Discovery of translation initiation elements enabled by a parallel arrayed screen of full-length viral UTRs in synthetic circular RNA

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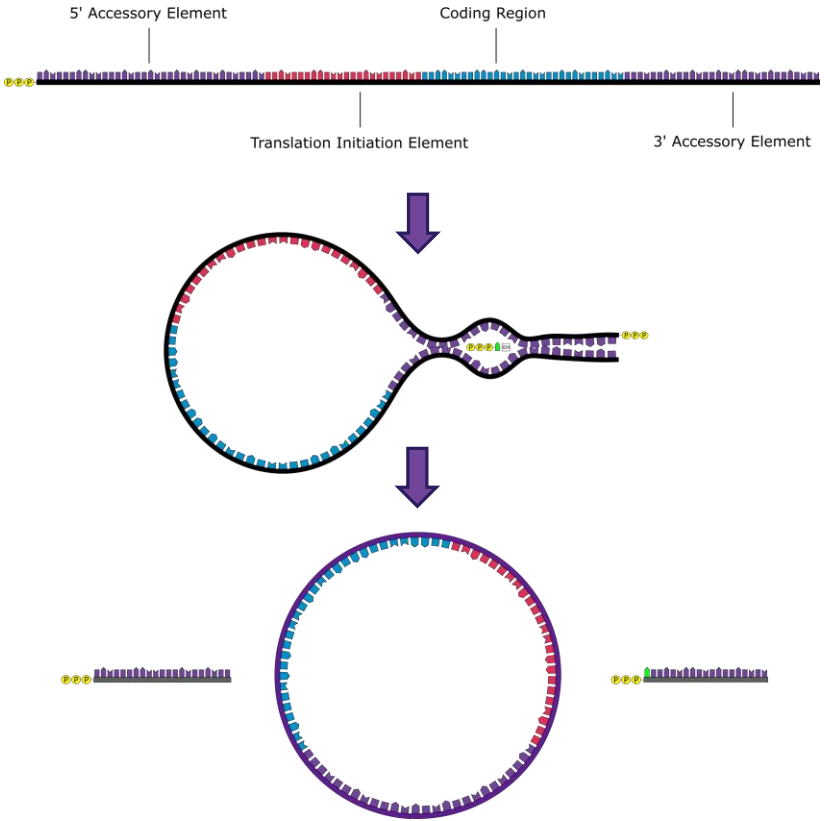
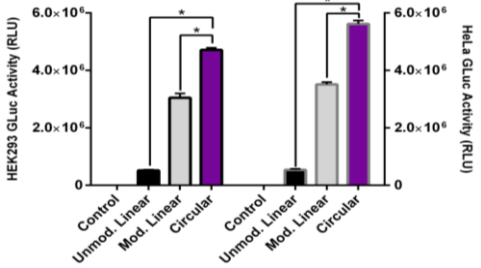
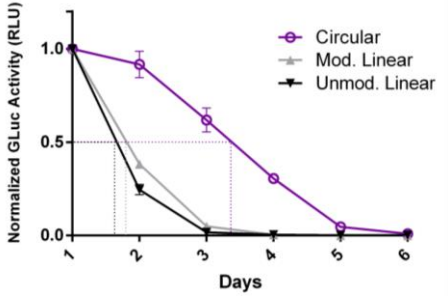
5/16/22

oRNA™: Synthetic circular RNA with therapeutic potential



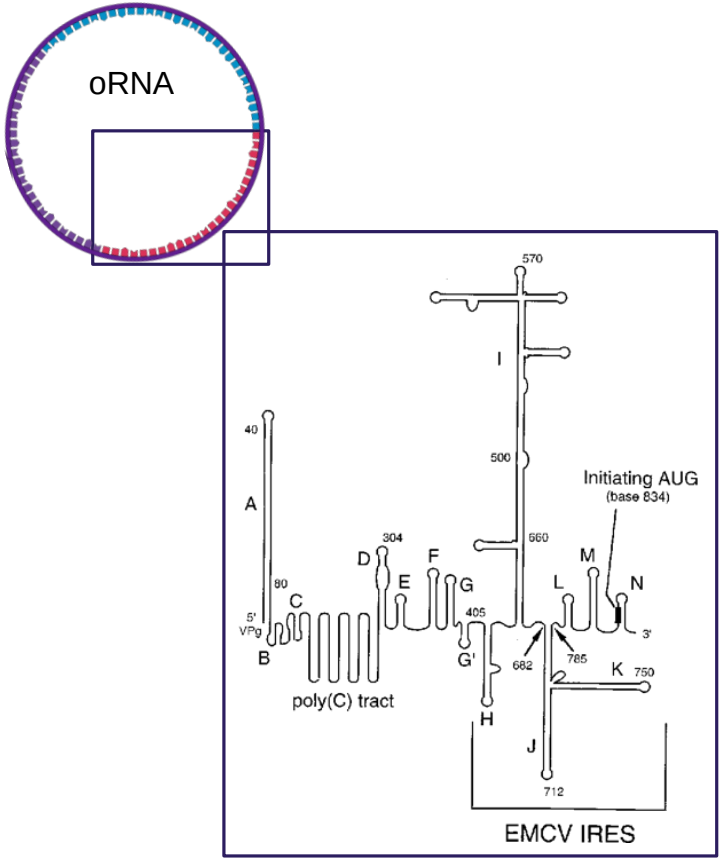
- oRNAs are a new class of long coding RNA with advantages over linear mRNA
- No 5’ cap or polyA tail required for translation
- Expression is driven by an IRES element
- High translation and stability without nucleoside modifications



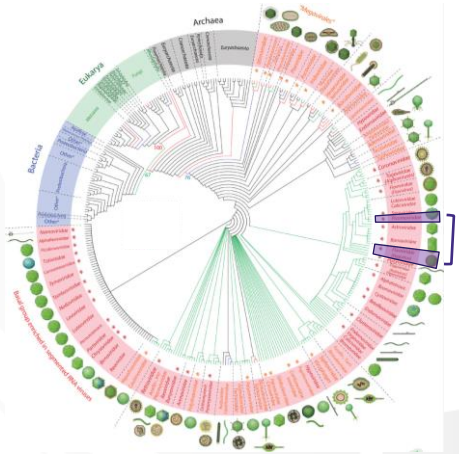


Wesselhoeft et al, 2018

The IRES drives translation from oRNA



- The IRES is a large RNA structure that drives translation
- Secondary and tertiary folding are both important for function
- IRES identification and development is critical for optimizing oRNA function, analogous to the identification of new cap structures
- IRES elements can be derived from viral UTRs, of which there are thousands of unique sequences
- Different types of IRESs exist



Nasir and Anolles, 2015

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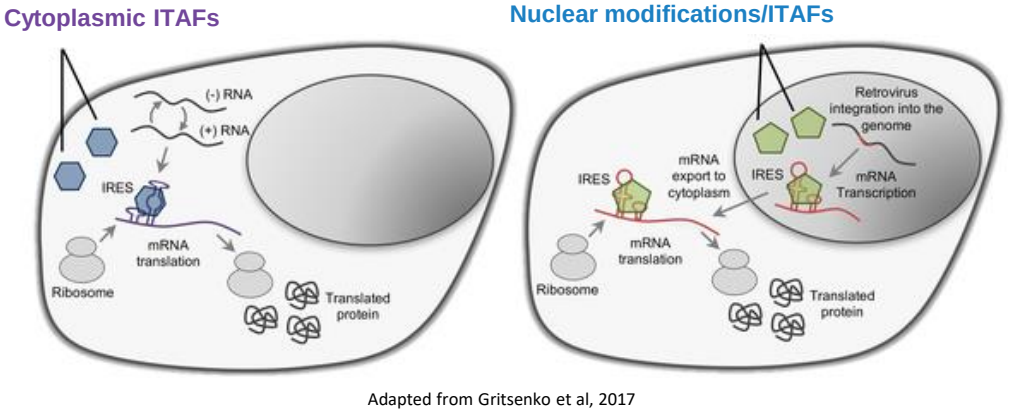
IRES activity depends on nucleic acid context



Adapted from Gritsenko et al, 2017

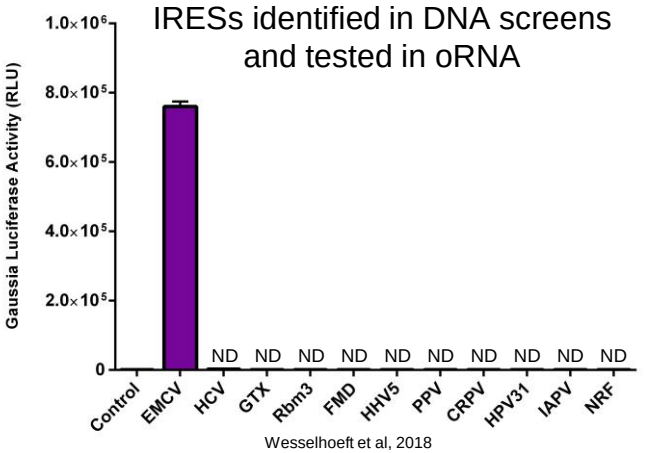
Viral/Cytoplasmic IRESs

- Translation dependent on cytoplasmic ITAFs
- Range from ~400-1000+ bases
- No need for modifications

Endogenous/Nuclear IRESs

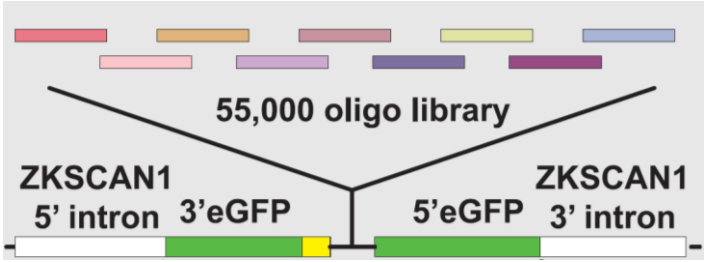
- Must have nuclear experience for translation
- Shorter sequences (<200 nt)
- Potential for base modifications and/or specific protein association

- IRESs identified using **plasmid screens** may or may not function in the context of **oRNA**
- The nuclear environment provides alternative mechanisms for cap-independent translation that are not available to cytosolic RNAs



Wesselhoeft et al, 2018

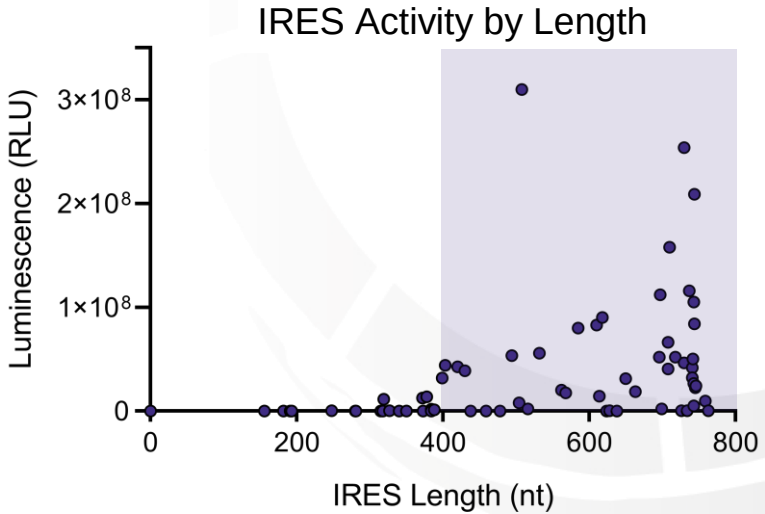
High-activity IRESs are long and complex



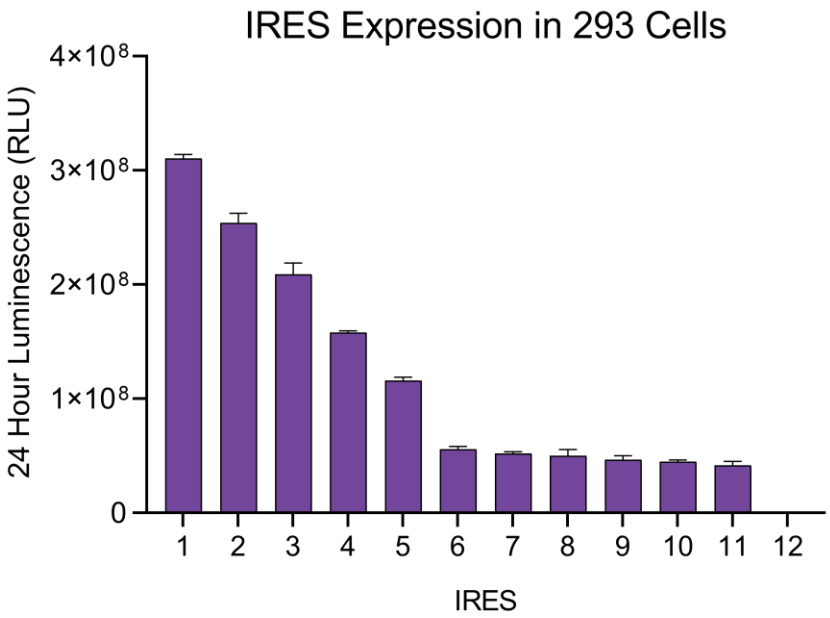
Chen et al, 2021

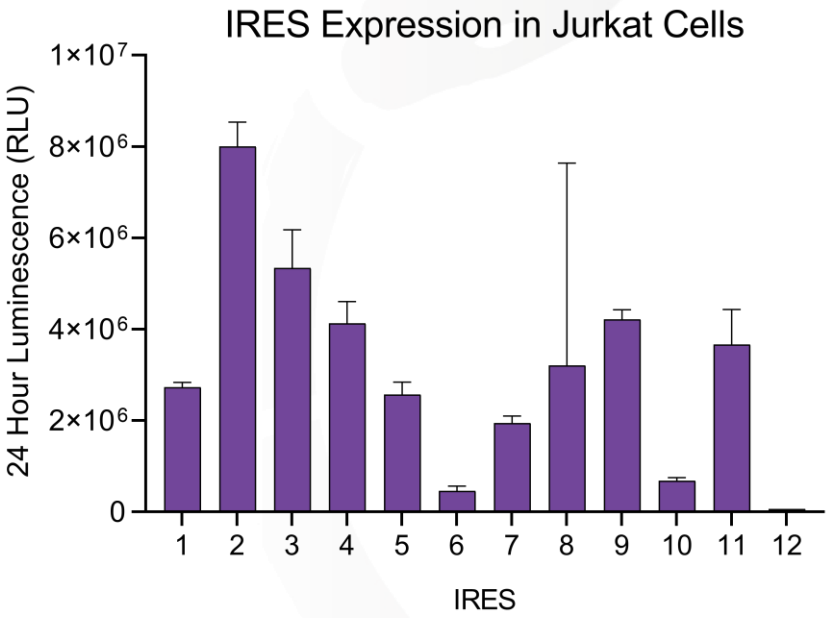
- Short oligo-based library approaches can be used to test large numbers of diverse sequences

- There is a correlation between IRES length and translation activity, with the 400-800nt UTR length range showing greatest activity
- Most active IRESs are out of reach of oligo-based libraries, and IRESs must be individually synthesized as full-sized 'genes'



IRES translation activity is cell type dependent





- Significant cell type tropism in IRES-mediated protein expression was observed
- A need emerged to characterize IRES elements in relevant contexts

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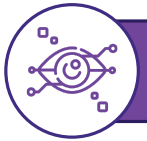
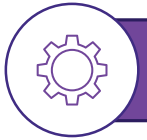
Many challenges await a robust IRES screen



There are thousands of putative IRES sequences we want to test

We need to test them in the oRNA context, and not plasmid DNA



They’re generally long (>500nt) and therefore not amenable to oligo-based libraries

We need to test them in relevant cell types to really understand their activity

Lipid nanoparticle formulation can impact IRES activity (or vice versa)

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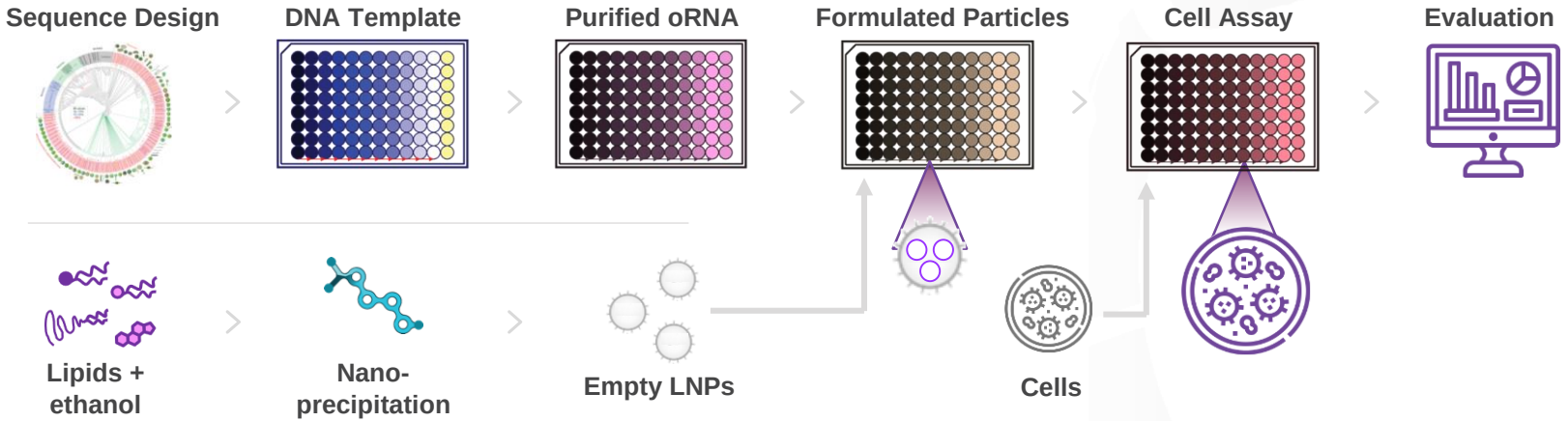
FoRCE™ high-throughput screening

Formulated oRNA Cell-based Evaluation

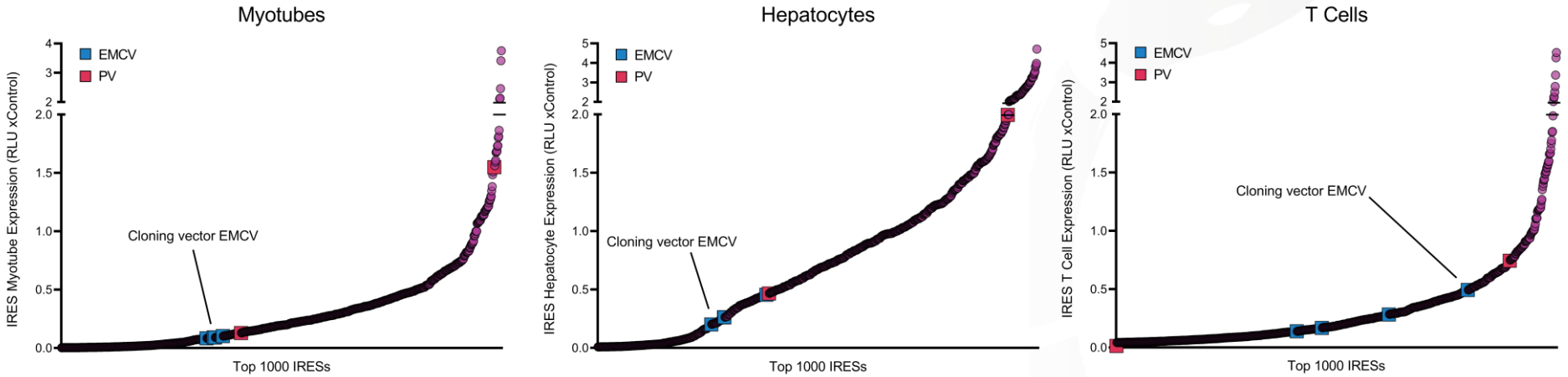


~3,000 IRES candidates were computationally identified

Novel oRNAs were synthesized and formulated into LNPs

IRESs were tested in primary human T cells, myotubes, and hepatocytes

Unlocking a new world of IRES elements



- We identified hundreds of new IRES elements highly active in oRNA
- Some IRESs are 10-40x stronger than the commonly used EMCV-type IRESs
- There can be significant differences even between IRESs from highly similar viruses, showing the importance of empirical approaches

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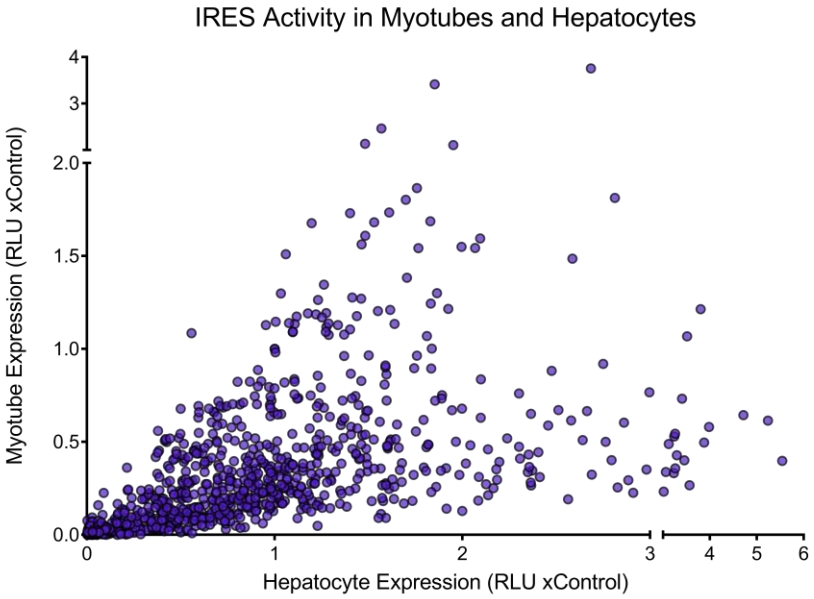
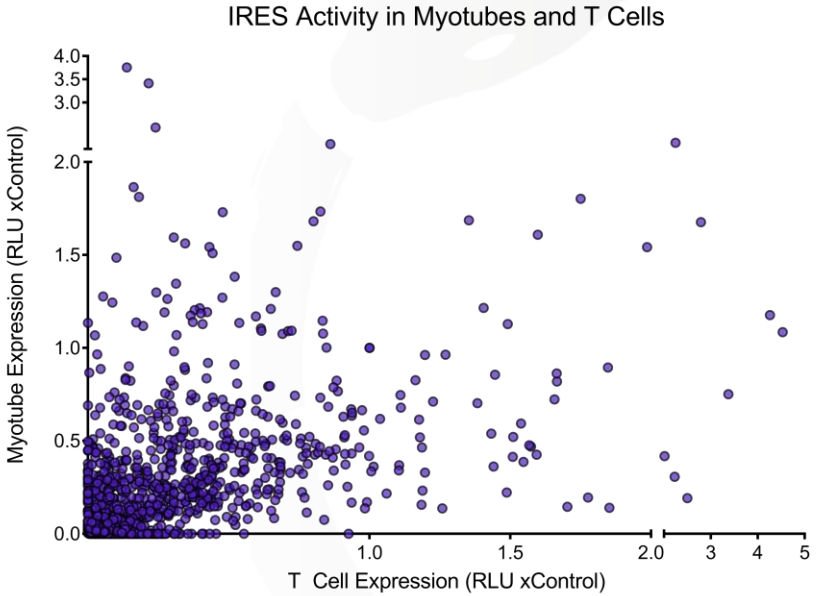
IRESs can have vastly different activities in different cell types





- Myotube and hepatocyte IRES activities often correlate
- T cell IRES activity often does not correlate with activity in myotubes and hepatocytes
- Few IRESs are strong in all cell types

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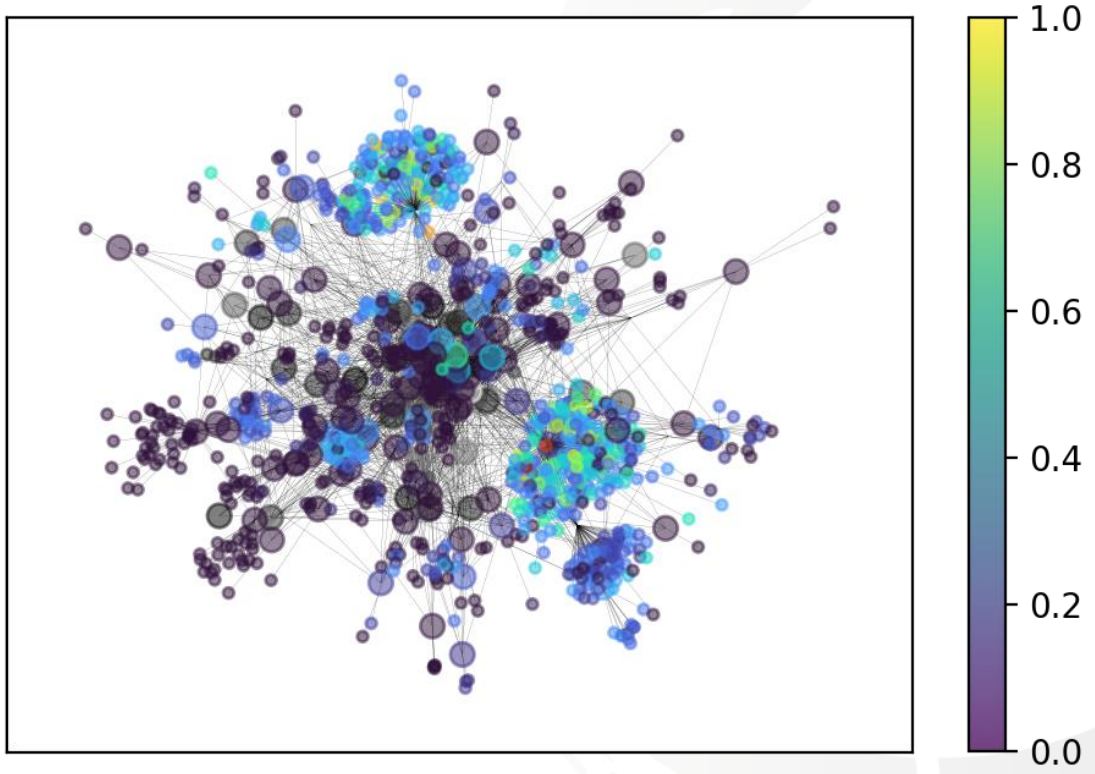
Phylogeny can be used to narrow the search

- UTRs cluster by sequence similarity
- Some clusters contain IRESs that are more active than others and are generally good places to go looking for IRESs
- Within clusters, there is a range of expression activity



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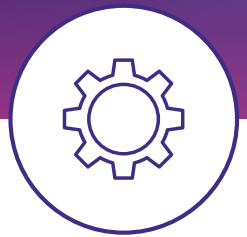
Orna has unlocked a new world of cap-independent translation



IRESs are challenging to screen, requiring:

- RNA context
- Long and complex sequences, 500nt+
- Relevant cell types
- Nanoparticle delivery

We created FoRCE to enable:

- Automated synthesis of thousands of oRNAs
- High throughput LNP formulation
- Material assessment in primary cells

Using FoRCE, we:

- Characterized thousands of putative IRESs
- Identified highly active IRESs superior to commonly used IRESs
- Gained an understanding of IRES tissue tropism

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Acknowledgements

Molecular Biology

Delivery Science

Biology

CMC

Safety

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