) & [Jørgen Kjems](#)

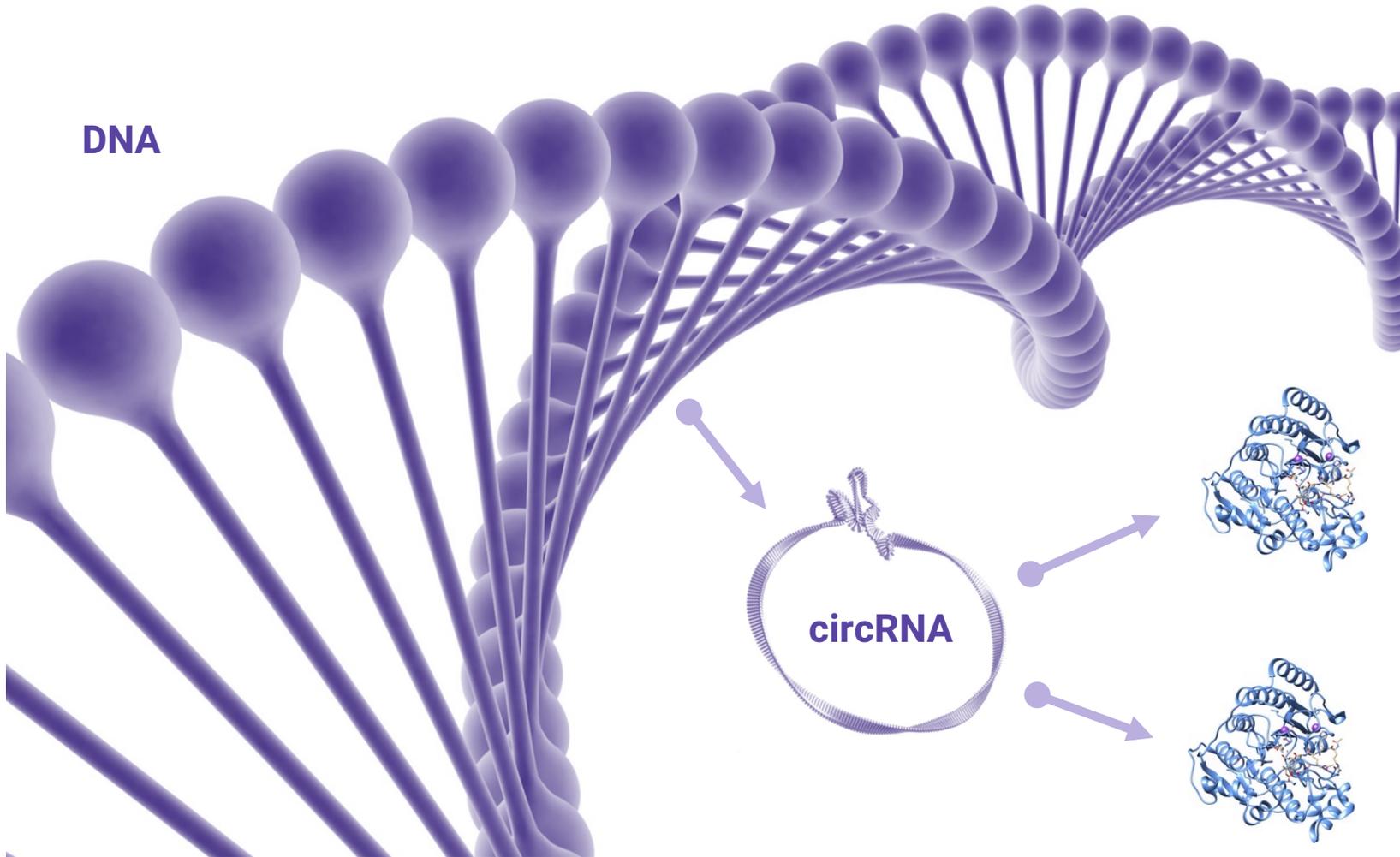


# circRNA is rapidly gaining momentum as an enhanced mRNA format



# circVec – Circio's proprietary vector system for in situ protein production

DNA



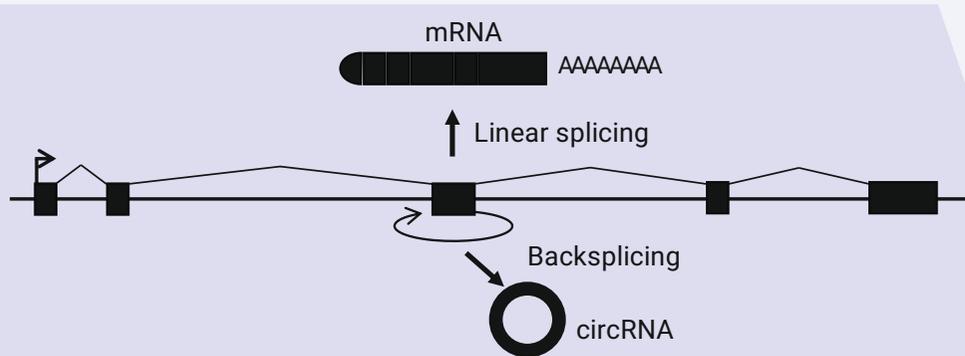
circVec  
DNA vector

Intracellular  
delivery

circRNA  
biogenesis

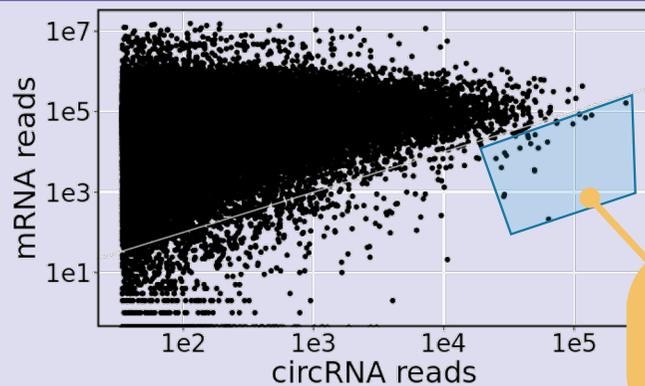
*in situ* protein  
production

# circVec starting point is based on nature's best design



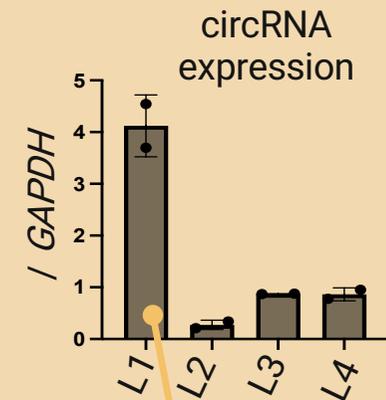
## Expression of endogenous circRNA

NGS analysis of 300+ RNAseq datasets



Maximum biogenesis-rate of endogenous circRNA

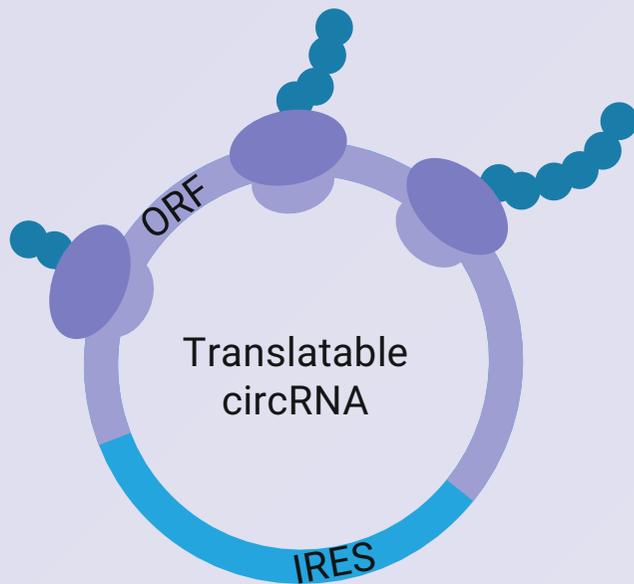
## Screening of most effective circRNA-producing sequences invented by nature



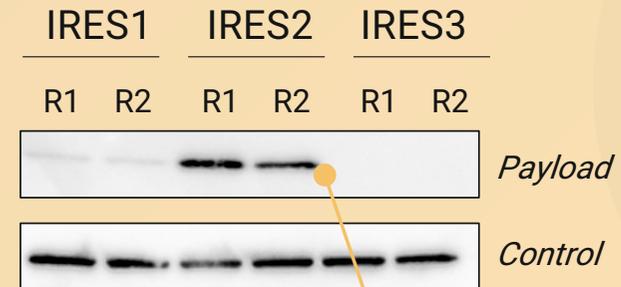
L1 cassette design shows best circRNA biogenesis

# circRNA as templates for protein production

**Internal Ribosomal Entry Site (IRES),** for cap-independent translation



**Dramatic difference in IRES efficacy,** Western blot, protein expression

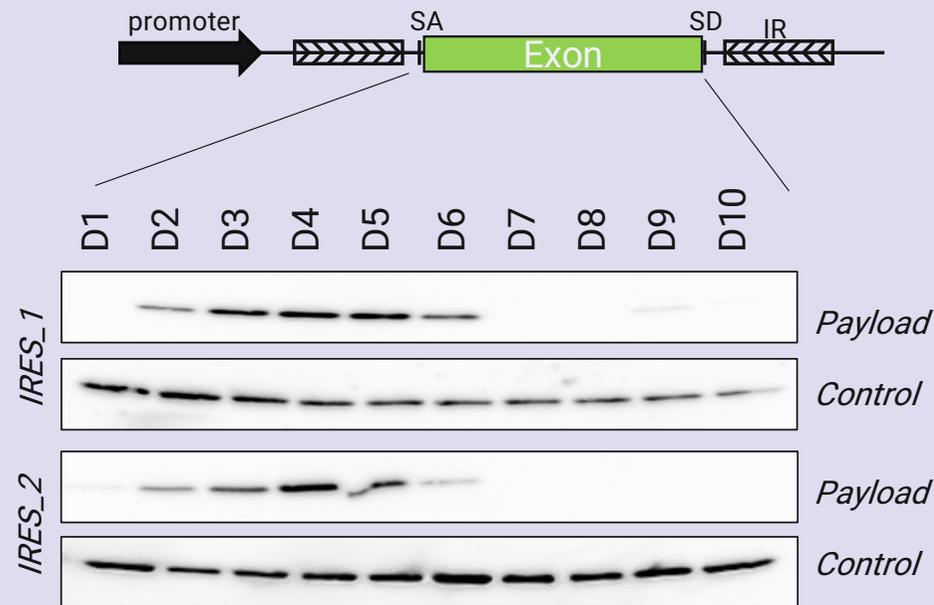


**IRES2 shows superior activity** compared to other IRESs tested.

# Optimizing yield from cassette – already competing with conventional mRNA-based designs

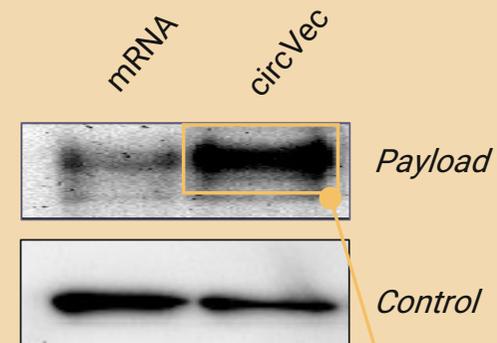
## Design optimization for protein expression

Western blot, circRNA protein payload



## Efficient circRNA designs outperform mRNA

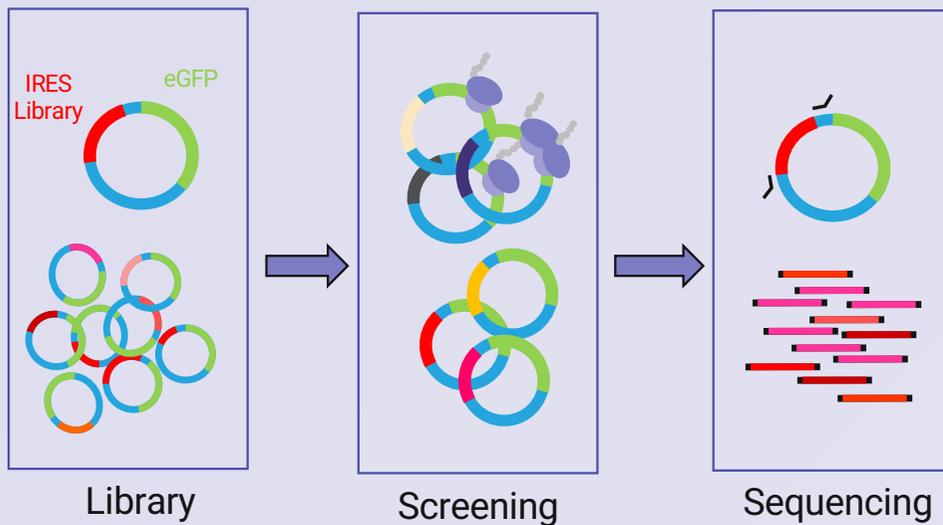
Western blot, protein expression



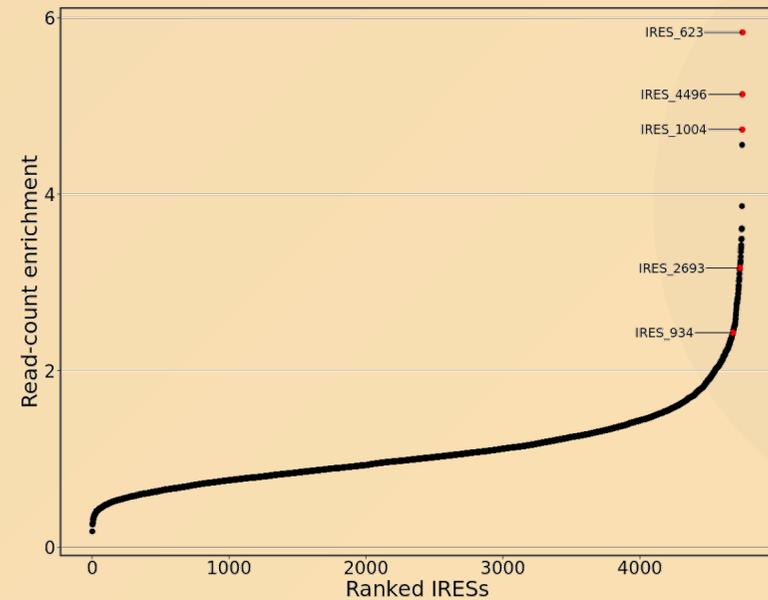
**circVec superiority over mRNA** achieved by optimal exon design

# High-throughput screen identified novel IRES with high translational potency

**IRES screen**, based on 1000+ putative IRES sequences, high-throughput setup to identify the most effective

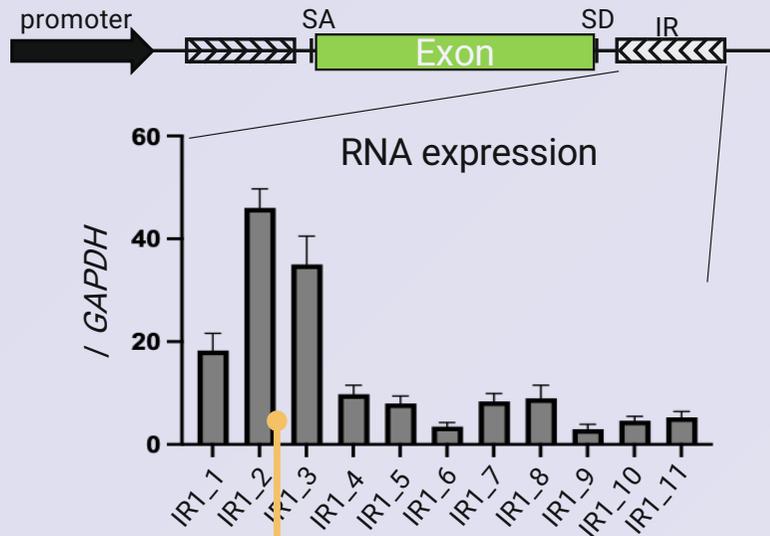


**Novel IRES identified**, pending further validation

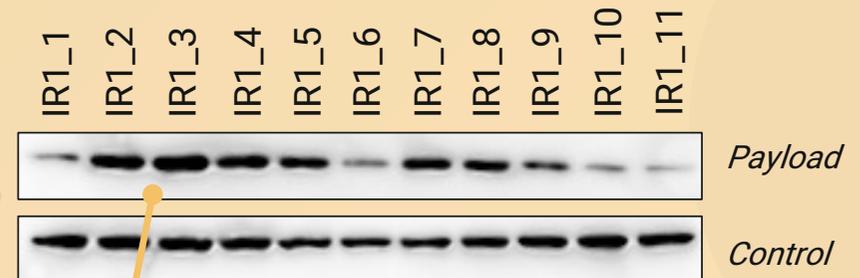


# Further optimizing of circRNA biogenesis

## circRNA expression, RT-qPCR



## eGFP expression from circRNA, western blot

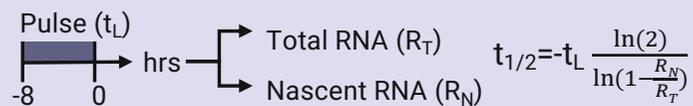


Modification of downstream inverted repeat (IR) shows **enhanced expression** on RNA and protein level compared to original design (IR1\_1)

# circRNA shows 15x prolonged RNA half-life and accumulated protein expression *in vitro*

## RNA stability

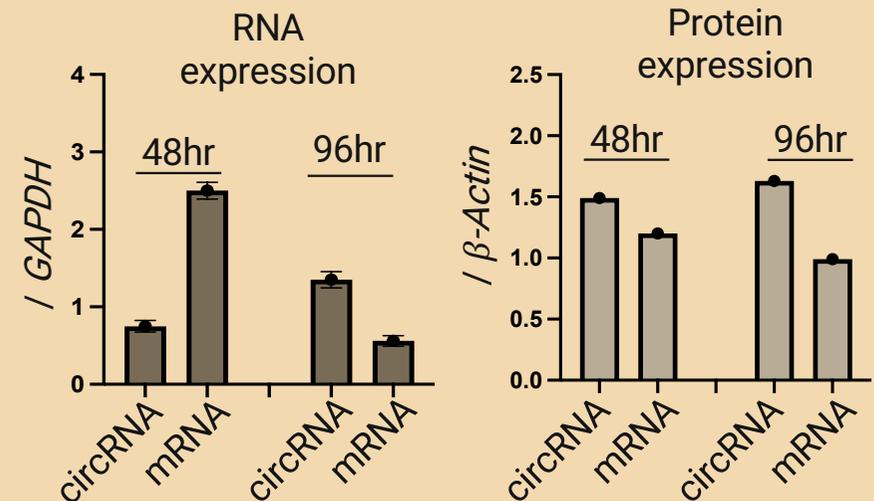
RT-qPCR, nascent vs. total RNA



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



## Accumulation of circRNA and protein payload over time, RT-PCR and Western blot



circRNA outperforms mRNA *in vitro* – confirmatory *in vivo* experiments ongoing



# circVec is a modular genetic cassette for *in situ* circRNA biogenesis and protein expression



Genetic cassette  
design



Multi-functional  
circRNA design

- Modular structure
- Efficient circRNA biogenesis
- In-built **regulatory functionality**
- **Vector agnostic** – applicable to a variety of DNA and viral systems
  
- Long-half life
- Enhanced protein expression
- microRNA sponging
- ...

# circVec-encoded circRNAs is broadly applicable and opens for new opportunities

## Oncology



Efficient, selective delivery and durable expression of therapeutic proteins in solid tumors

## Vaccines



Enhanced potency, single dose vaccine concept with simplified administration

## Rare disease



Durable protein replacement, without need for genome integration

Designed for **intra-cellular circRNA production, durable protein expression and targeted regulatory functionality**

**Thank you for  
your attention**



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