



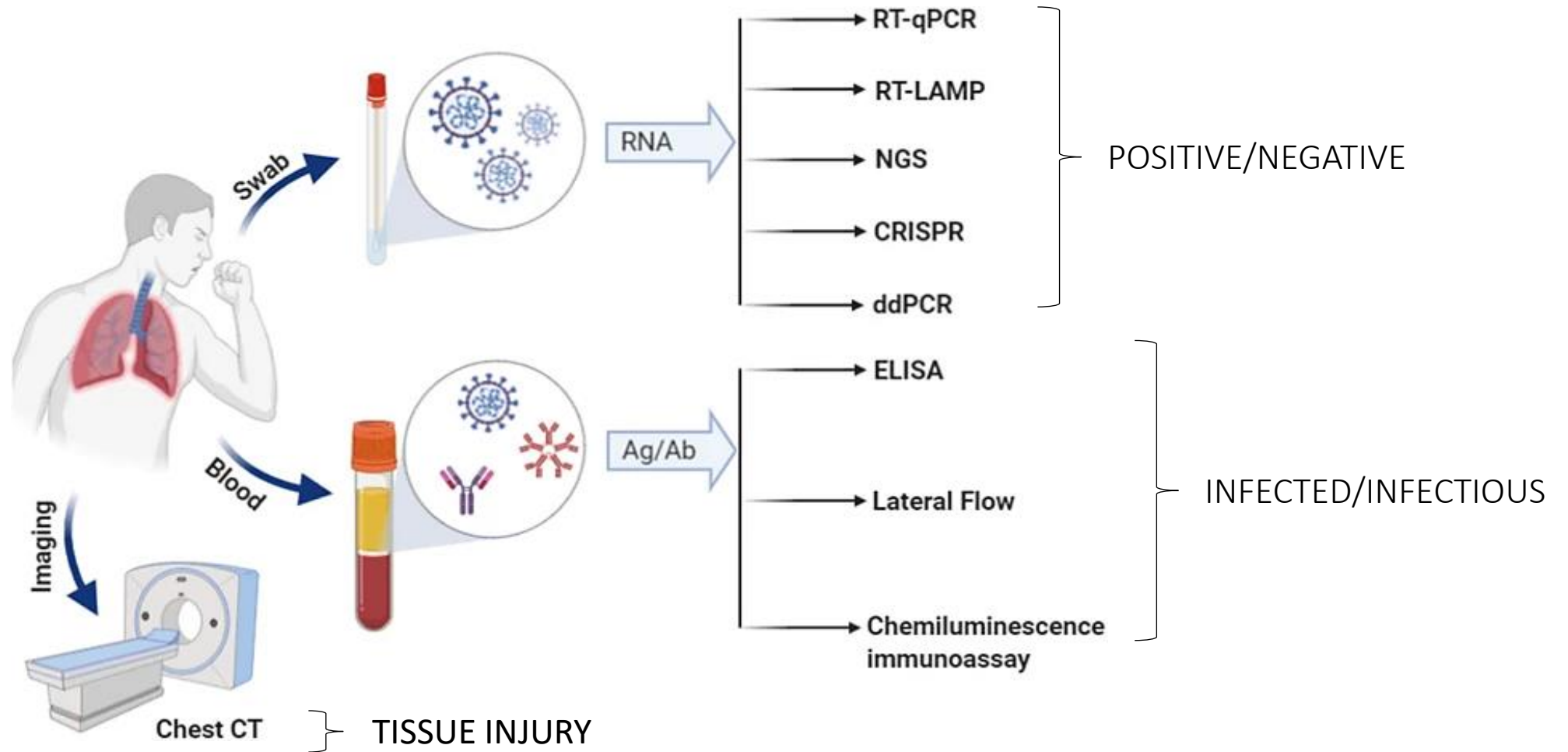
**PRECISION
DIAGNOSTICS
EUROPE 2021**

MSc. Veronika Grešáková, PhD

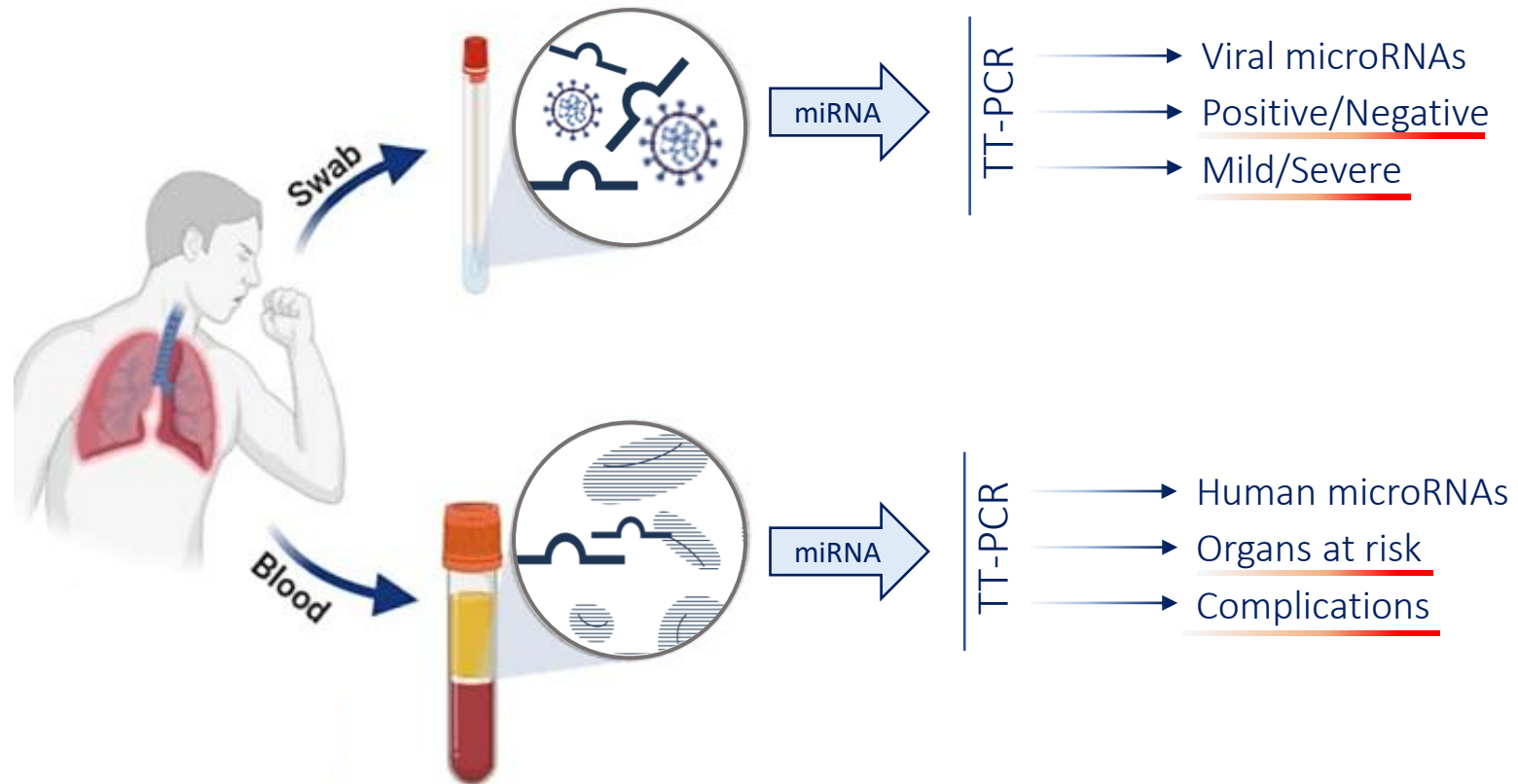
Detection of SARS-CoV-2 microRNA panel using Two-Tailed qPCR method



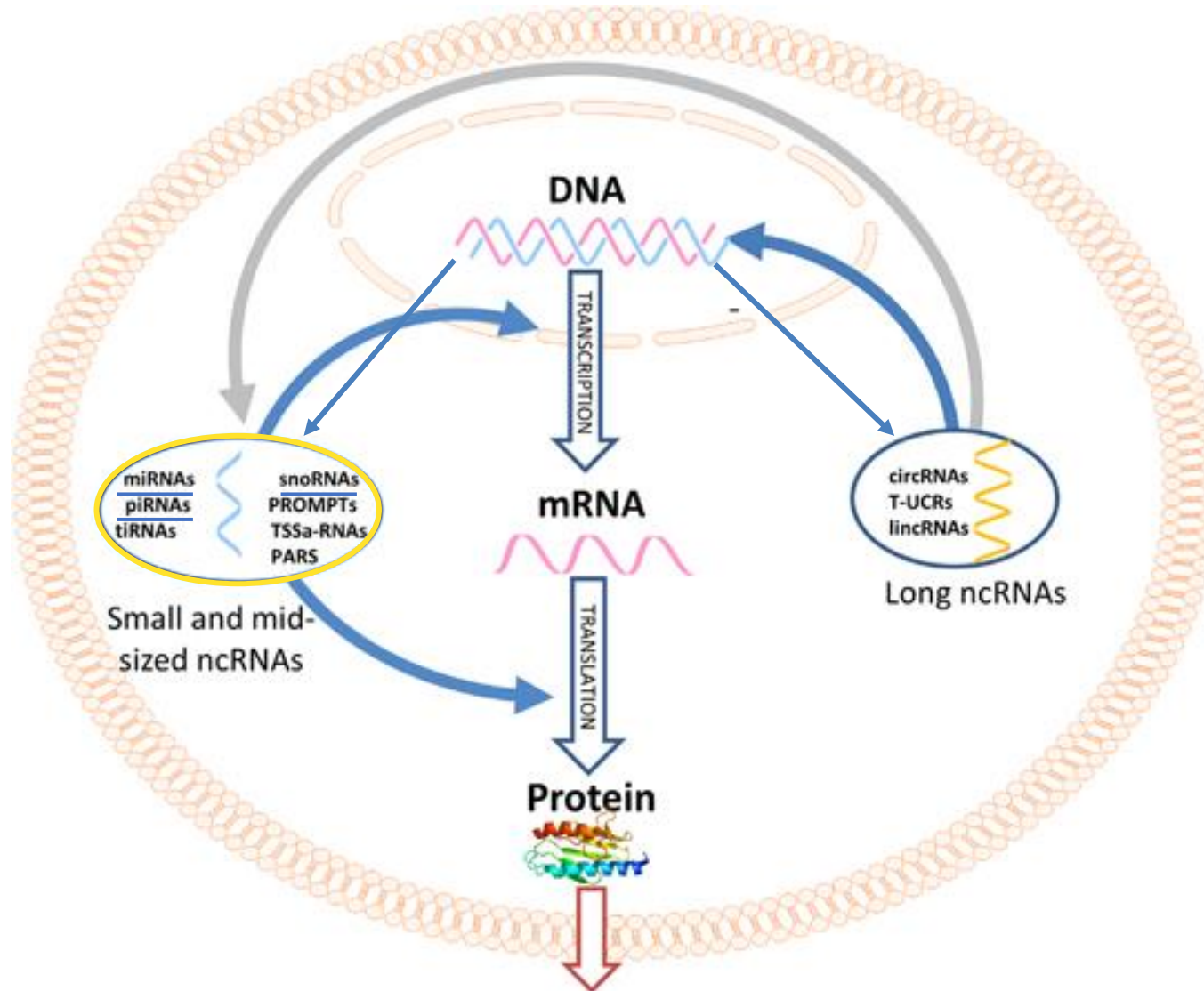
Current approaches for the detection of SARS-CoV-2



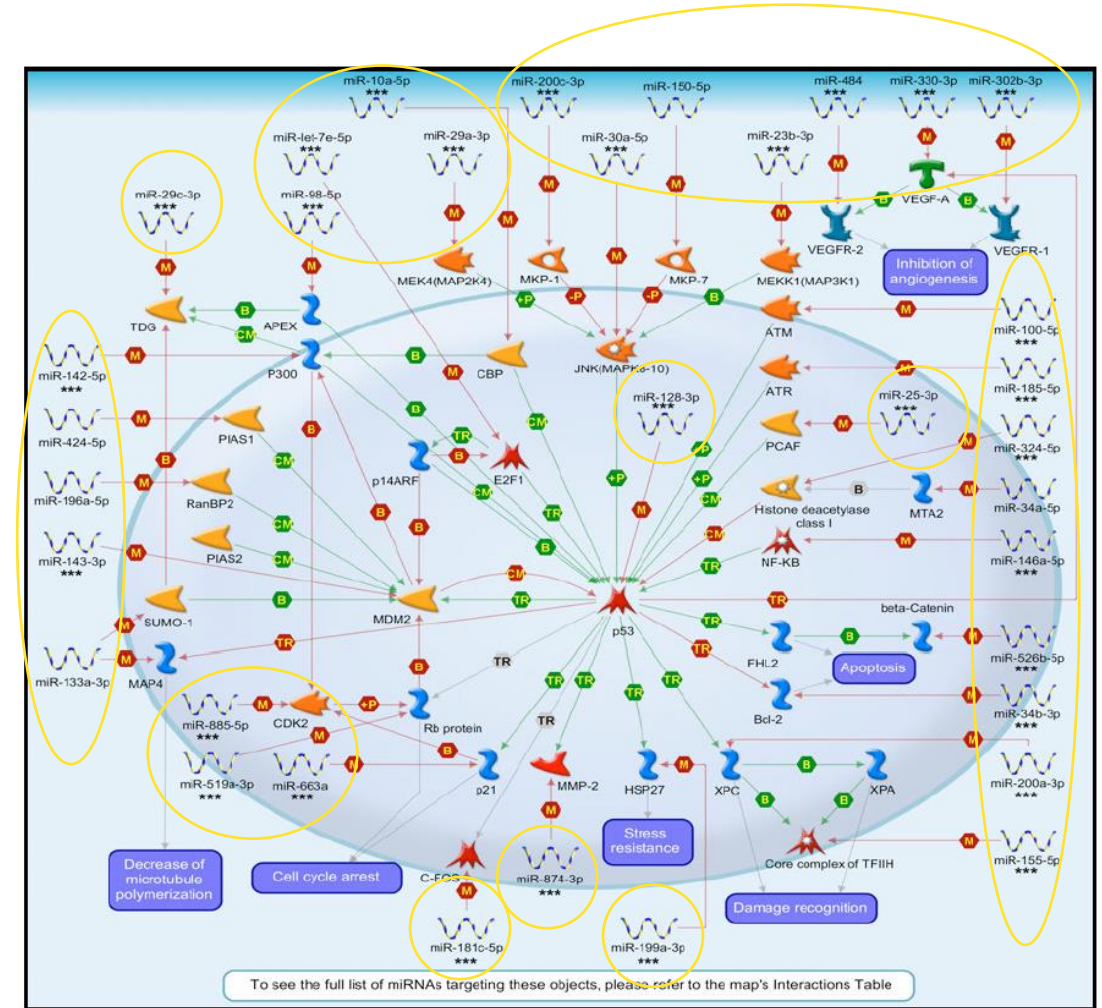
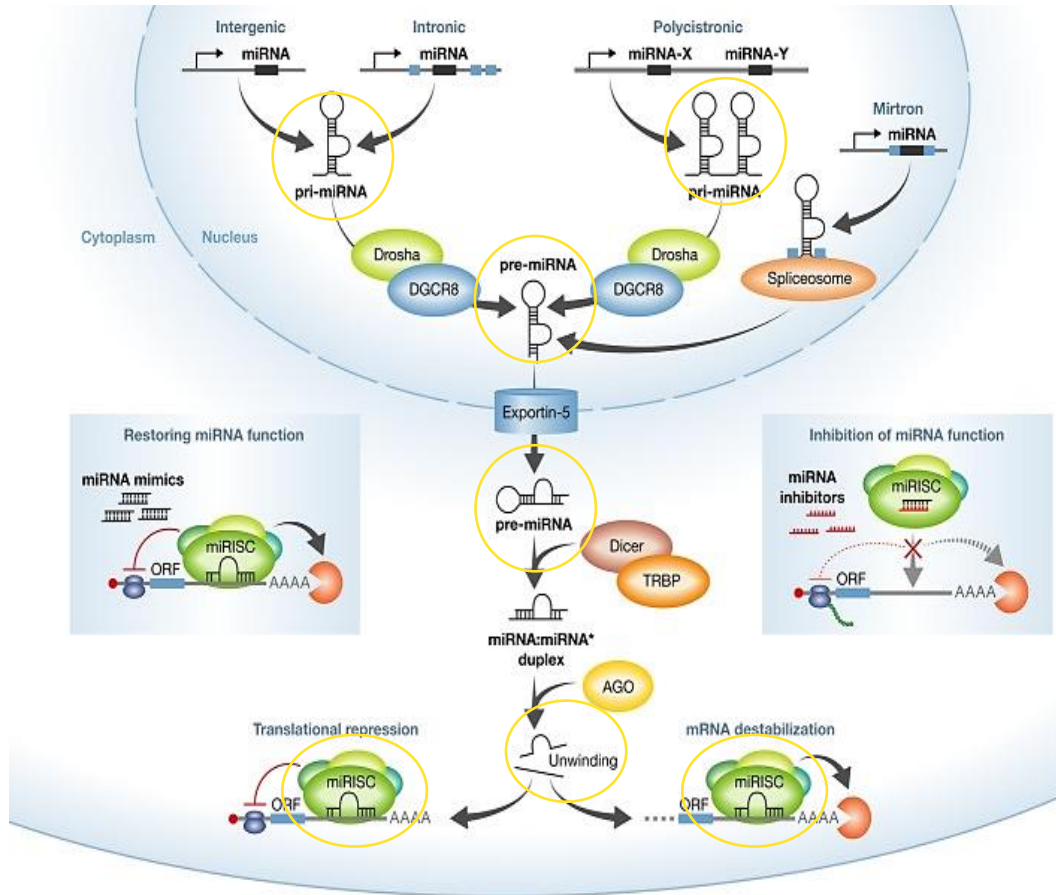
Novel approach for the detection & prediction of SARS-CoV-2



New universe of Biomarkers – non-coding RNAs

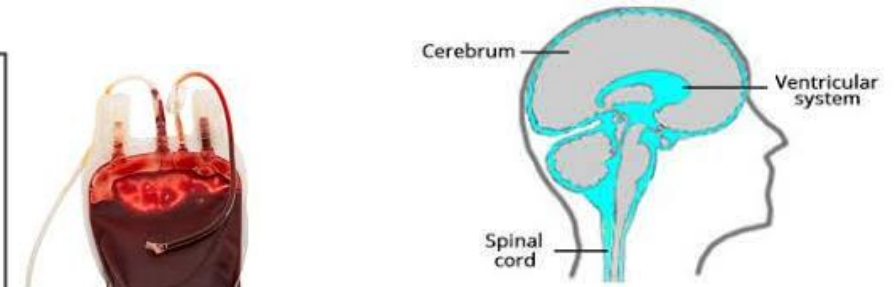
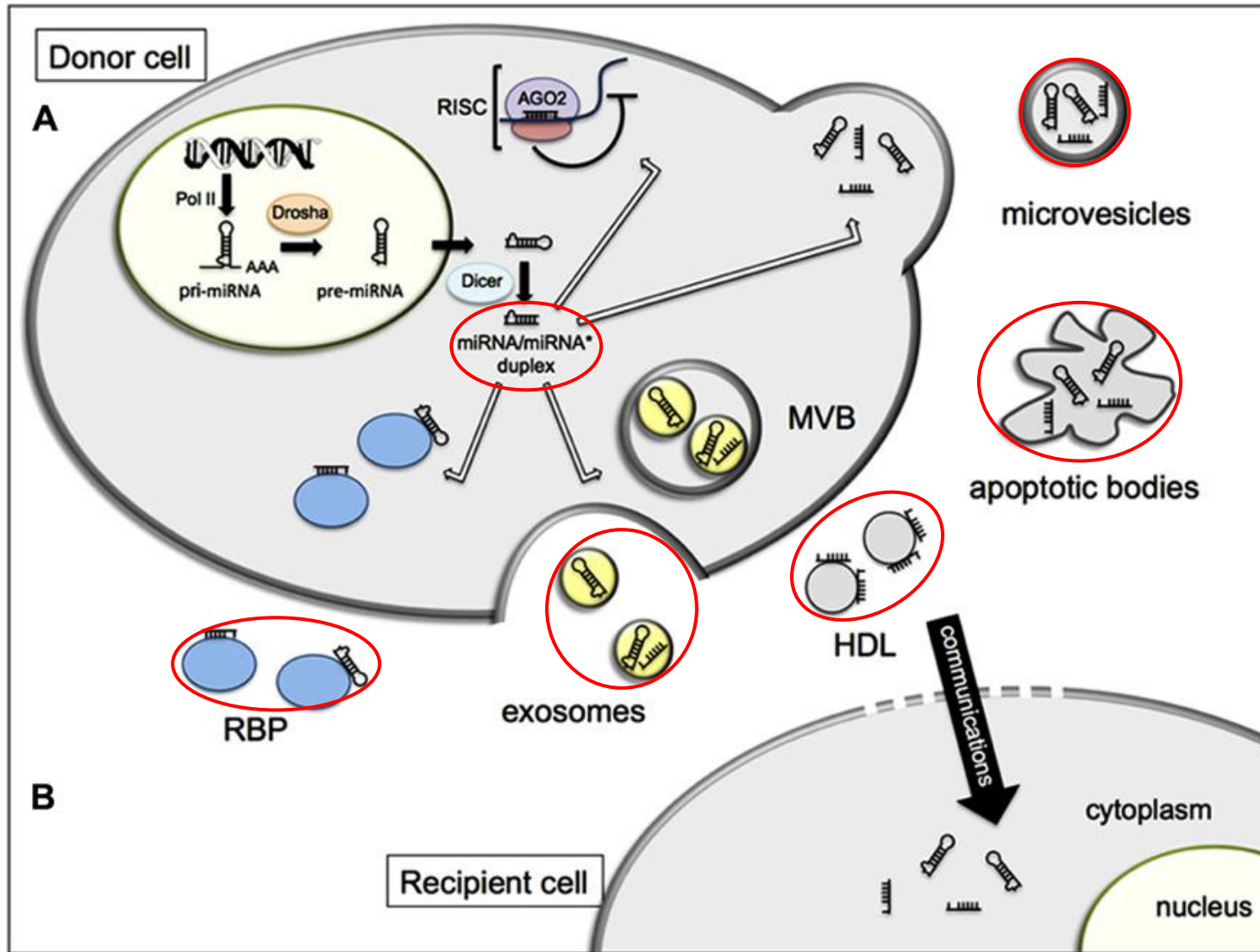


microRNAs – What? Why?

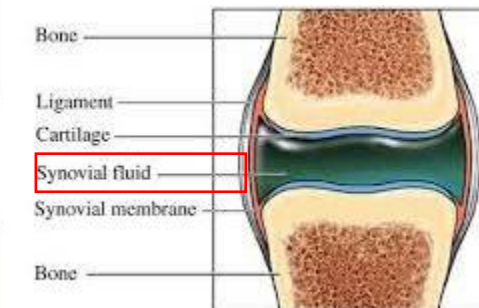


To see the full list of miRNAs targeting these objects, please refer to the map's Interactions Table

microRNAs – How?



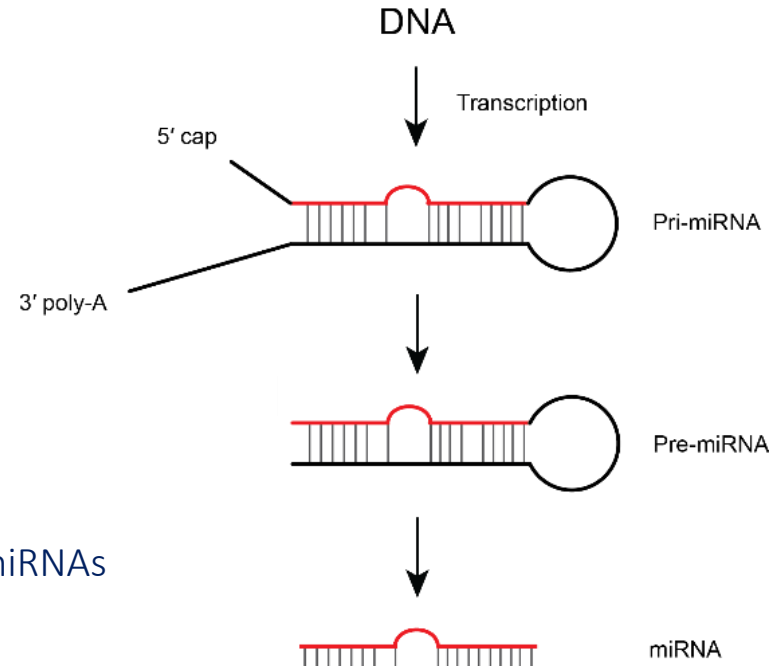
- ✓ Blood
- ✓ Urine
- ✓ SOF
- ✓ CSF
- ✓ Tears
- ✓ Sweat
- ✓ Milk
- ✓ Feaces



<https://doi.org/10.3389/fgene.2013.00214>

Challenges in miRNA quantification

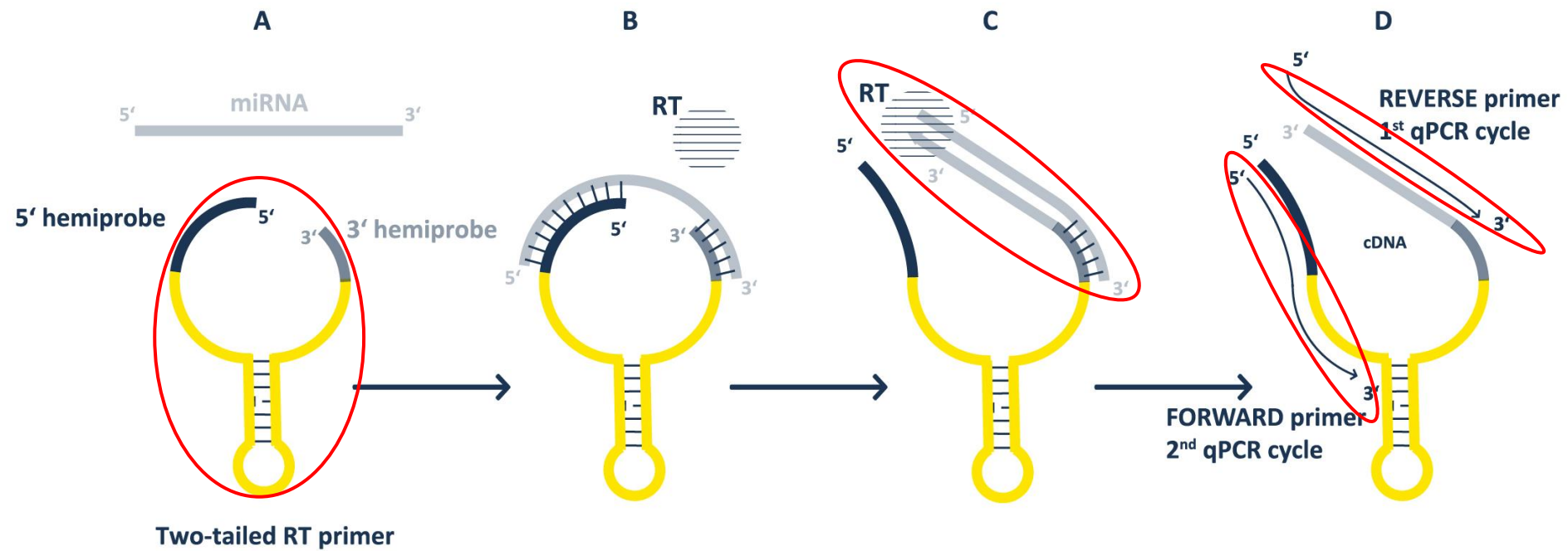
- Short length (~22nt)
- Lack of common sequences (e.g., poly-A)
- Variable GC content
- Mature sequences can be nearly identical (e.g., let-7 family)
- Biochemical modifications of terminal nucleotides
- The mature miRNA sequence is present also in the pre- and the pri-miRNAs
- miRNA isoforms (isomiRs) might evade capture, due to terminal heterogeneity



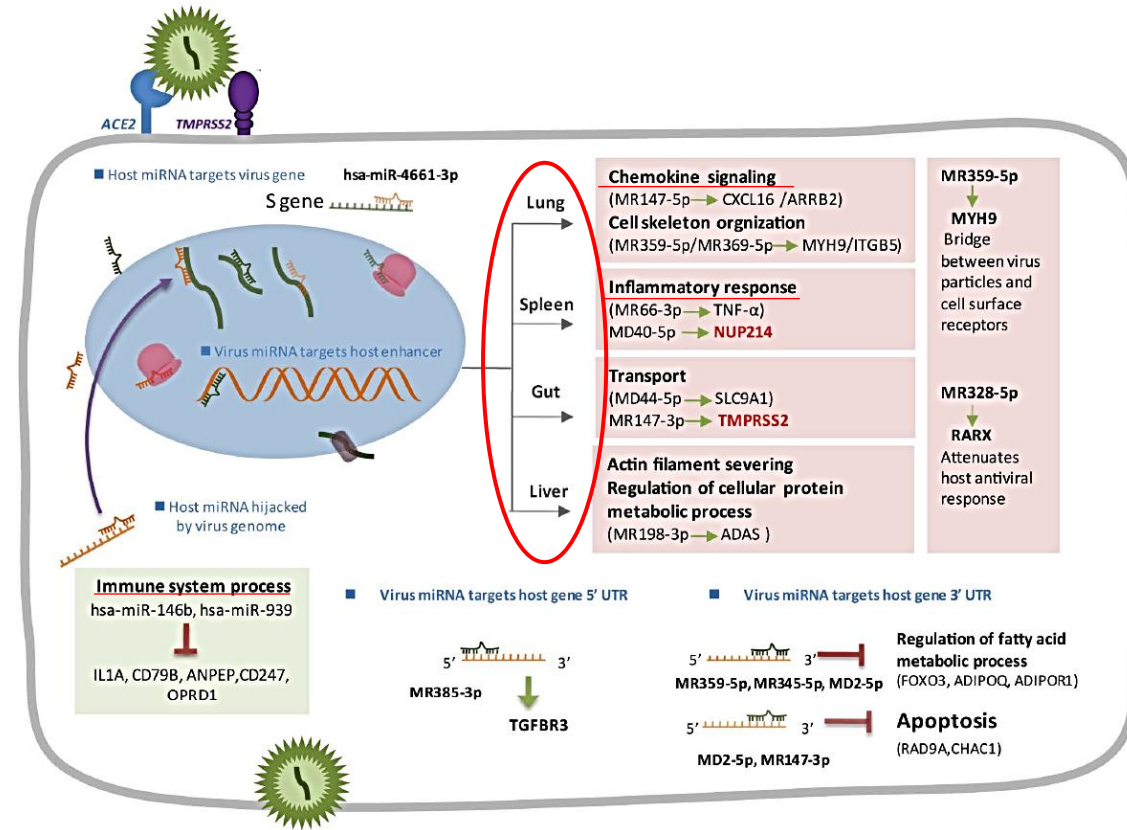
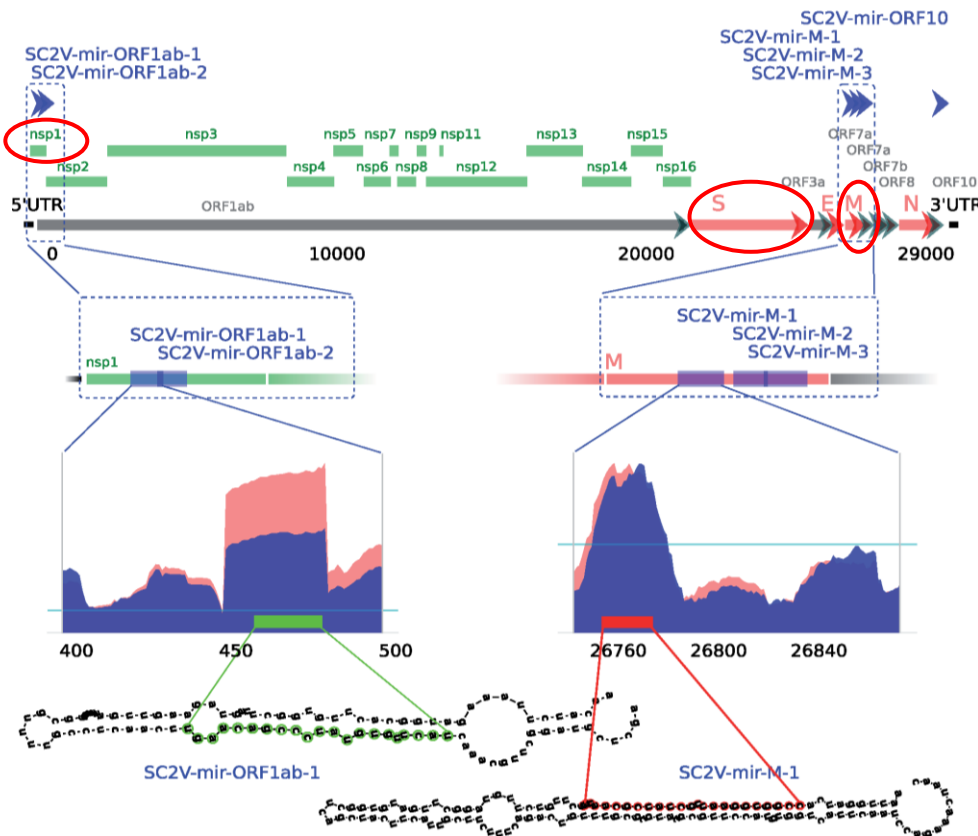
name	sequence
let-7a	UGAGGUAGUAGGUUGUAUAGUU
let-7b	UGAGGUAGUAGGUUGUGUGGUU
let-7c	UGAGGUAGUAGGUUGUAUGGUU
let-7d	AGAGGUAGUAGGUUGCAUAGUU
let-7e	UGAGGUAGGAGGUUGUAUAGUU
let-7f	UGAGGUAGUAGAUUGUAUAGUU
let-7g	UGAGGUAGUAGUUUGUACAGUU
let-7i	UGAGGUAGUAGUUUGUGCUGUU

Innovative quantification assay : TT-PCR

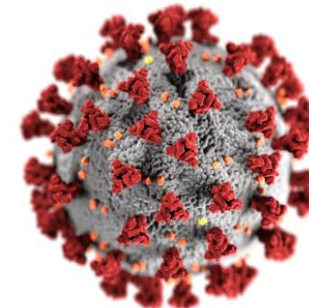
- Two-Tailed RT- qPCR assay
- miRNA - specific RT primer
- miRNA/cDNA - specific qPCR



Literature data mining for SARS-CoV-2 miRNAs



- miR-MD-2-5p
- miR-MD-147-3p
- miR-M-3-3p
- miR-ORF10-5p
- miR-ORF10-3p
- miR-ORF1ab-1-3p
- miR-M-1-5p

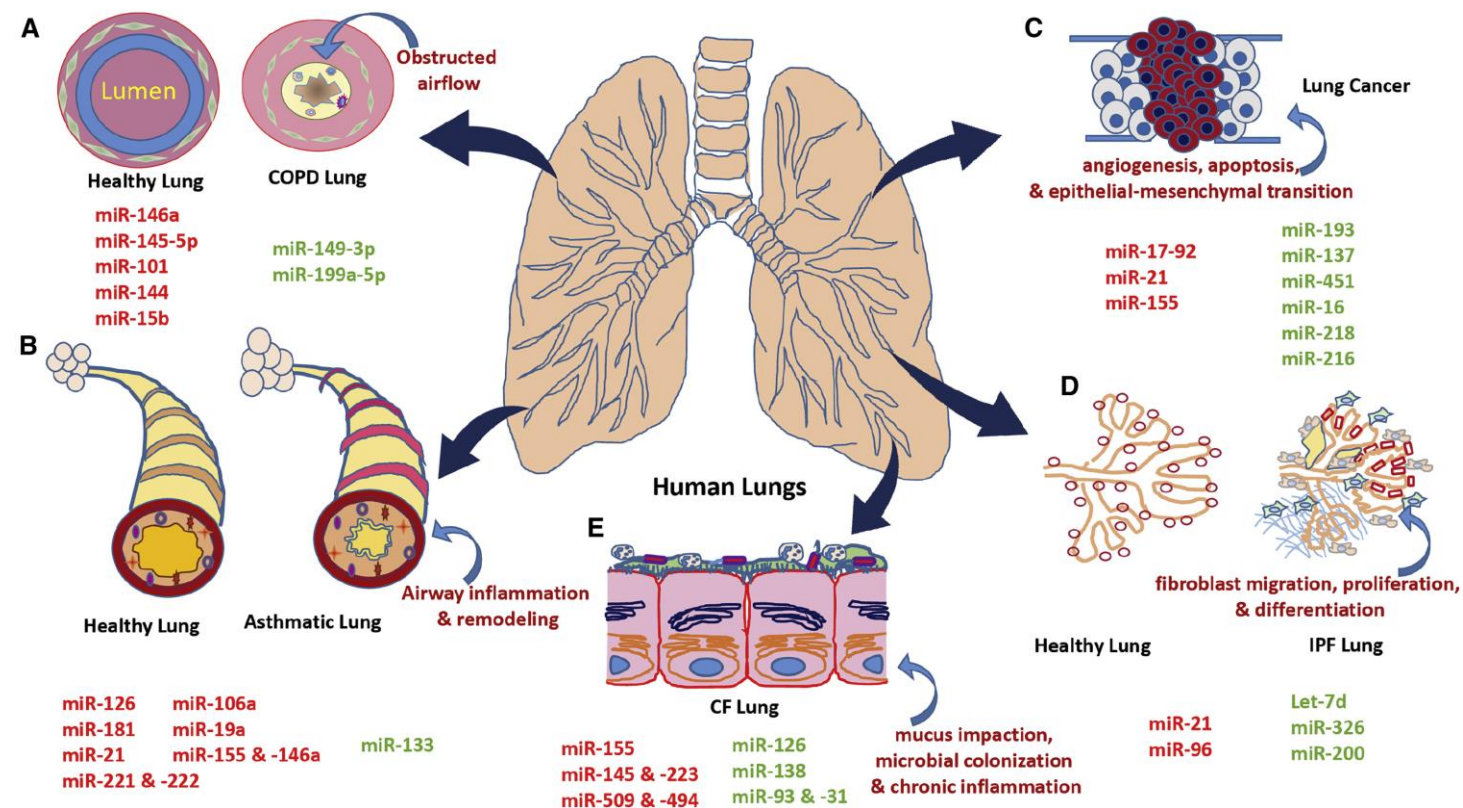


doi: 10.5812/archcid.104140

<https://doi.org/10.1093/bioinformatics/btaa1002>

Literature data mining for human miRNAs

- miR-451b
- miR-4288
- miR-4661-3p
- miR-3613-5p
- miR-214-3p
- miR-222-3p
- miR-124-3p
- miR-125a-3p
- miR-126-3p
- miR-146a-5p
- miR-423-5p
- miR-223-3p
- miR-20b-5p
- miR-21-5p
- miR-191-5p
- miR-122-5p
- miR-451a
- miR-23a-3p
- miR-31-5p
- miR-449c-5p



TT-PCR at global anti-COVID service

CARDIOLOGICAL RISK

Cat. No.	miRNA
RDTT0010251PRI	hsa-miR-449c-5p
RDTT0000076PRI	hsa-miR-21-5p

THROMBOSIS RISK

Cat. No.	miRNA
RDTT0000076PRI	hsa-miR-21-5p
RDTT0000440PRI	hsa-miR-191-5p
RDTT0000421PRI	hsa-miR-122-5p
RDTT0001631PRI	hsa-miR-451a
RDTT0000078PRI	hsa-miR-23a-3p

HOST CELL INFECTION

Cat. No.	miRNA
RDTT0019840PRI	hsa-miR-451b
RDTT0016918PRI	hsa-miR-4288
RDTT0019730PRI	hsa-miR-4661-3p
RDTTMD25PPRI	sc2v-miR-MD-2-5p
RDTTORF1AB13PPRI	sc2v-miR-ORF1ab-1-3p
RDTTORF1AB15PPRI	sc2v-miR-ORF10-5p

VIRUS FIGHTING

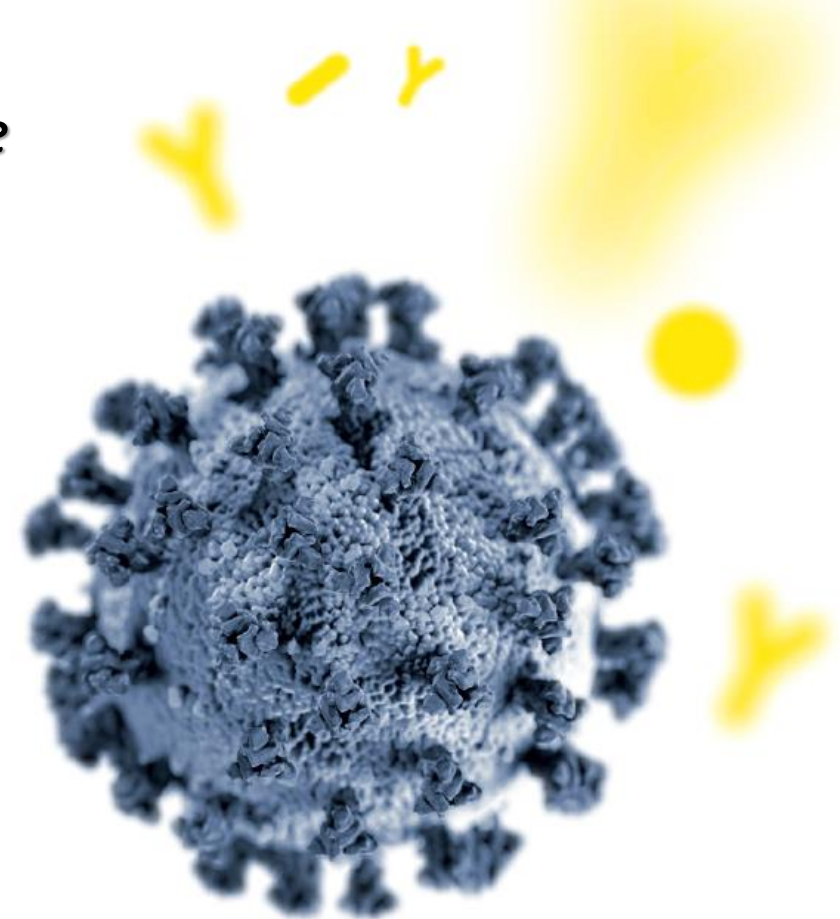
Cat. No.	miRNA
RDTT0019840PRI	hsa-miR-451b
RDTT0016918PRI	hsa-miR-4288
RDTT0019730PRI	hsa-miR-4661-3p
RDTT0017990PRI	hsa-miR-3613-5p
RDTT0000271PRI	hsa-miR-214-3p
RDTT0000279PRI	hsa-miR-222-3p
RDTT0000422PRI	hsa-miR-124-3p
RDTT0001413PRI	hsa-miR-20b-5p

HOST IMMUNITY IMPAIRMENT

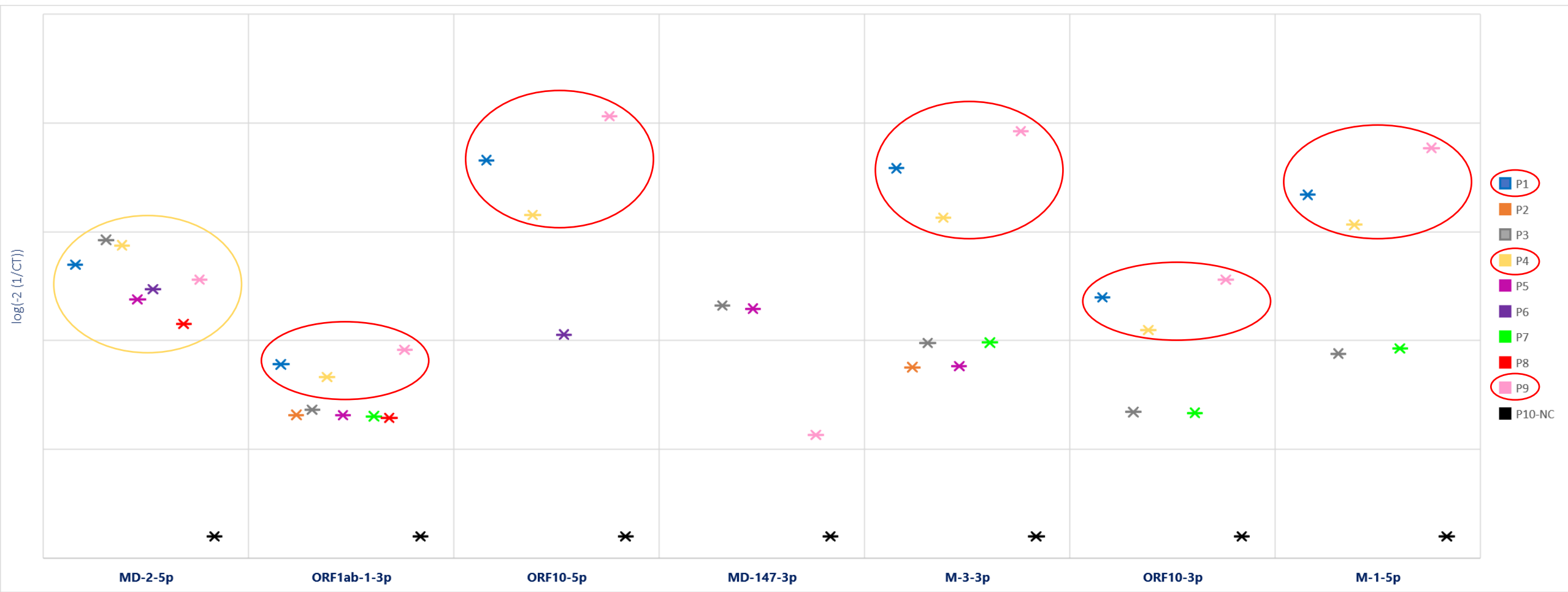
Cat. No.	miRNA
RDTTORF1AB13PPRI	sc2v-miR-ORF1ab-1-3p
RDTTORF1AB15PPRI	sc2v-miR-ORF10-5p
RDTTMD25PPRI	sc2v-miR-MD-2-5p

GUT CELLS INVASION, LUNG INVASION

Cat. No.	miRNA
RDTTMD1473PPRI	sc2v-miR-MD-147-3p

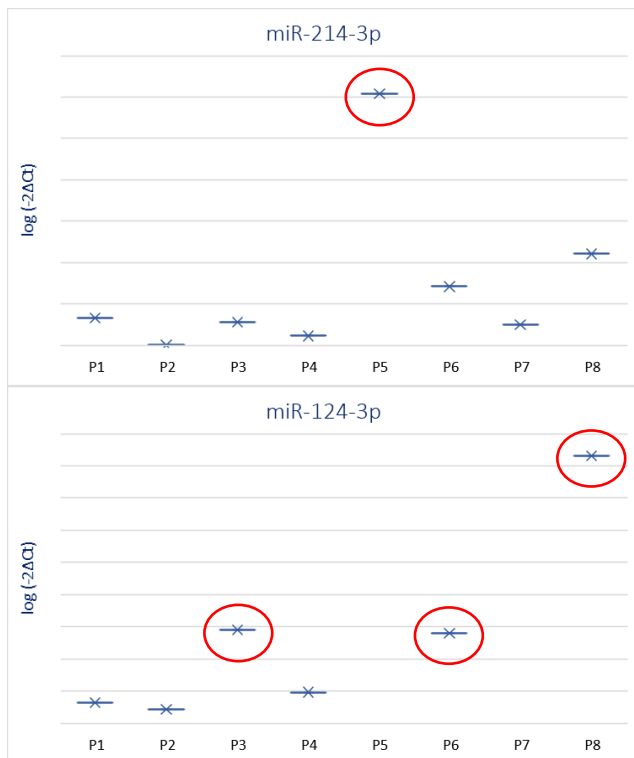


Trial SARS-CoV19 miRNA measurements



Trial human miRNA measurements

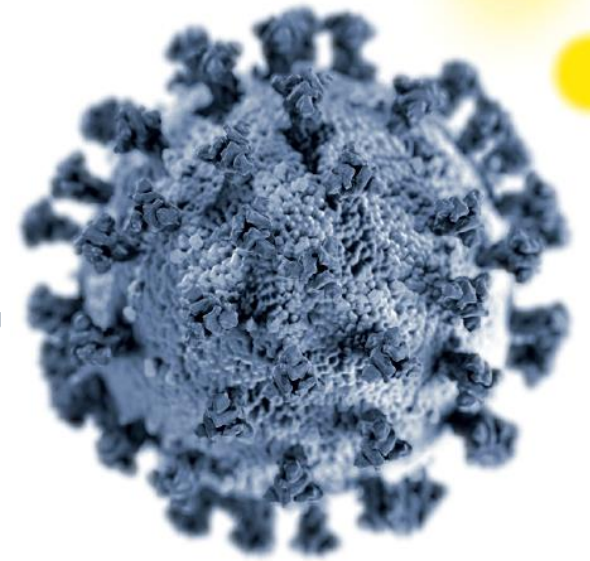
- P1 → COV+, bilateral lung inflammation
- P2 → COV+, British mutation
- P3 → COV+, bilateral lung inflammation + thrombosis : miR124-3p
- P4 → COV+, ICHS+HT, liver transplantation
- P5 → COV+, multiplex sclerosis : miR214-3p
- P6 → COV+, bilateral lung inflammation + Chronic Lymphatic Leukemy
- P7 → COV+, water on the lungs
- P8 → COV+, severe viral pneumonia



Aim : diagnostic tool

- Reasonable number of targets
- Easy to prepare (multiplexing)
- Easy to evaluate (simple data processing, final report)
- Validated on large number of patients
- Make all TT-PCR assays available for RUO

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Thank you for your attention!



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