

MicroRNA based diagnostic of infectious diseases in human and animals

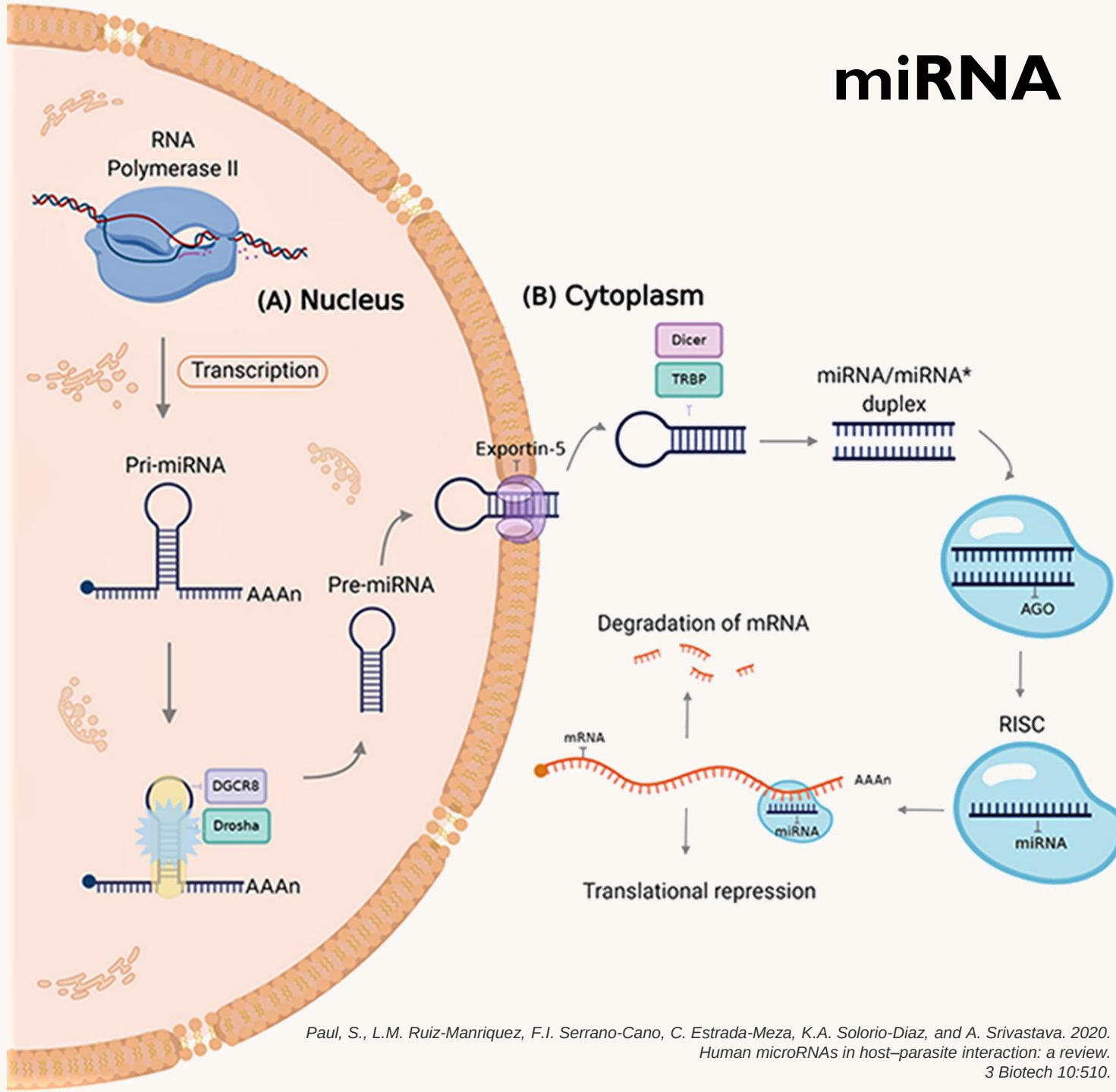
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Donia Macartney-Coxson², Miguel E. Quiñones-Mateu³, Axel Heiser¹

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²Human Genomics, Institute of Environmental Science and Research (ESR)

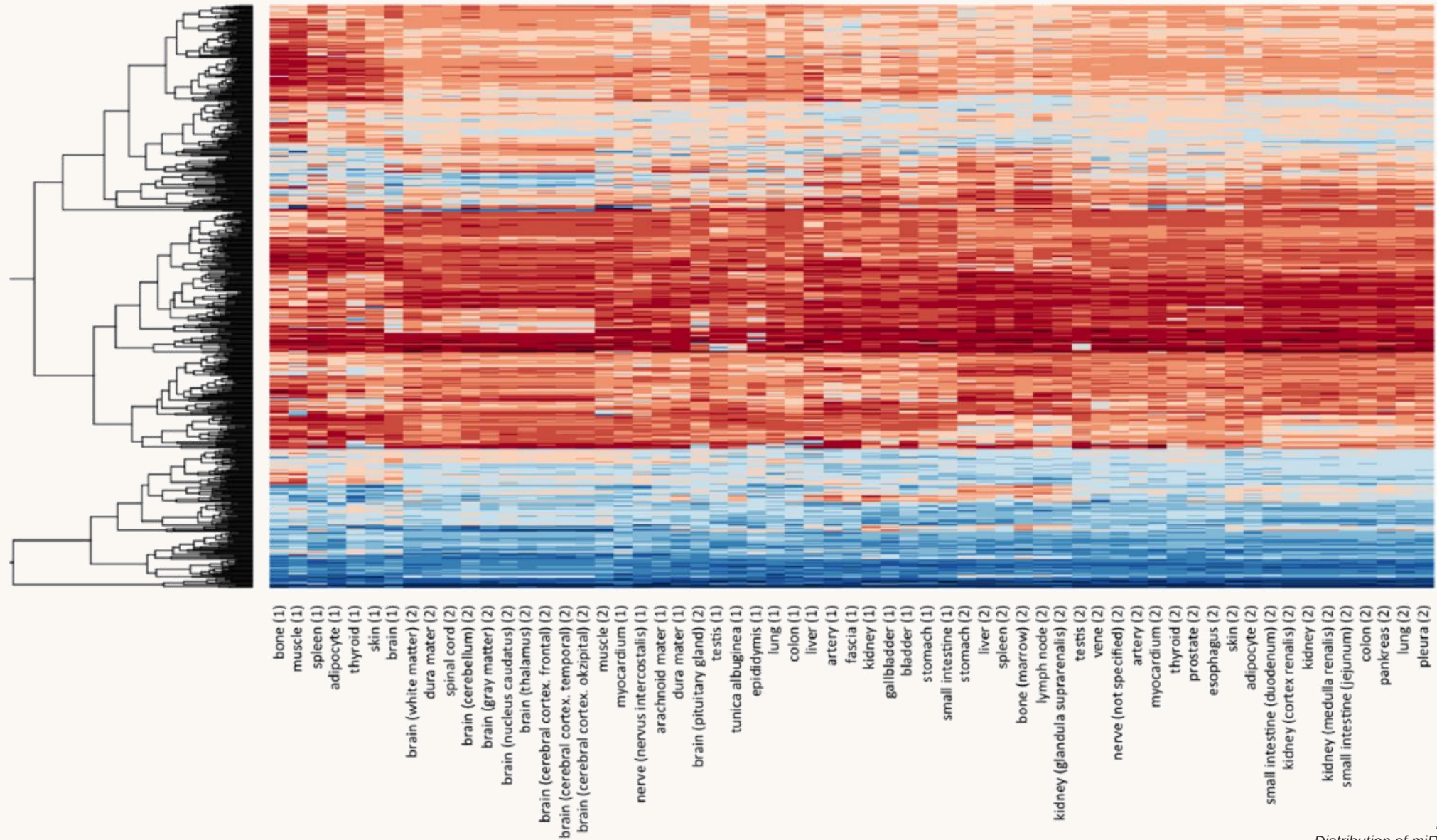
³Department of Microbiology and Immunology, University of Otago

miRNA

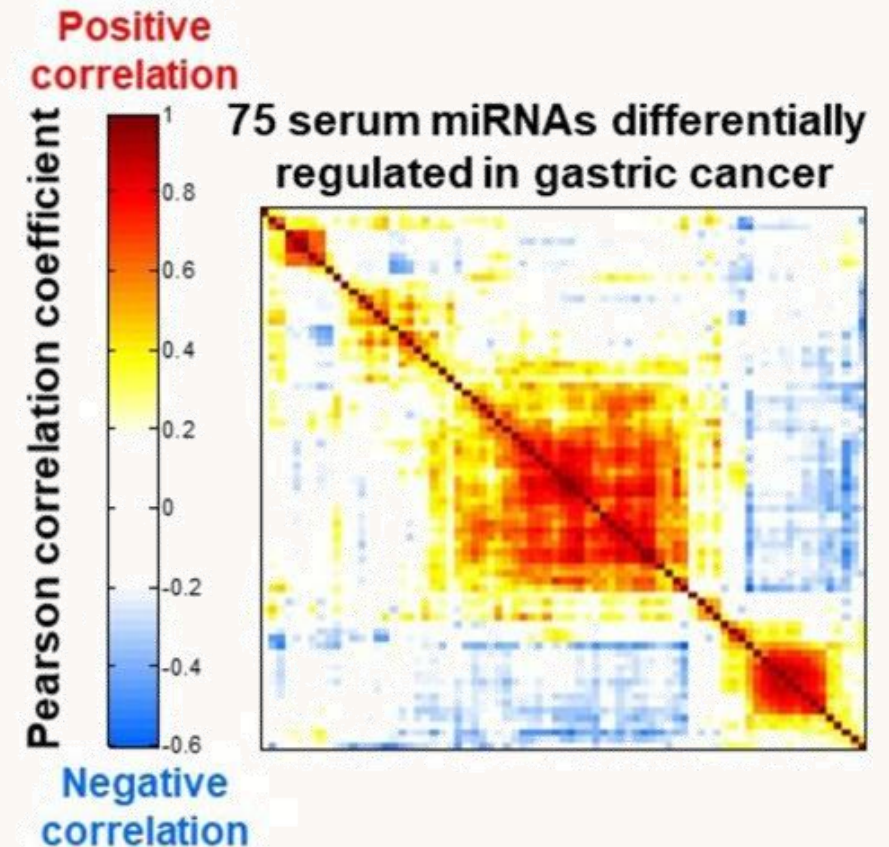
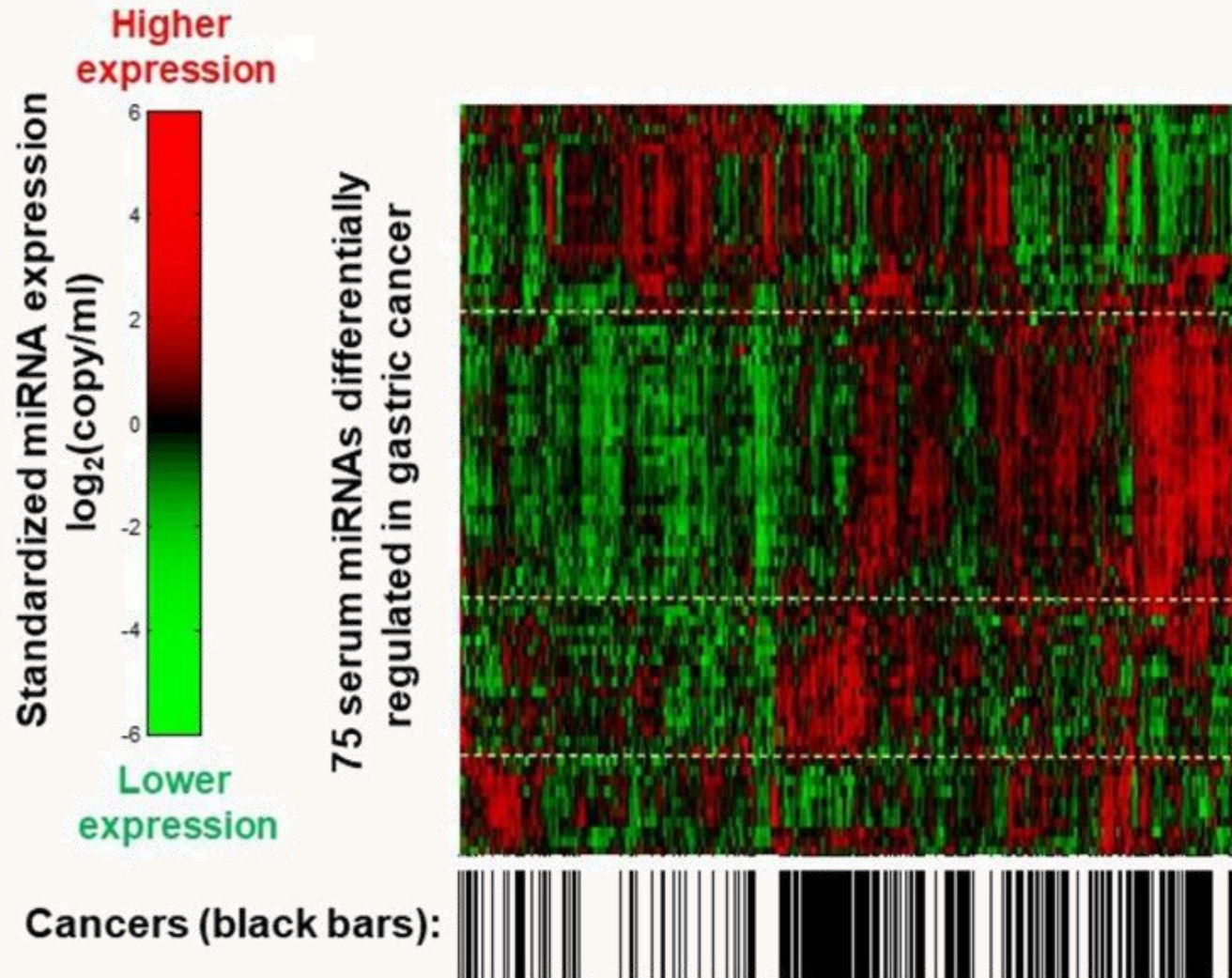


- transcribed by RNA polymerase II
- fold into a long pri-miRNA with a hairpin structure.
- cleaved by the microprocessor, generating a precursor miRNA (pre-miRNA).
- Exportin-5 facilitates its export to the cytoplasm.
- type III RNase Dicer complex cleaves the hairpin
- duplex is isolated by a helicase enzyme
- functional miRNA strand is loaded together with the Argonaute (AGO) protein into the RNA-induced silencing complex (RISC)
- RISC targets mRNAs by sequence complementary binding
- mediate gene suppression through
 - translational repression or
 - mRNA degradation

miRNA are expressed in many tissues and cell types

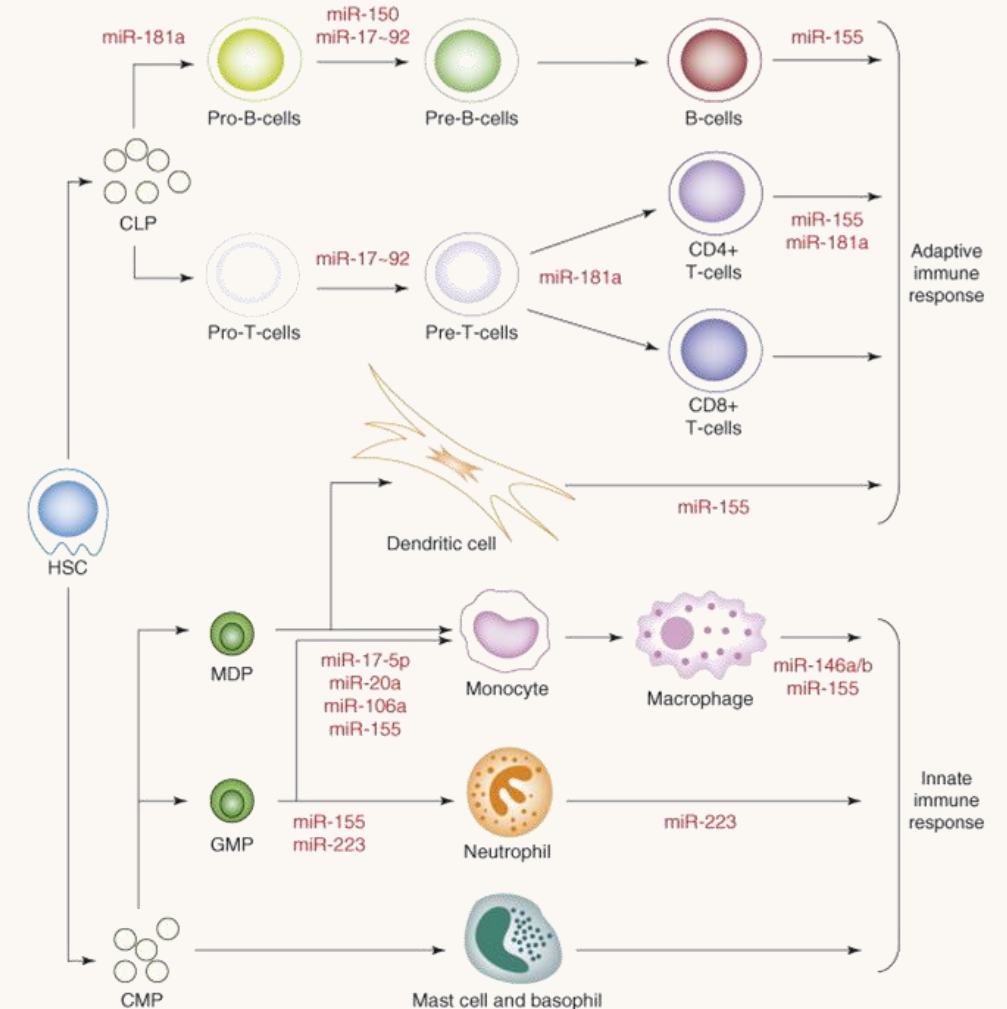
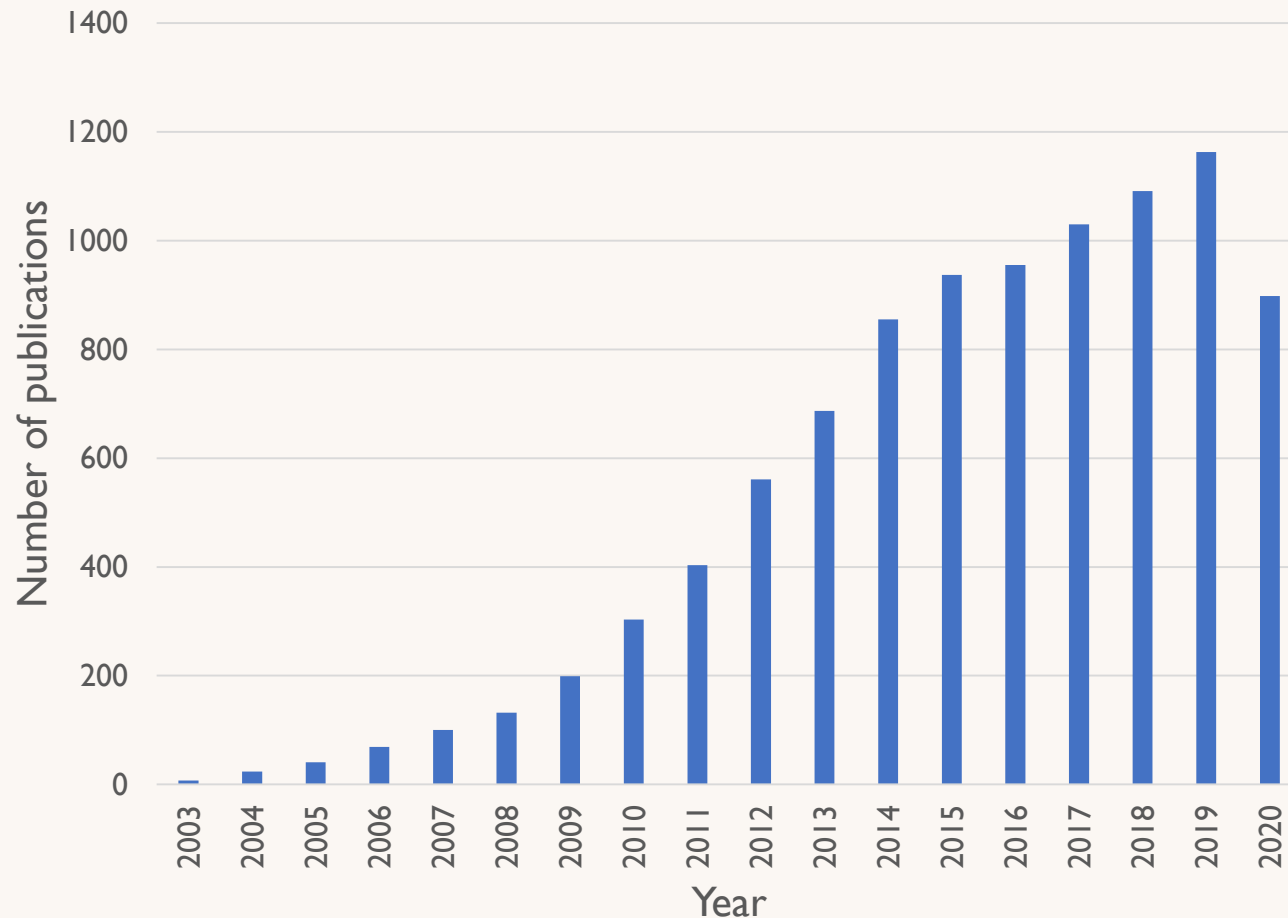


Infection and disease cause specific miRNAs profiles (“fingerprints”)



miRNAs have a role in responses to infectious diseases

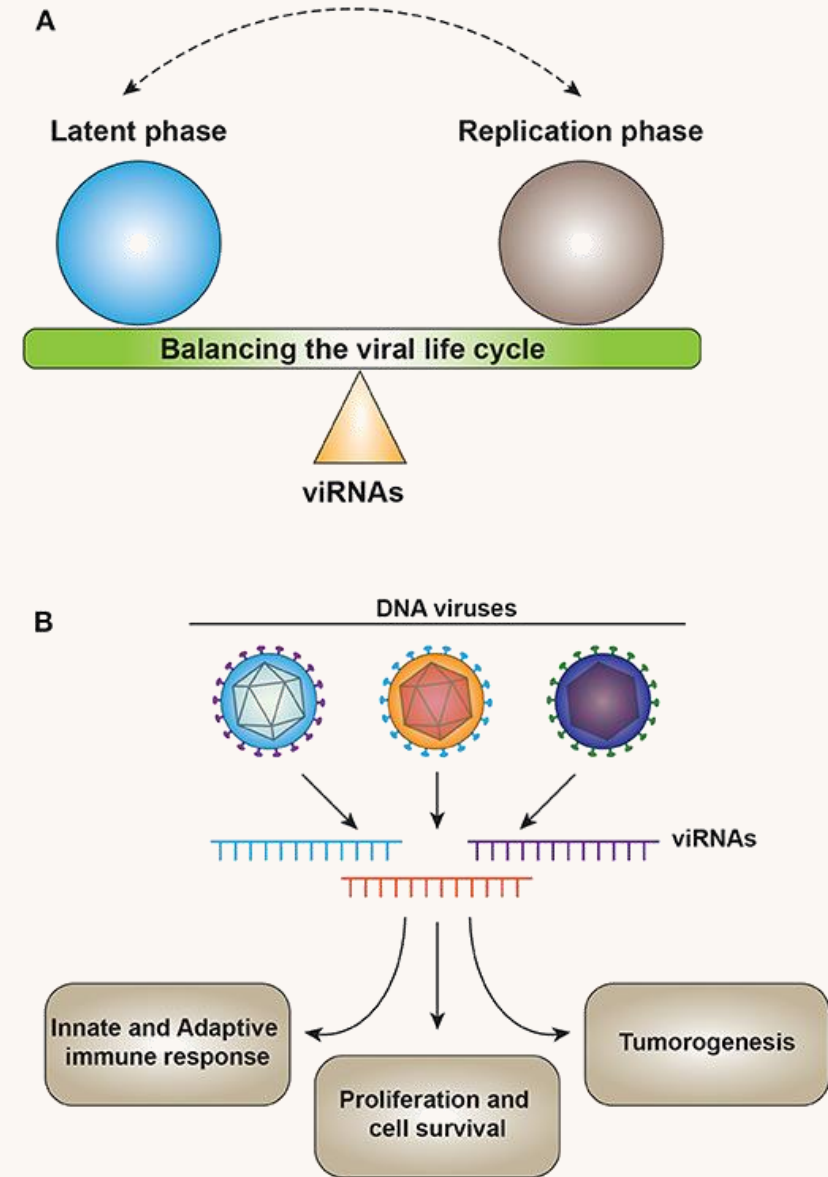
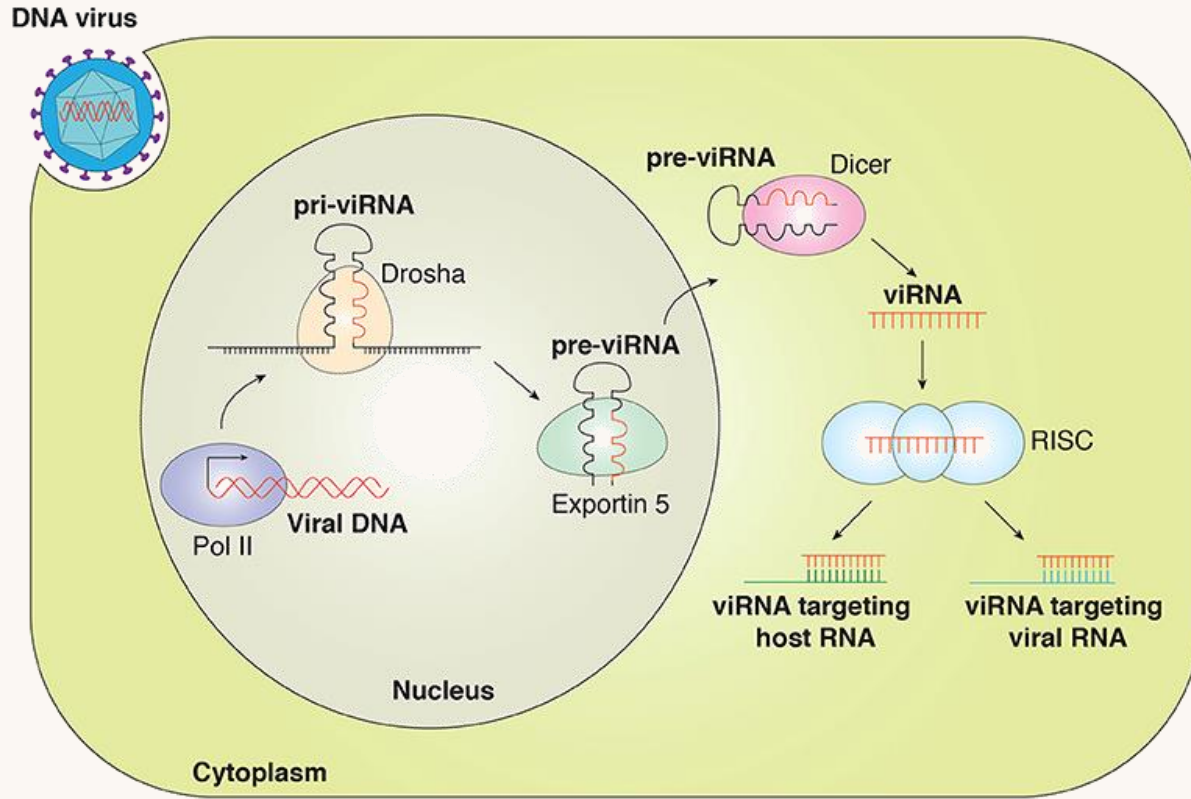
PubMed Search: miRNA + Infection



TRENDS in Immunology

Lindsay, M.A.
microRNAs and the immune response.
Trends in Immunology (2008); 29:343–351

Viruses also express miRNA

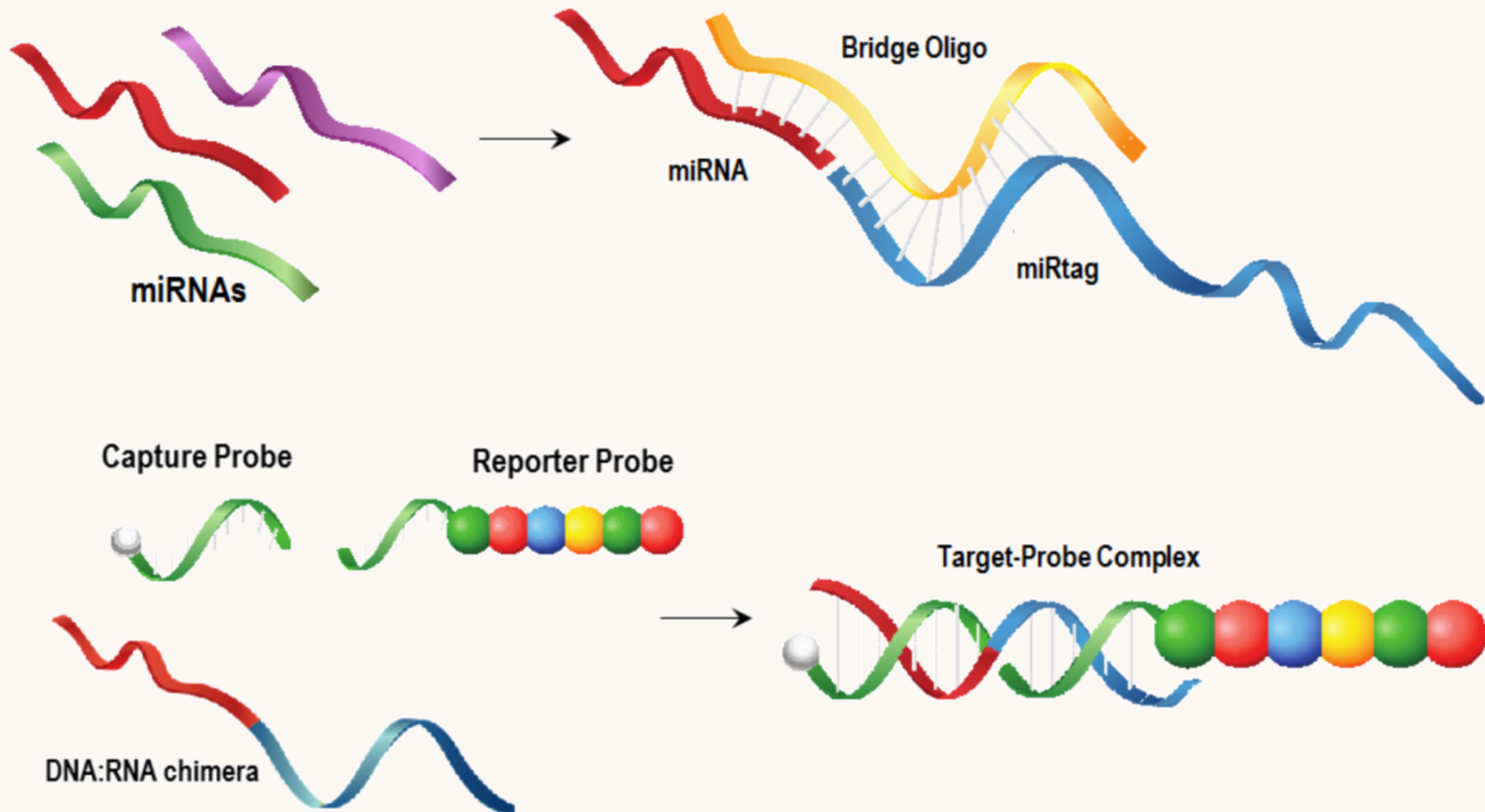


miRNA can be detected and quantified

- Northern blotting
- Real-time qPCR
- Microarray technology
- RNAseq
- NanoString
- New
 - Nanomaterial-based detection
 - Non-PCR nucleic acid amplification
 - Rolling circle amplification (RCA)
 - duplex-specific nuclease (DSN)
 - loop-mediated isothermal amplification (LAMP)
 - Strand-displacement amplification (SDA)
 - Enzyme-free amplification

Ye, J., M. Xu, X. Tian, S. Cai, and S. Zeng. 2019. Research advances in the detection of miRNA. Journal of Pharmaceutical Analysis 9:217–226

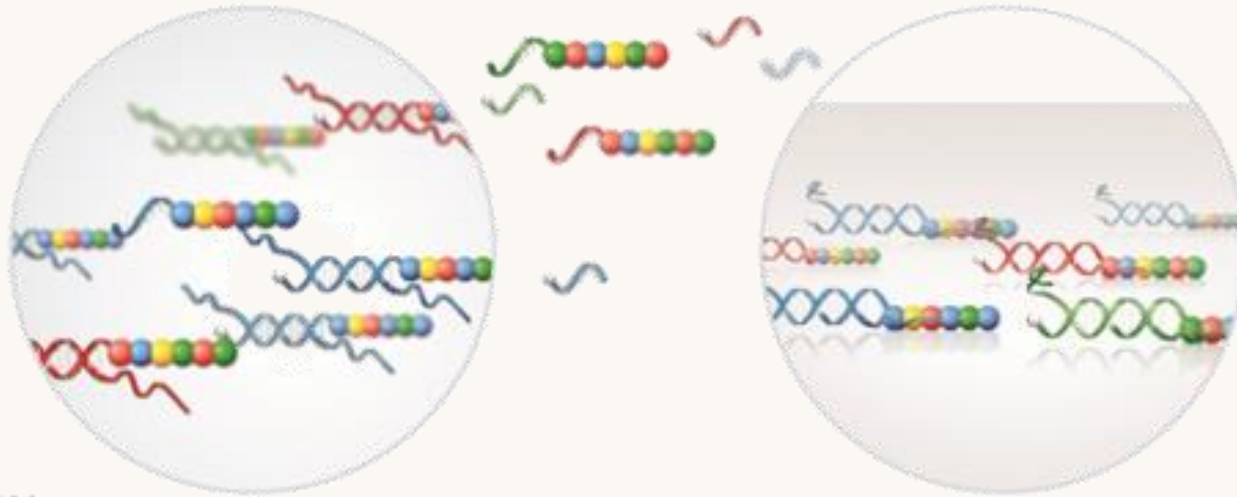
miRNA detection with Nanostring



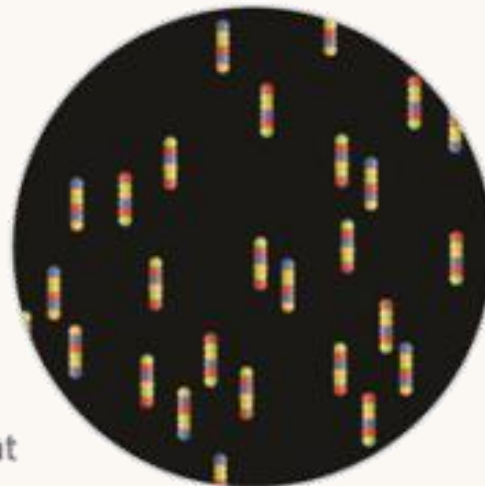
miRNA detection with Nanostring



The Prep Station removes excess CodeSet and immobilizes CodeSet/RNA complexes in the nCounter Cartridge for data collection.



Sample cartridges are placed in the Digital Analyzer for data collection. Fluorescent barcodes on the surface of the cartridge are counted and a running total of each target is tabulated.

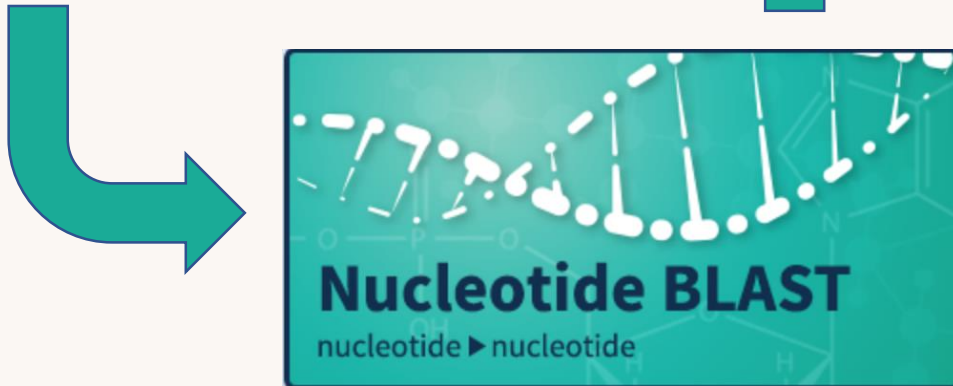


Barcode	Counts	Identity
	3	miRNA #1
	2	miRNA #2
	1	miRNA #3

Detection of bovine miRNA

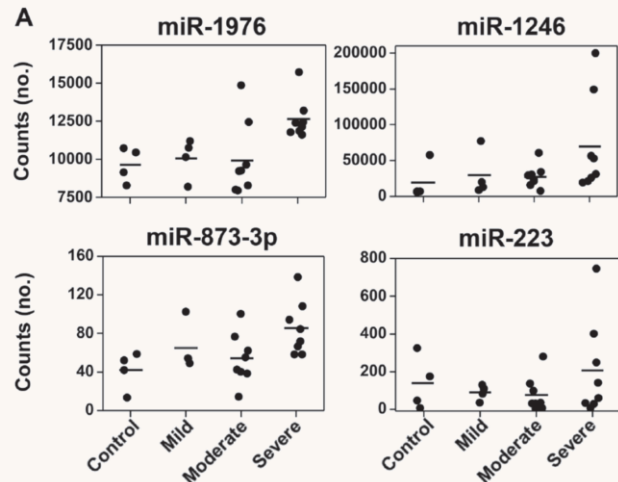
- nCounter Human v3 miRNA Assay
829 human miRNA sequences

- miRBase (www.mirbase.org)
1085 bovine miRNA sequences

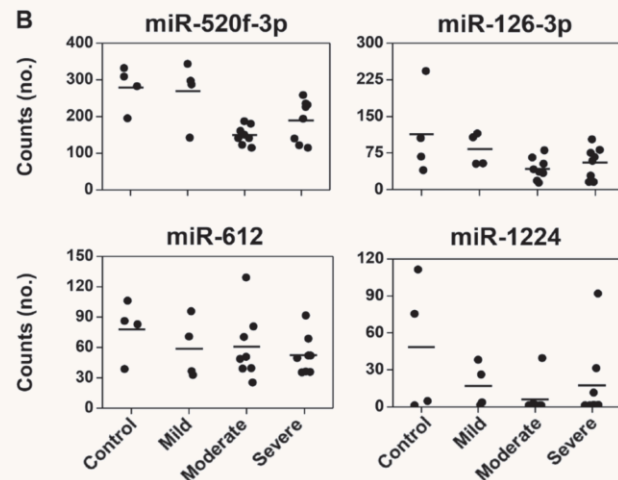


- exact match for 180 bovine miRNA
- 26 detected in cattle serum
 - 5 identical
 - 8 homologues ≤ 2 nt mismatch
- 13 “new” bovine miRNAs

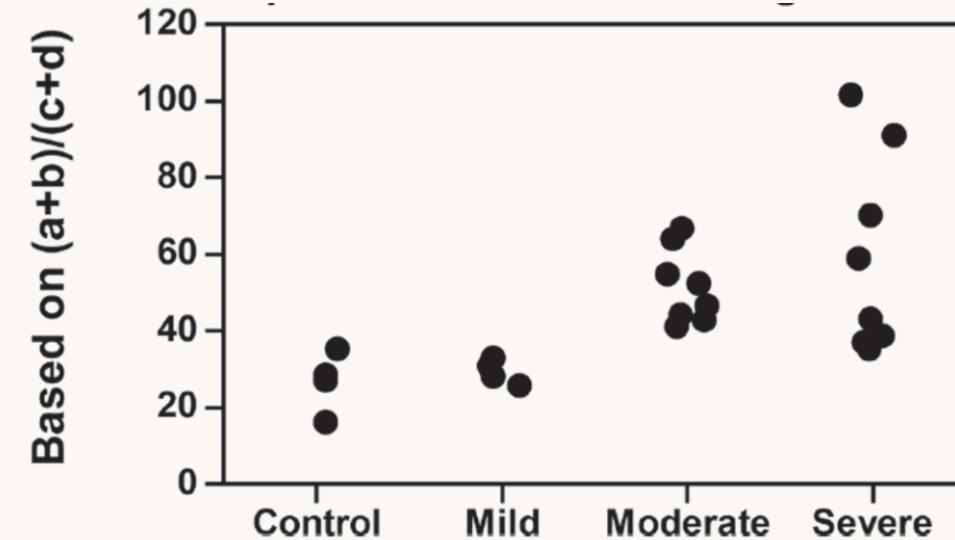
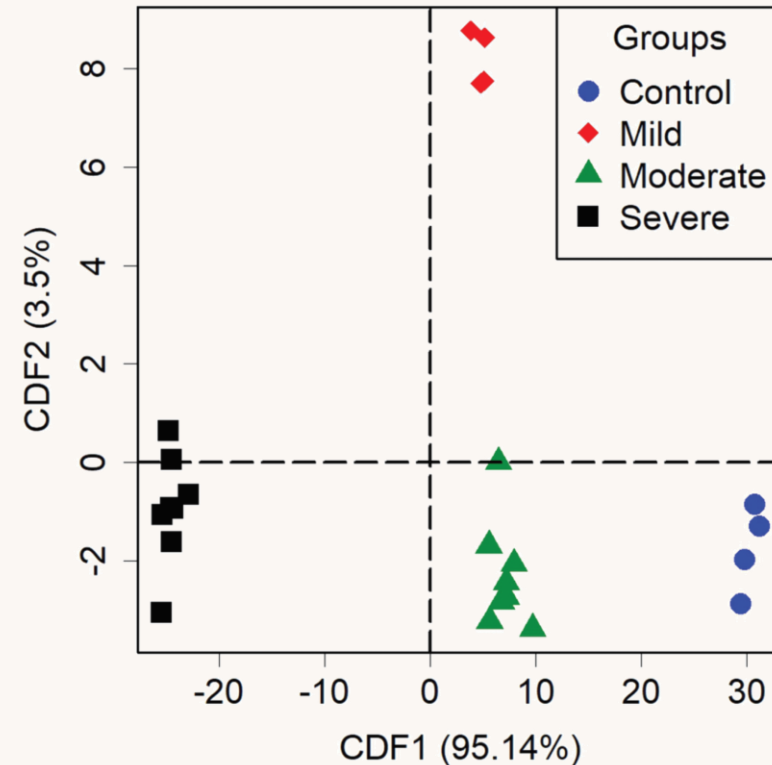
Diagnosis of severity of Johne's disease in cattle based on miRNA profiles



Upregulated miRNA in cattle serum



Downregulated miRNA in cattle serum



MicroRNA-based diagnosis of COVID-19 in asymptomatic people

COVID-19 Innovation Acceleration Fund



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**Sandeep
Gupta**



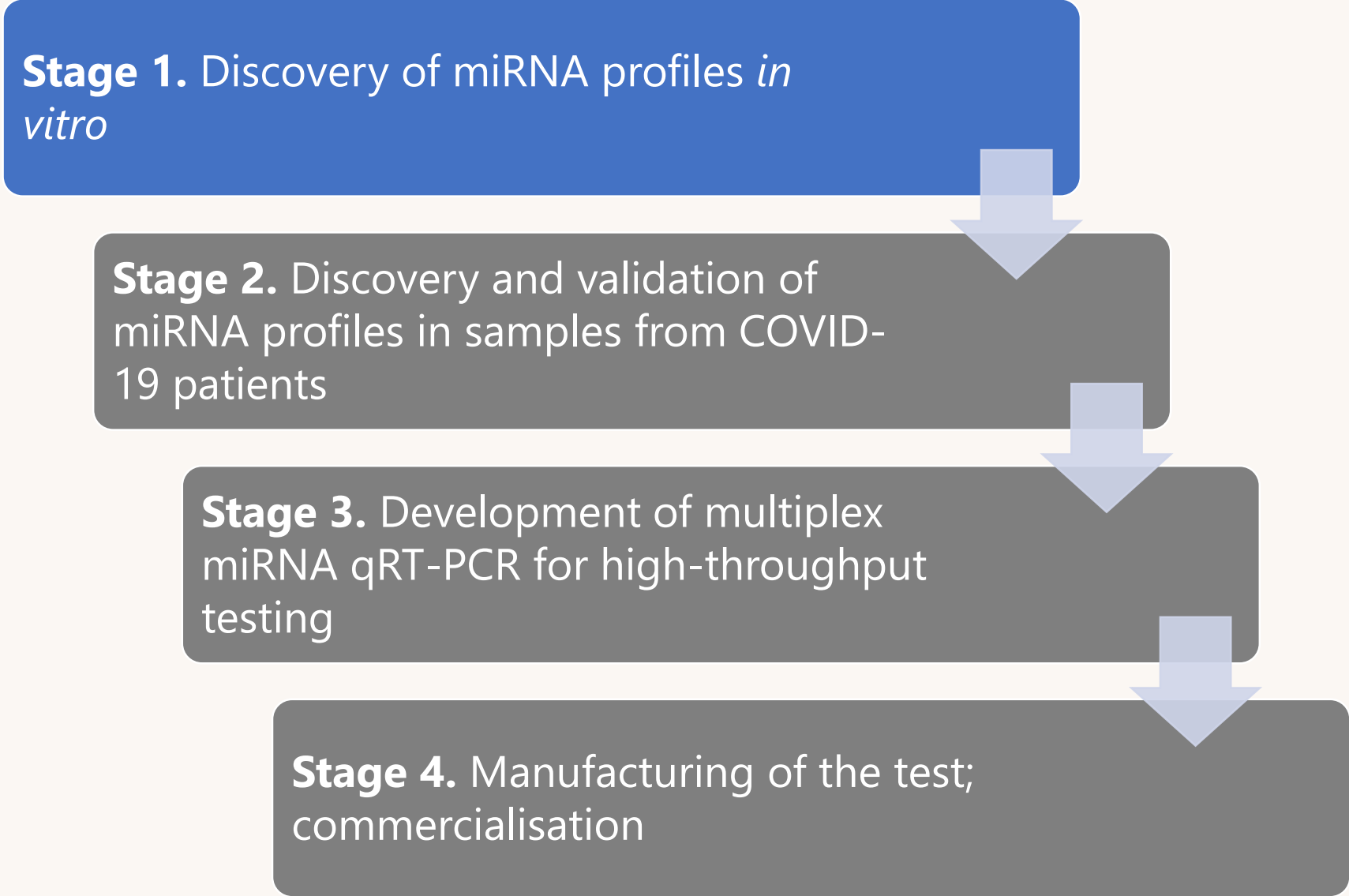
**Miguel
Quiñones-Mateu**



**Donia
Macartney-Coxson**

MicroRNA-based diagnosis of COVID-19 in asymptomatic people

Stage 1. Discovery of miRNA profiles *in vitro*



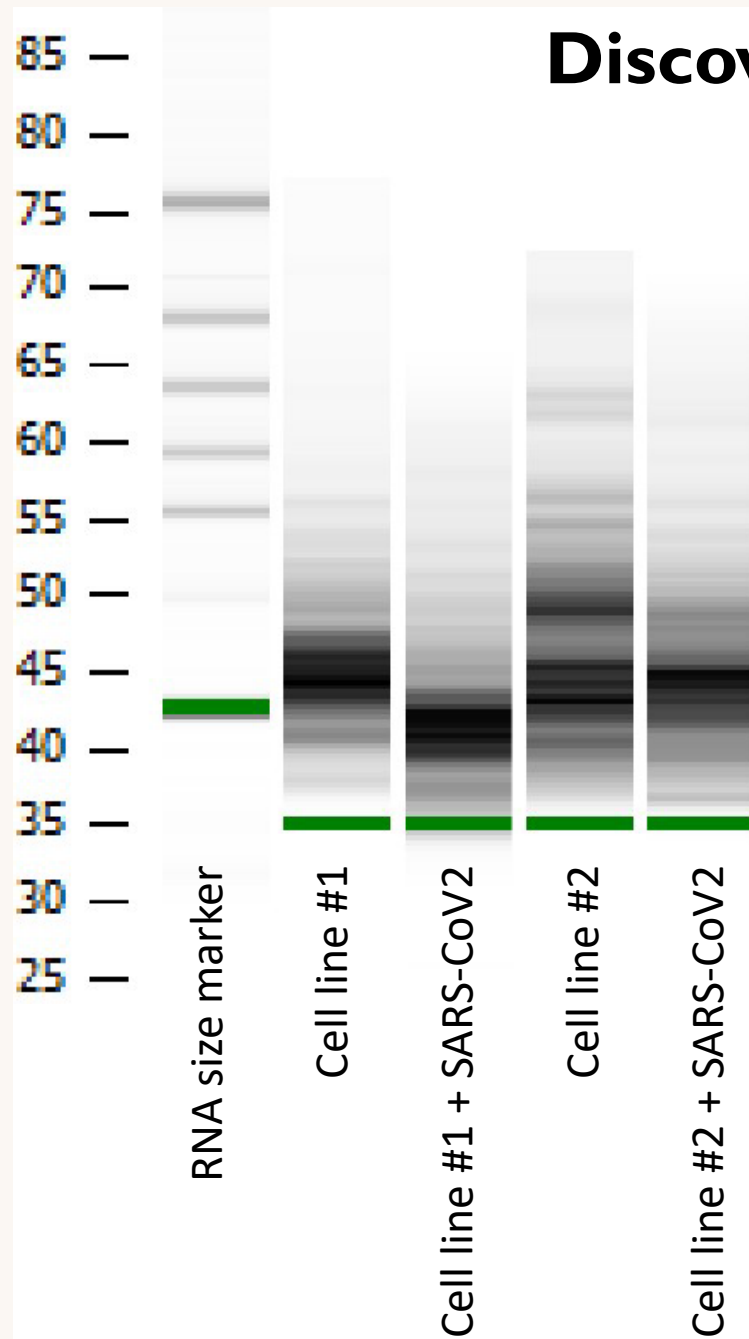
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graph TD; S1[Stage 1. Discovery of miRNA profiles in vitro] --> S2[Stage 2. Discovery and validation of miRNA profiles in samples from COVID-19 patients]; S2 --> S3[Stage 3. Development of multiplex miRNA qRT-PCR for high-throughput testing]; S3 --> S4[Stage 4. Manufacturing of the test; commercialisation];
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Stage 2. Discovery and validation of miRNA profiles in samples from COVID-19 patients

Stage 3. Development of multiplex miRNA qRT-PCR for high-throughput testing

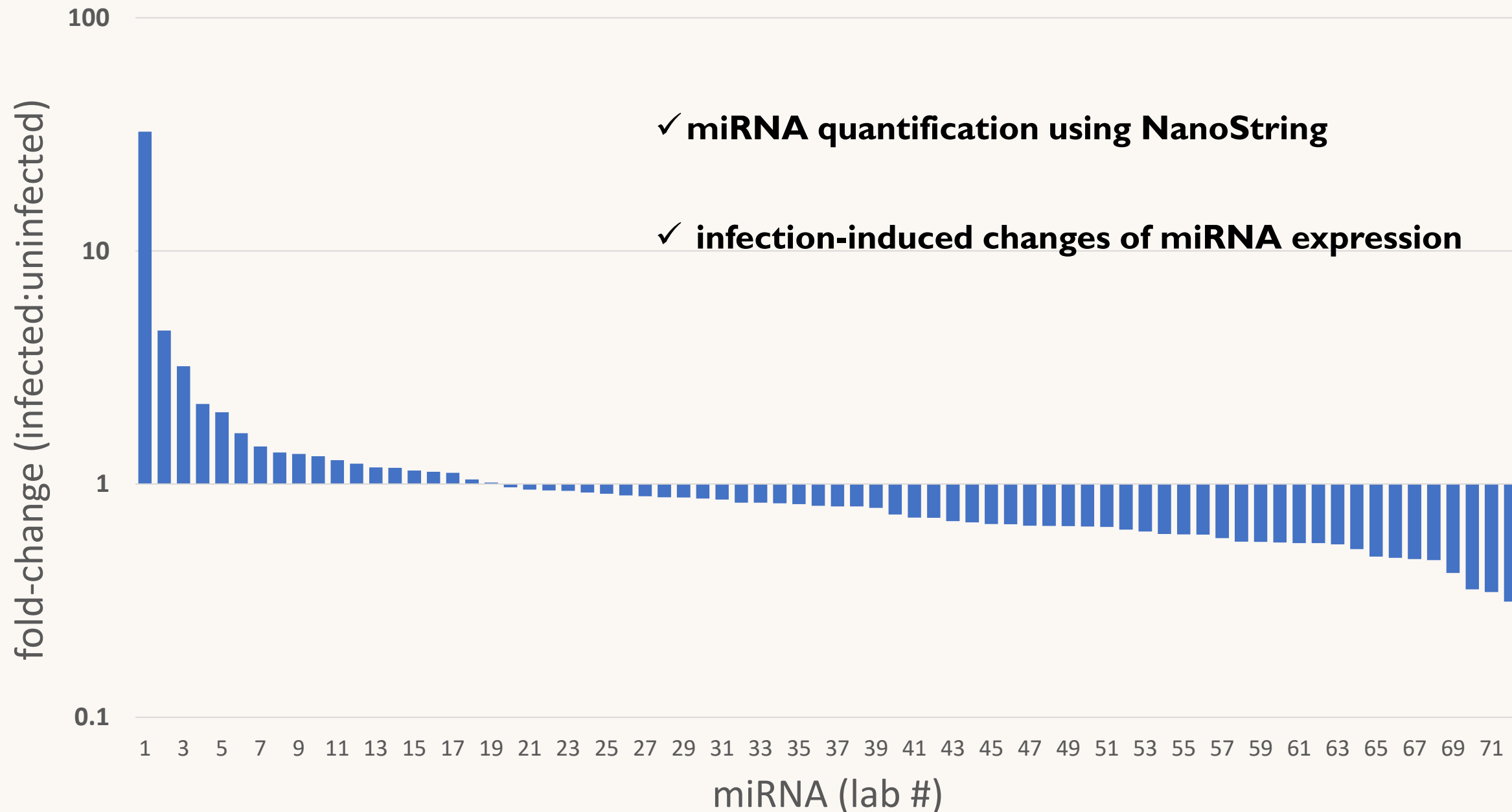
Stage 4. Manufacturing of the test; commercialisation

Discovery of miRNA profiles *in vitro*



✓ Isolation of miRNA
from cell culture supernatant

Discovery of miRNA profiles *in vitro*



Discovery of miRNA profiles *in vitro*

Next steps

- ☐ virus dose titration
- ☐ virus infection time course
- ☐ Influenza A control

- ☐ RNAseq
- ☐ Identification of SARS-CoV-2-specific miRNA profiles

- ☐ Develop qRT-PCR for selected miRNAs

MicroRNA-based diagnosis of COVID-19 in asymptomatic people

Stage 1. Discovery of miRNA profiles *in vitro*

Stage 2. Discovery and validation of miRNA profiles in samples from COVID-19 patient

Stage 3. Development of multiplex miRNA qRT-PCR for high-throughput testing

Stage 4. Manufacturing of the test; commercialisation



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Thank you