



Serum non-coding RNA profiling as a promising diagnostic approach

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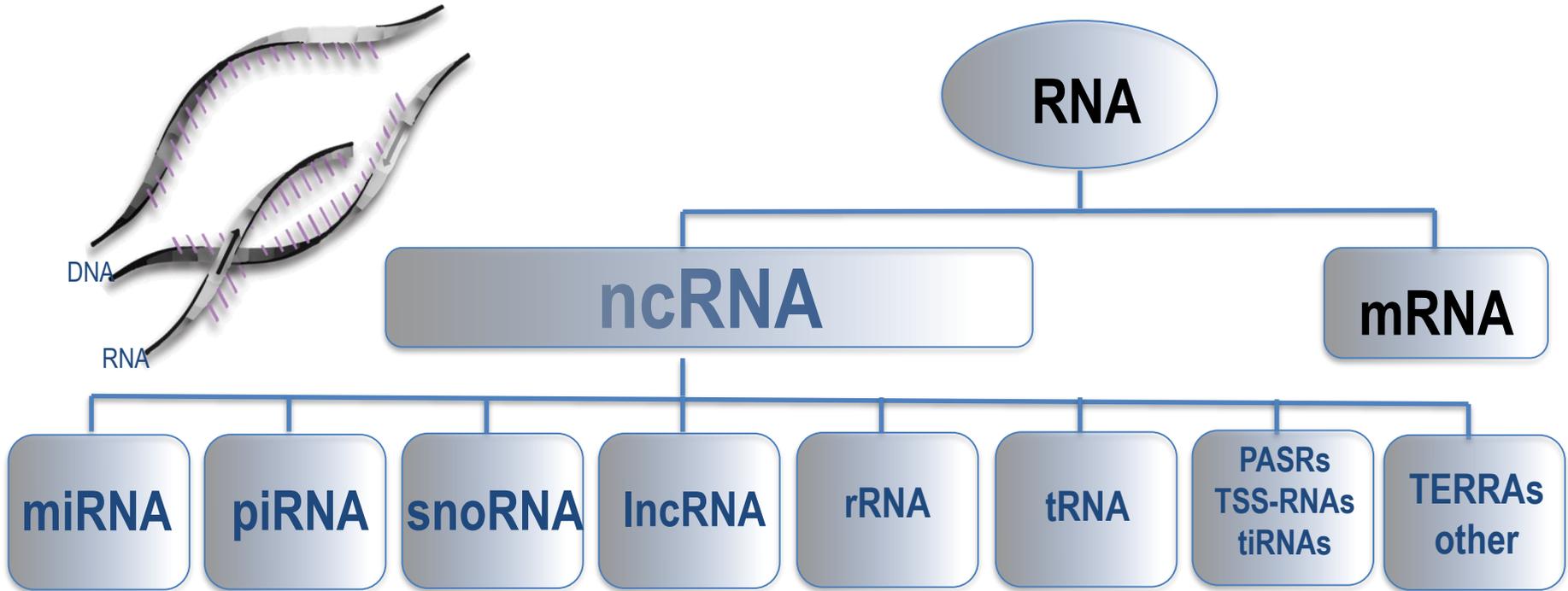
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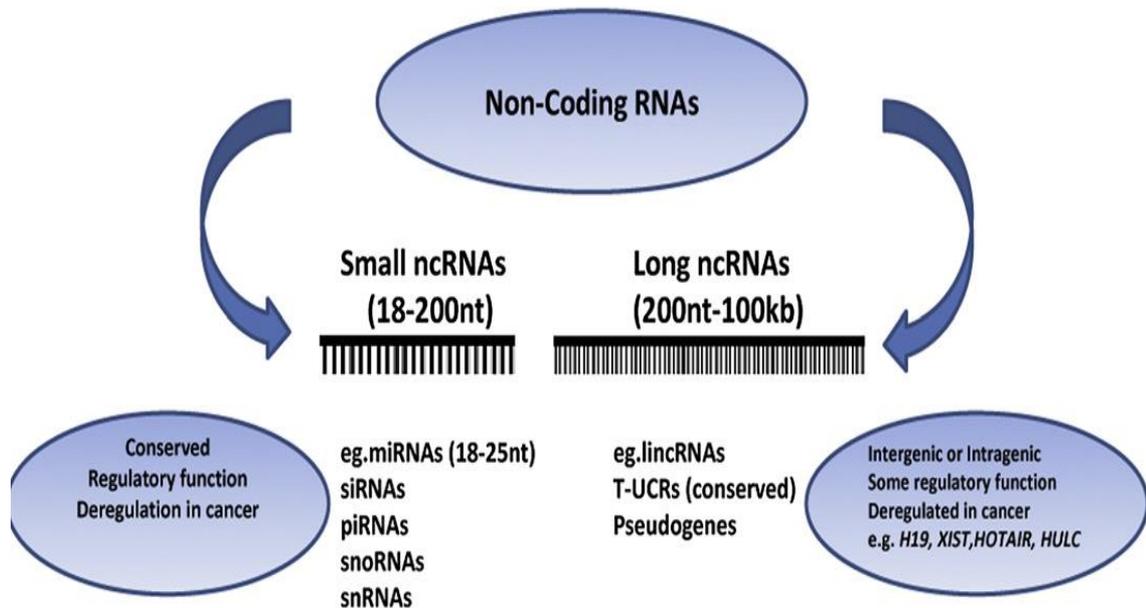
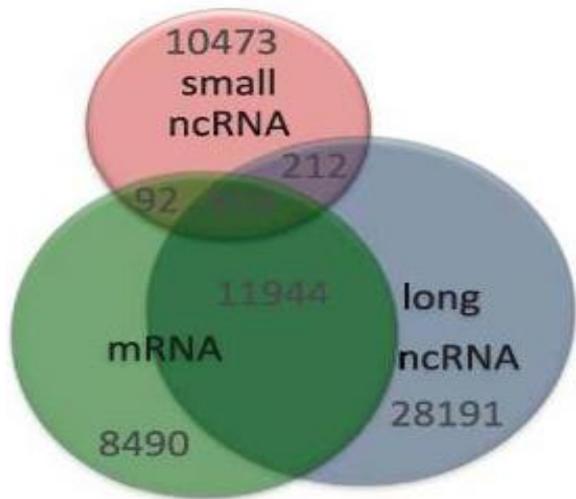
Non-protein-coding RNAs





Non coding RNAs: biomarkers and therapeutic targets

Number of transcripts in human transcriptome



MicroRNAs: regulators of mRNA expression

Endogenous, non protein coding, small RNAs

Exhibit tissue specific or developmental stage specific expression

Regulate translation and stability of mRNAs

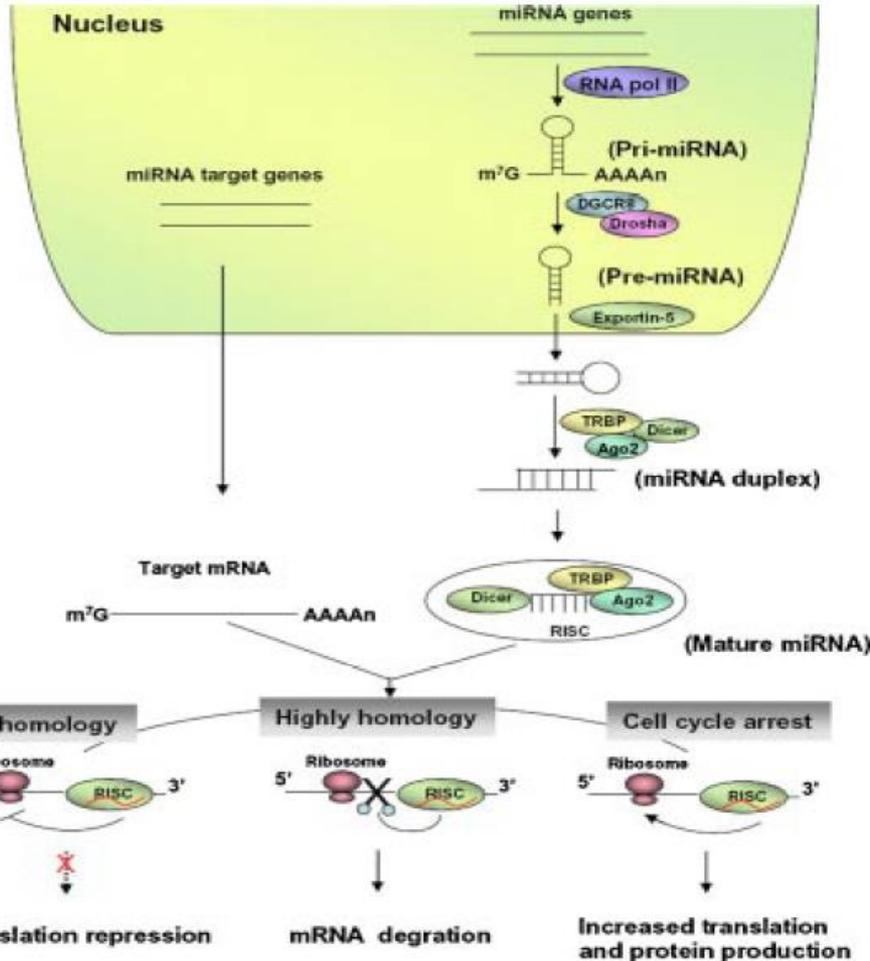
Role

in hematopoiesis

In control of cell survival /proliferation

In chromatin regulation

In immunity





- Methods of ncRNA profiling-advantages
- Types of ncRNAs and their function
- Example1: the impact of renal dialysis in serum ncRNAs
- Example2: serum ncRNA profiling for identification of biomarkers for male fertility



ncRNA profiling for disease diagnosis and prediction

- Serum ncRNAs
 - diurnal variation
 - limited characterization of ncRNA status in health
- Tissue ncRNAs
 - cell-type complexity



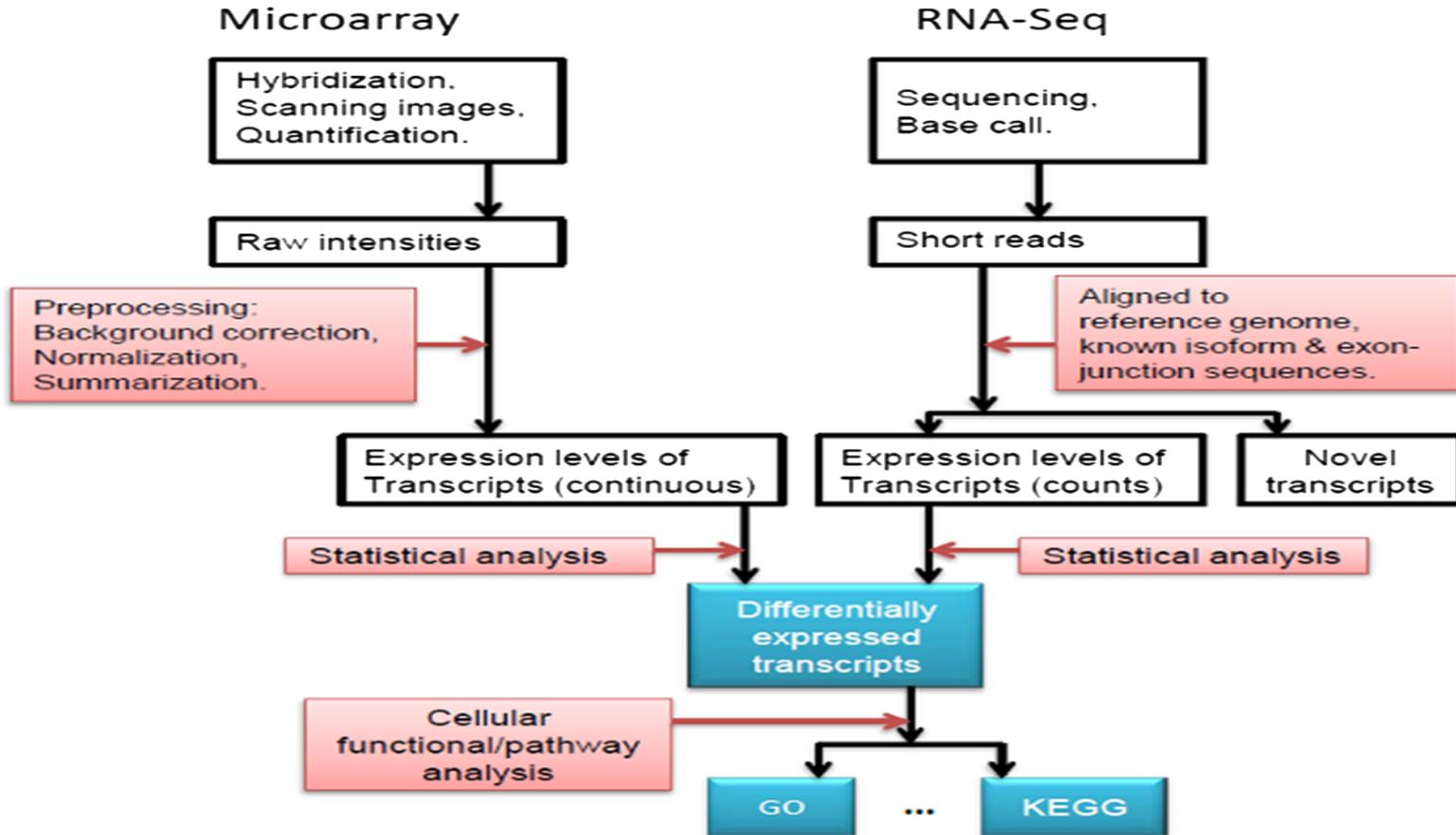
Available technologies

- single or multiple ncRNA detection: biochip technologies
- Profiling:
 - array technologies
 - RNA sequencing platforms



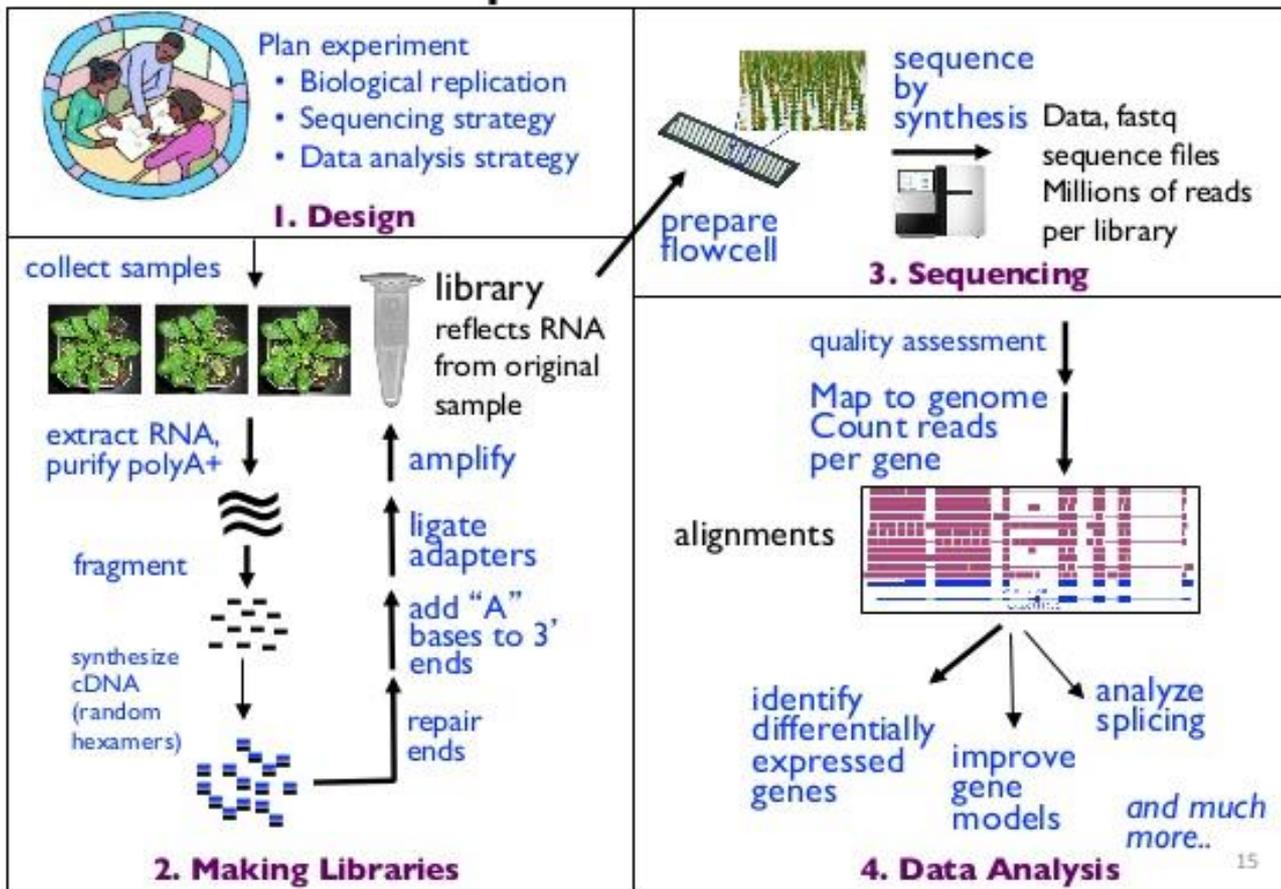


Comparison of microarray and RNAseq profiling methods





RNA-Seq Overview - Illumina





Advantages of RNA profiling

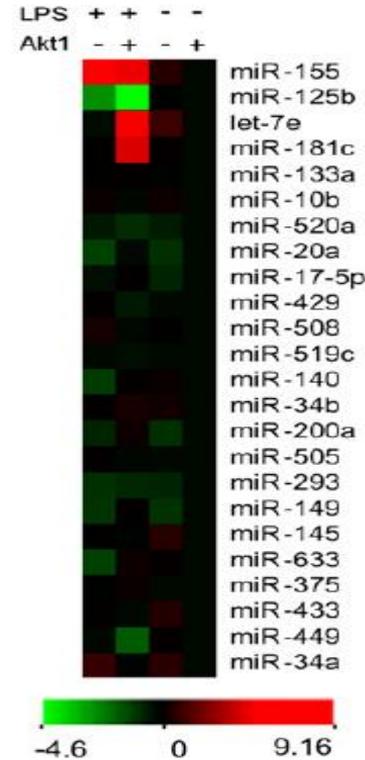
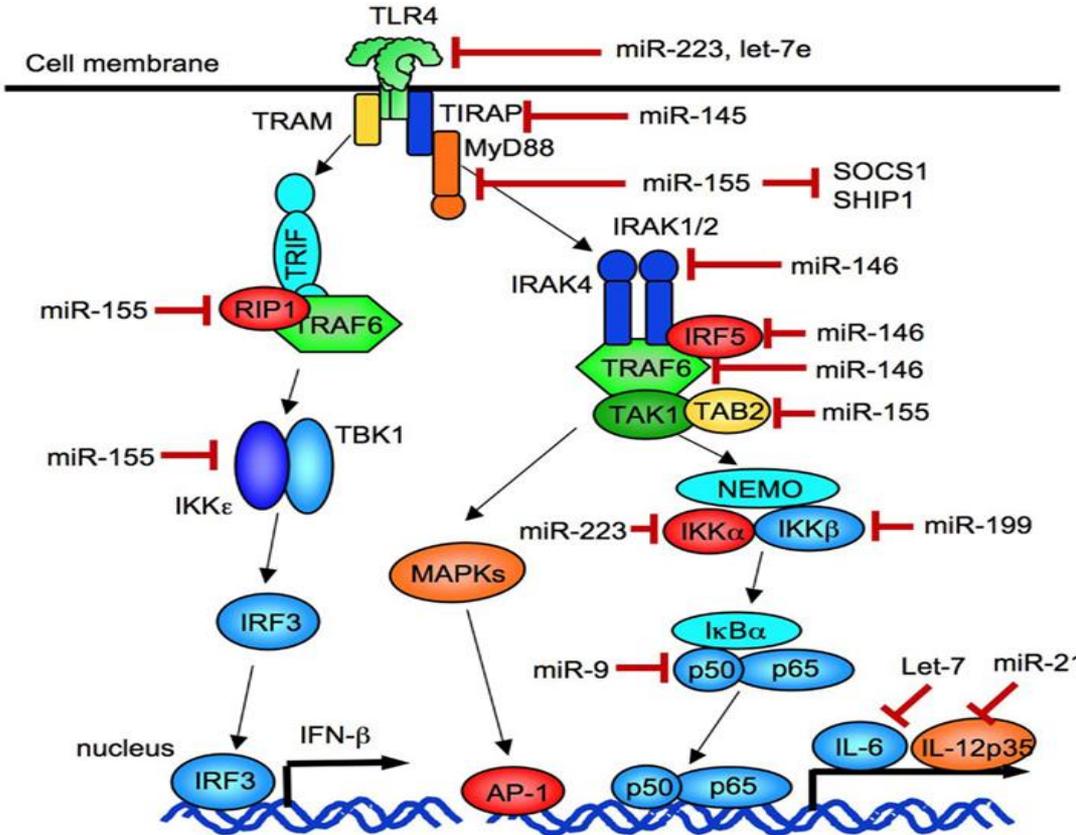
- Determination of the RNAome of each individual and potential unique changes
- Given the complexity of disease pathogenesis identification of pathways affected rather than individual markers can provide safer diagnosis
- Allows identification of novel biomarkers



Intracellular ncRNA profiling: determine pathogenetic mechanisms and cell biomarkers



miRNAs regulate TLR signalling and inflammation



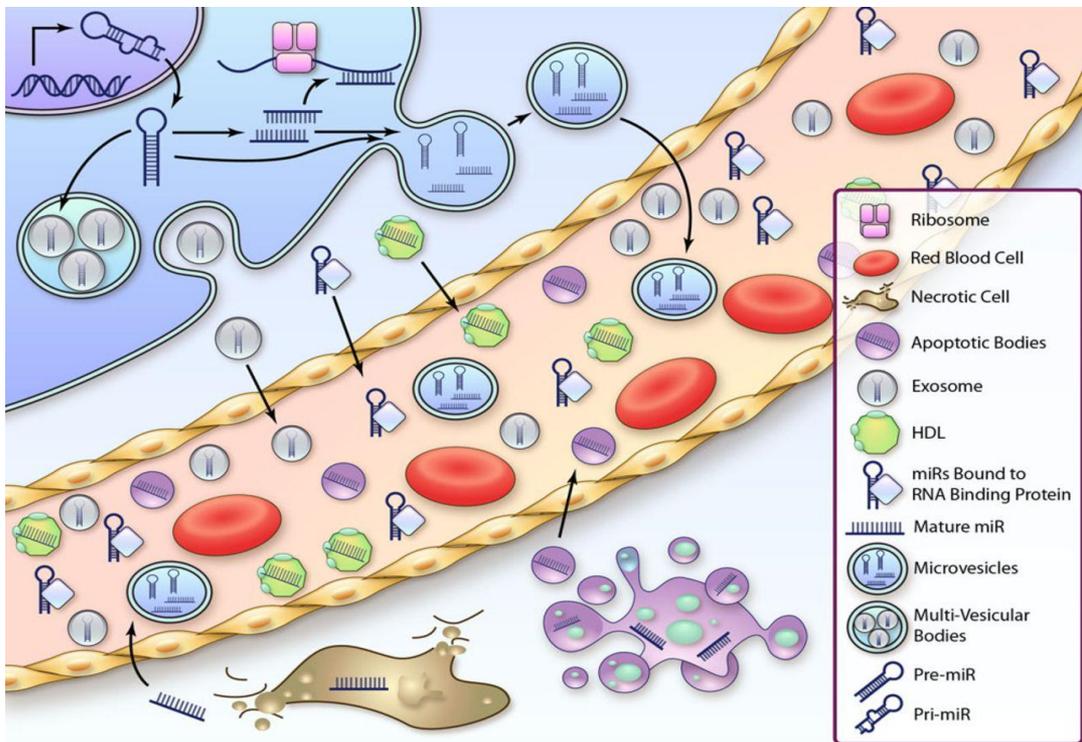
Androulidaki et al, Immunity 2009

Arranz et al, PNAS 2012

Vergadi et al, J Immunol 2014



ncRNAs are secreted in the serum and play the role of second messengers; potential serum biomarkers



Sources:

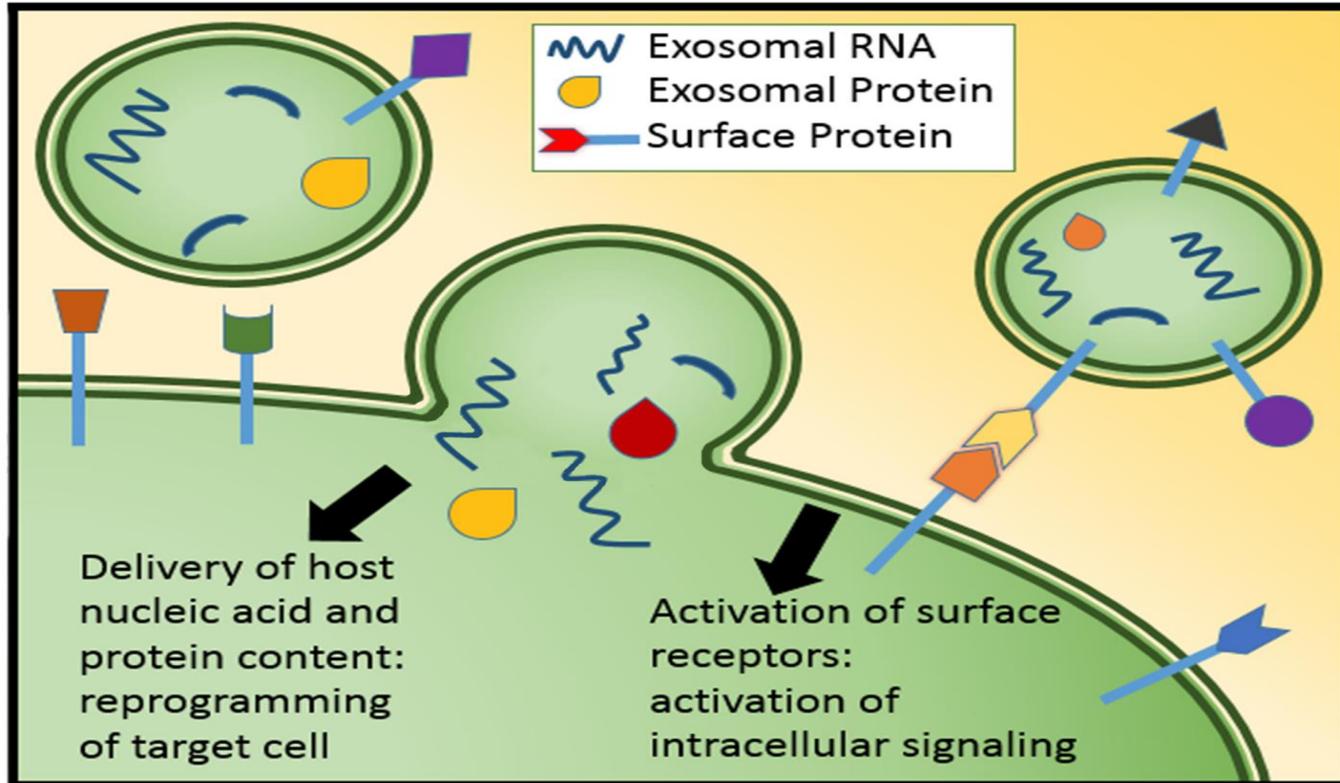
1. ncRNAs actively secreted
2. Released from apoptotic cells
3. Released from necrotic cells

Modes of transport

1. in exosomes
2. Bound on RNA/protein complexes
3. bound on lipoproteins
4. Engulfed in apoptotic bodies
5. Free (unbound)

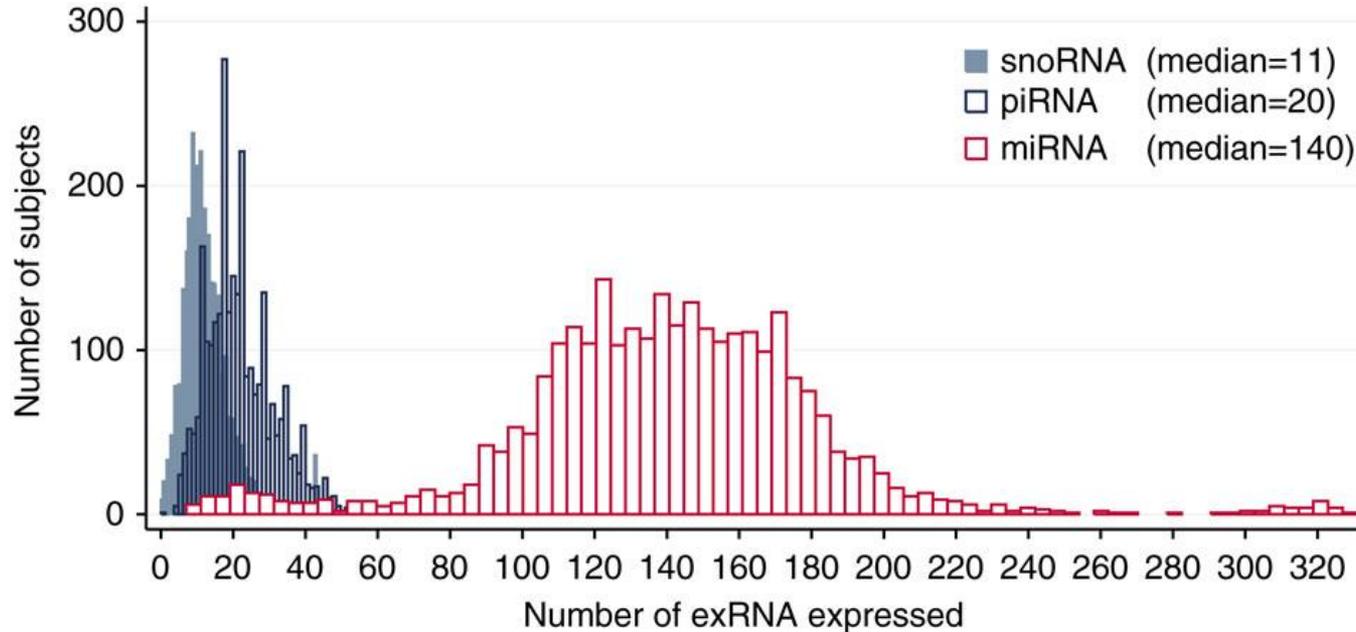


Exosomal RNAs are delivered in a cell-specific manner utilizing receptor recognition and affect target tissue function



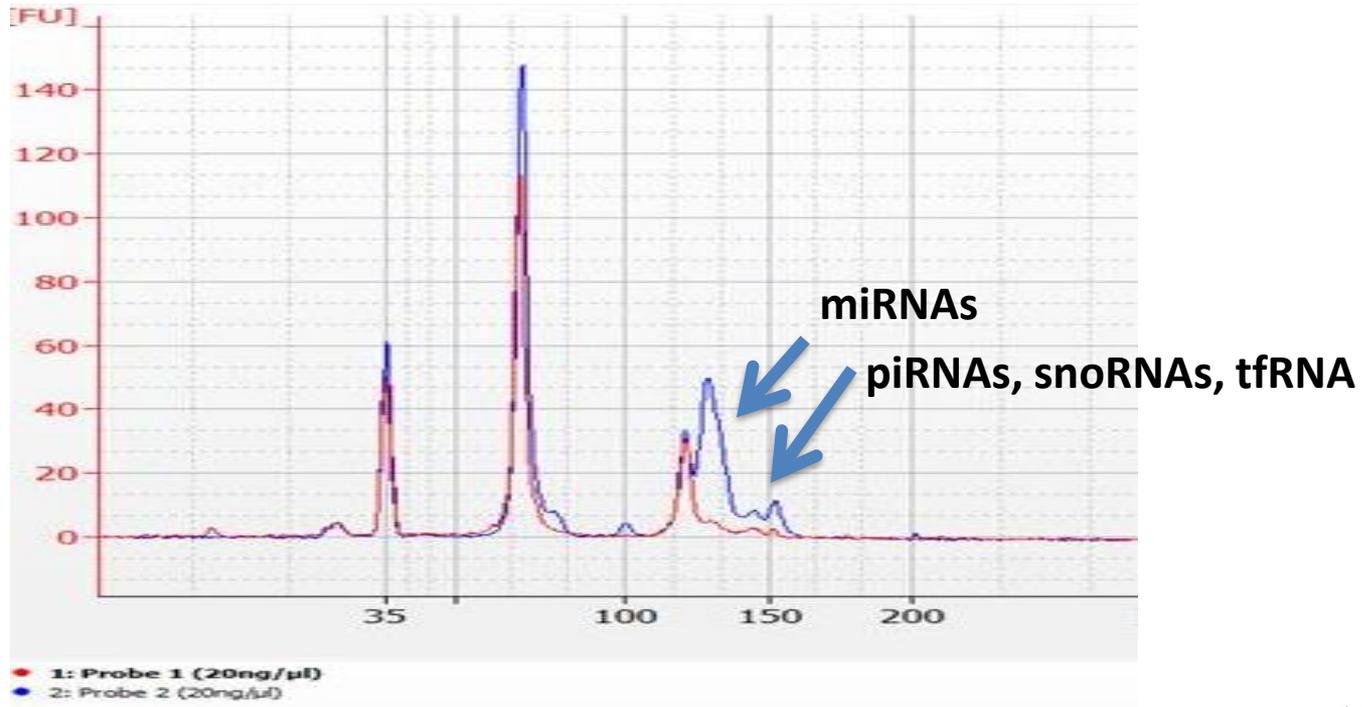


Presence of ncRNAs in the serum





Serum RNA profile





Examples of RNA profiling for determining biomarkers

- The case of Renal dialysis
- piRNAs in the serum as markers of male subfertility

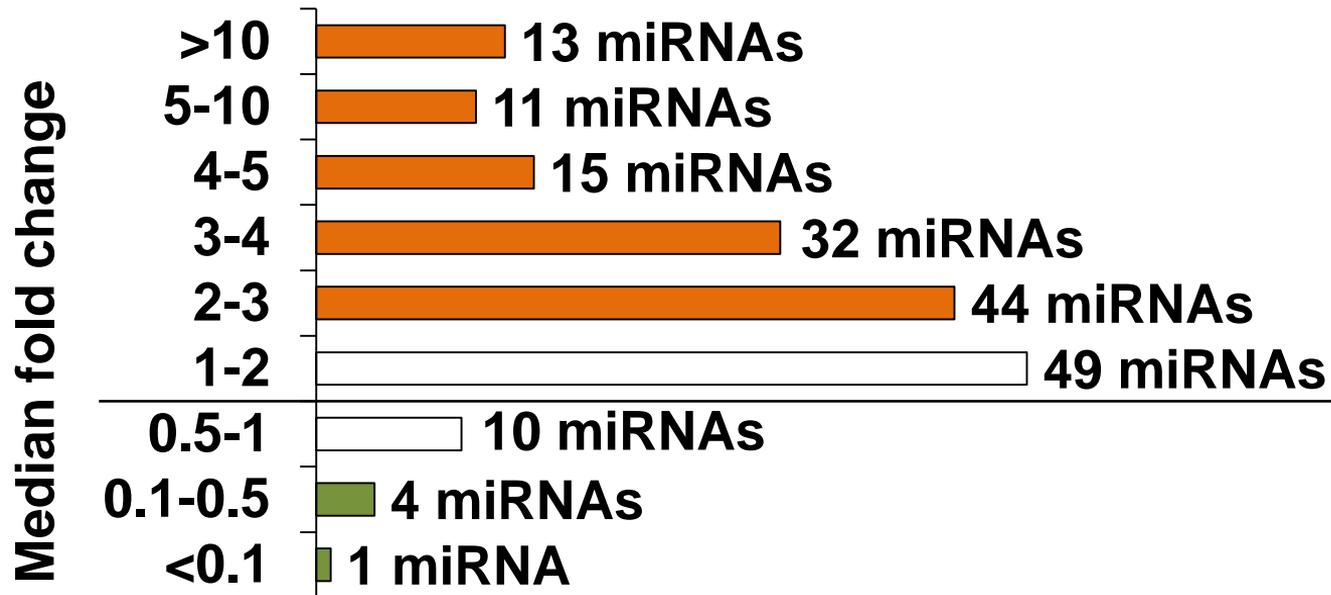


ncRNAs as functional biomarkers of kidney disease and associated pathologies

- Is serum miRNA profile altered due to hemodialysis?
 - collection of serum samples before and right after hemodialysis; extract RNA; analyze using miRNA arrays



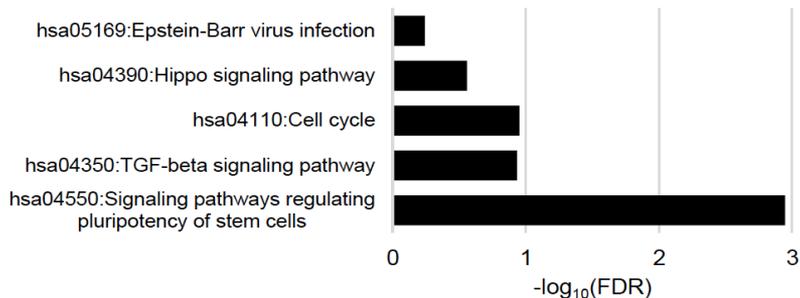
Does hemodialysis alter the miRNA profile in the serum?



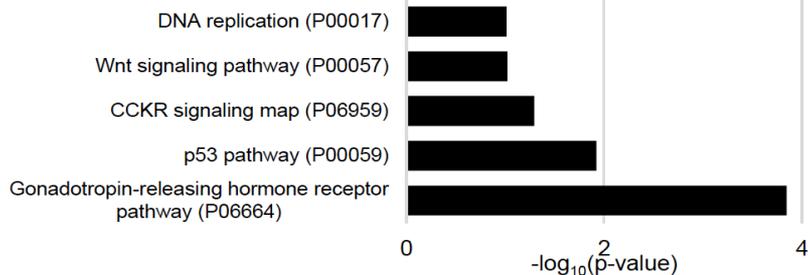


Gene families affected by miRNAs differentially expressed after hemodialysis-potential link to associated pathologies

A KEGG pathways



B PANTHER pathways



C Gene Ontology terms

Biological processes

GO:0045893~positive regulation of transcription, DNA-templated

GO:0006351~transcription, DNA-templated

GO:0006413~translational initiation

GO:0098609~cell-cell adhesion

GO:0016032~viral process

GO:0043161~proteasome-mediated ubiquitin-dependent protein cat. process

GO:0006614~SRP-dependent cotranslational protein targeting to membrane

GO:0045892~negative regulation of transcription, DNA-templated

GO:0051301~cell division

GO:0000184~nuclear-transcribed mRNA catabolic process



- The profile of serum miRNAs is altered during hemodialysis
 - Implications in the pathogenesis of hemodialysis associated diseases (cardiovascular disease; cancer; subfertility)

Products already available utilizing miRNA profiling in diagnosis/prognosis.. and more to come



thrombomiR™

biomarkers of platelet function

osteomiR™

validated bone biomarkers



PANArray™
miRNA Expression Profiling Kit



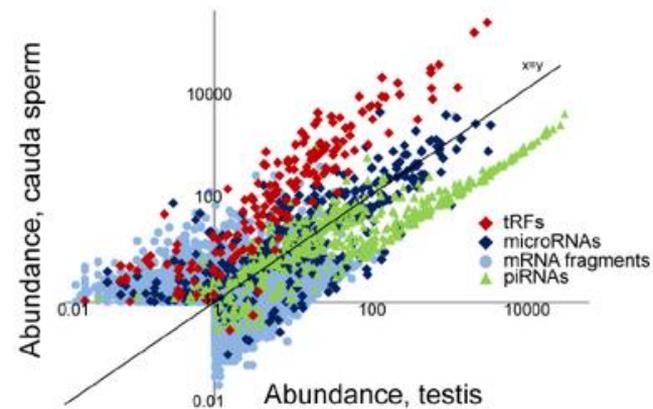
piRNAs

- Are expressed predominantly in male germ cells in mammals (and some tumor cells)
- They function as components of adaptive immune systems that protect host (sperm) from foreign nucleic acids such as transposons

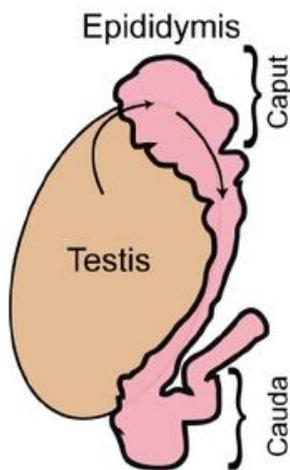


piRNAs are predominant in the testis and present in sperm and seminal fluid

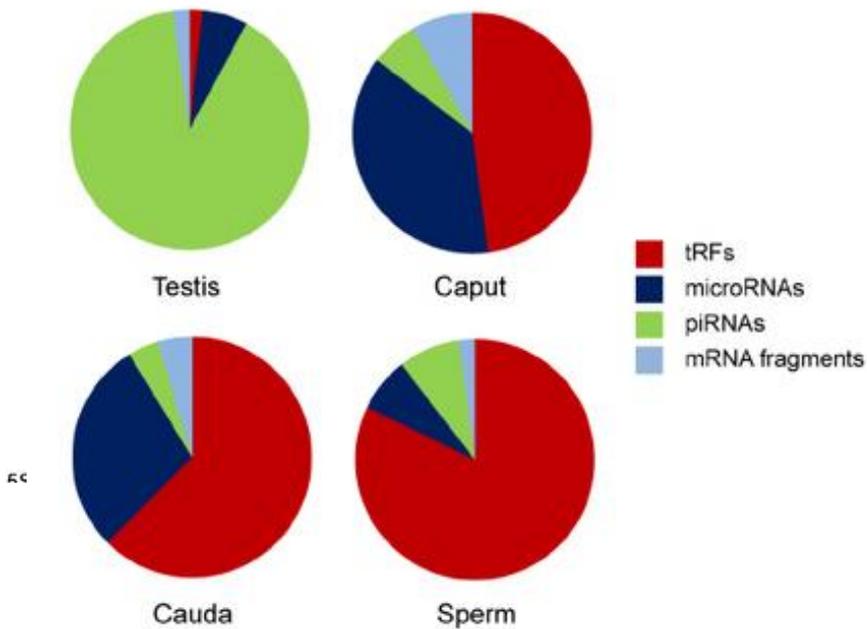
A



B



C





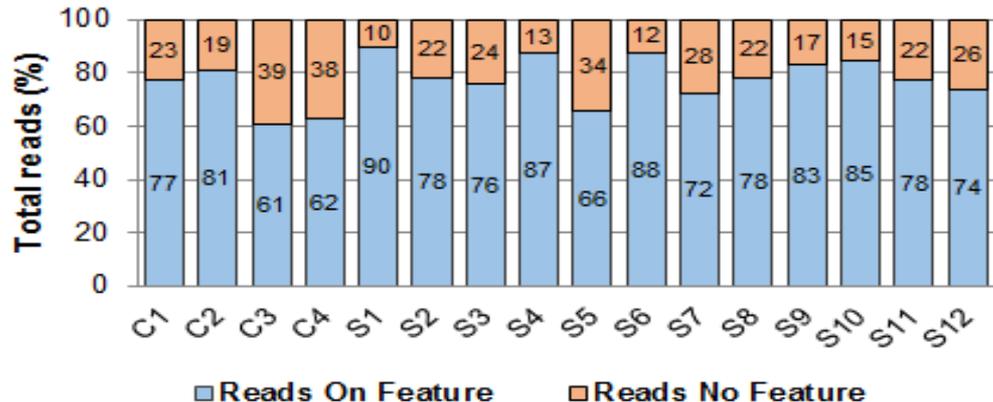
Serum RNA sequencing to identify novel non-coding RNAs associated with male subfertility

- Control population normal TT and normal FSH (**Control**)
- Subfertile men with normal TT and normal FSH (**Subfertile 1**)
- Subfertile men with normal TT and high FSH (**Subfertile 2**)
- Subfertile men with low TT and normal FSH (**Subfertile 3**)

Illumina Next Generation Sequencing



Reads mapped in database detection rate ~80%

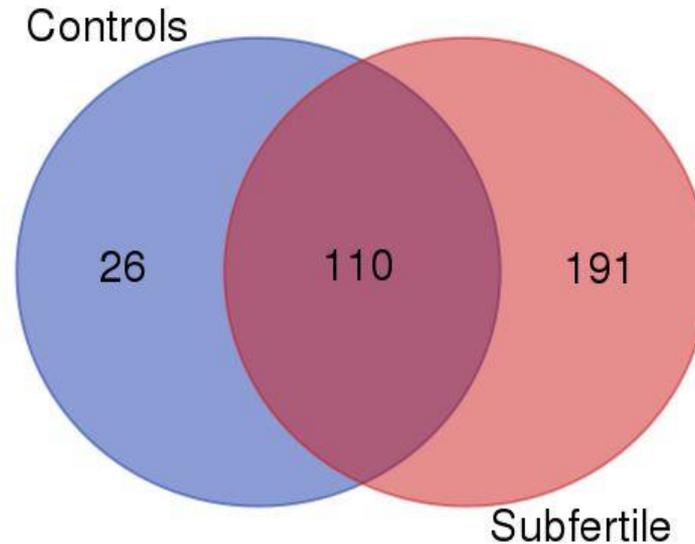
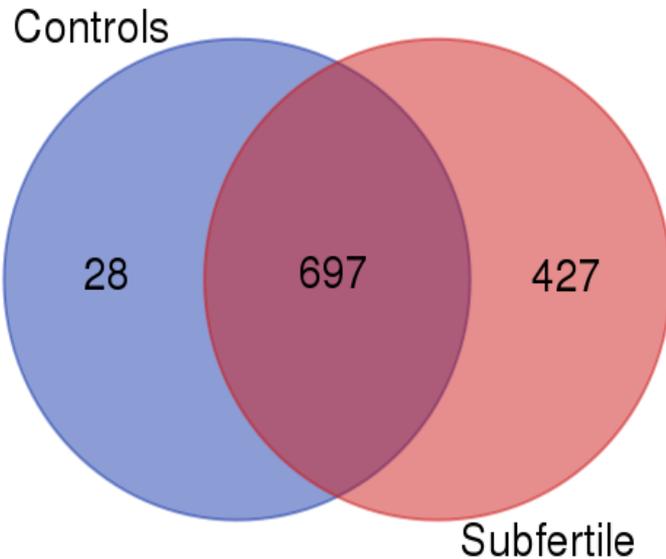




Comparison between Control and all Subfertile groups tested

miRNAs

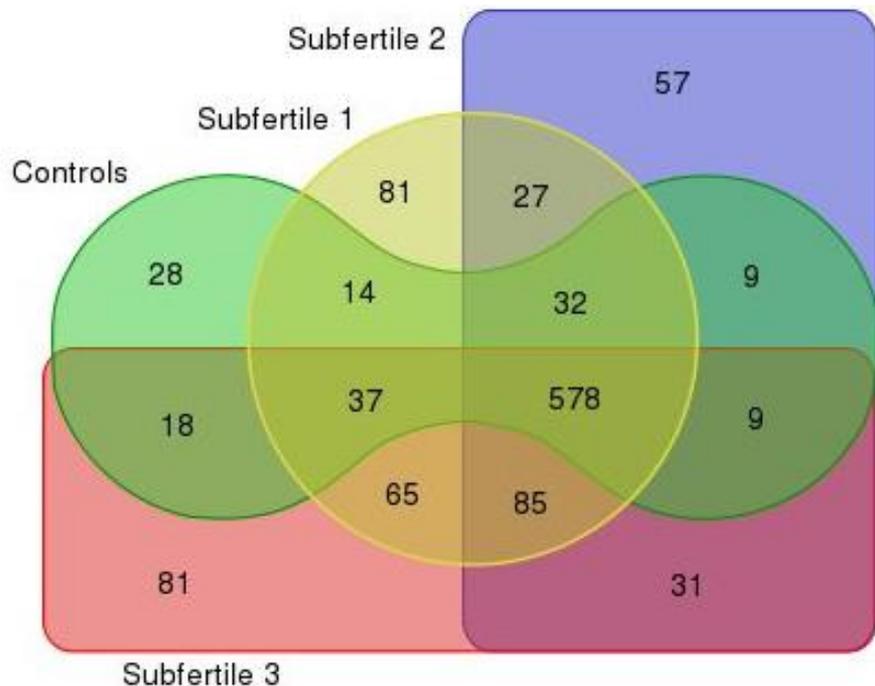
piRNAs



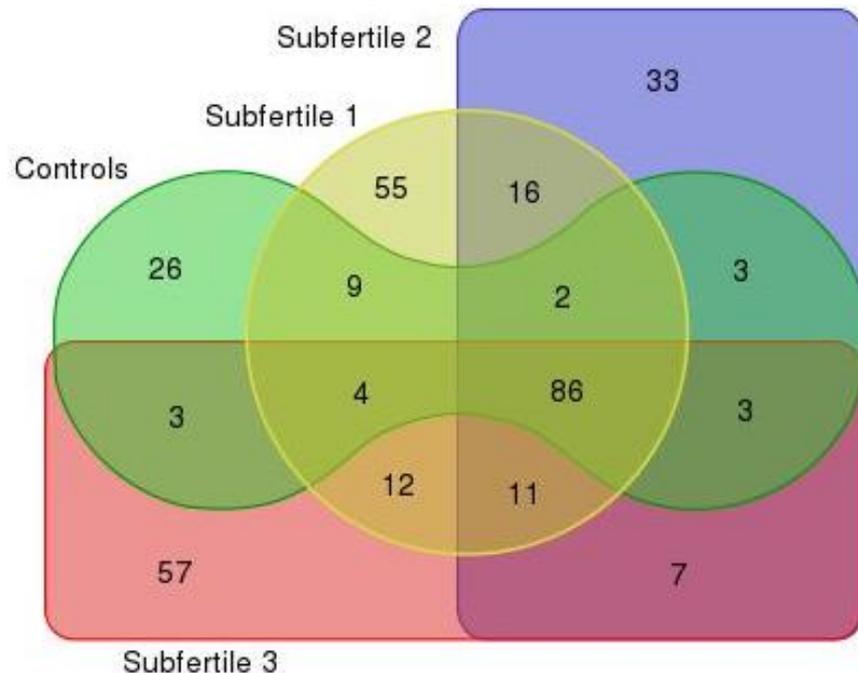


Differential expression of miRNAs and piRNAs in the serum

miRNAs

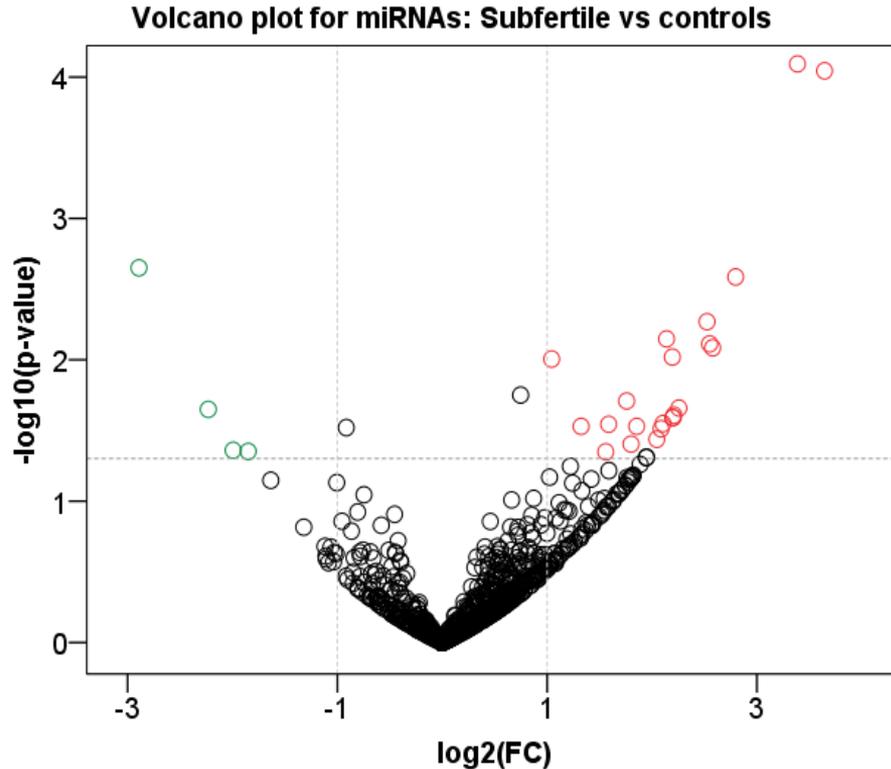


piRNAs



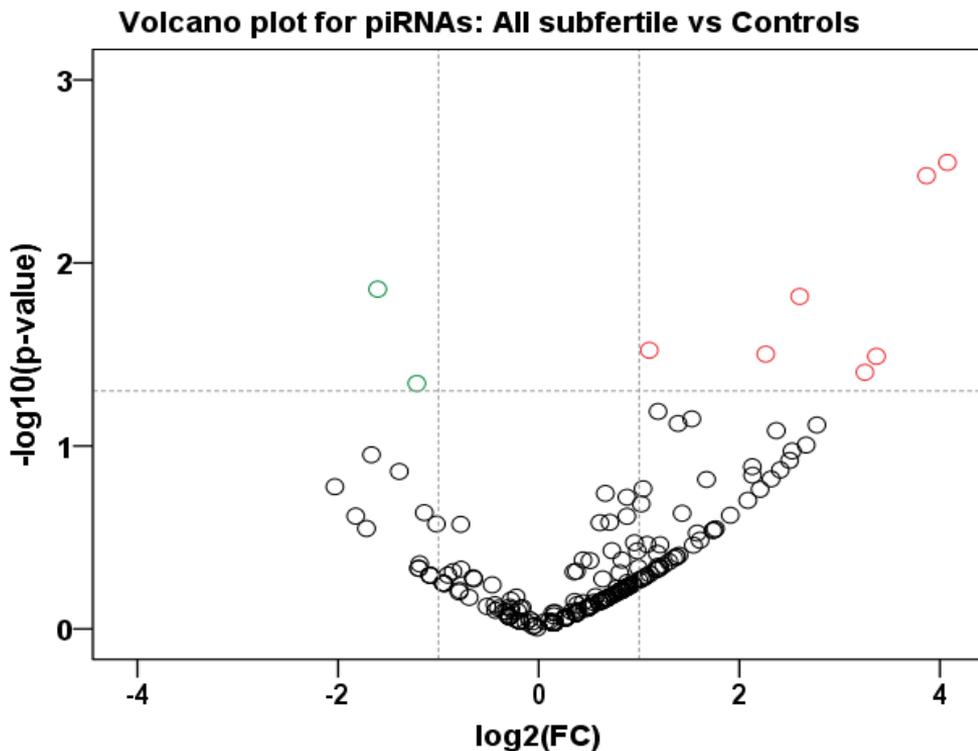


miRNA profile in the serum significantly differs between Subfertile and Control men

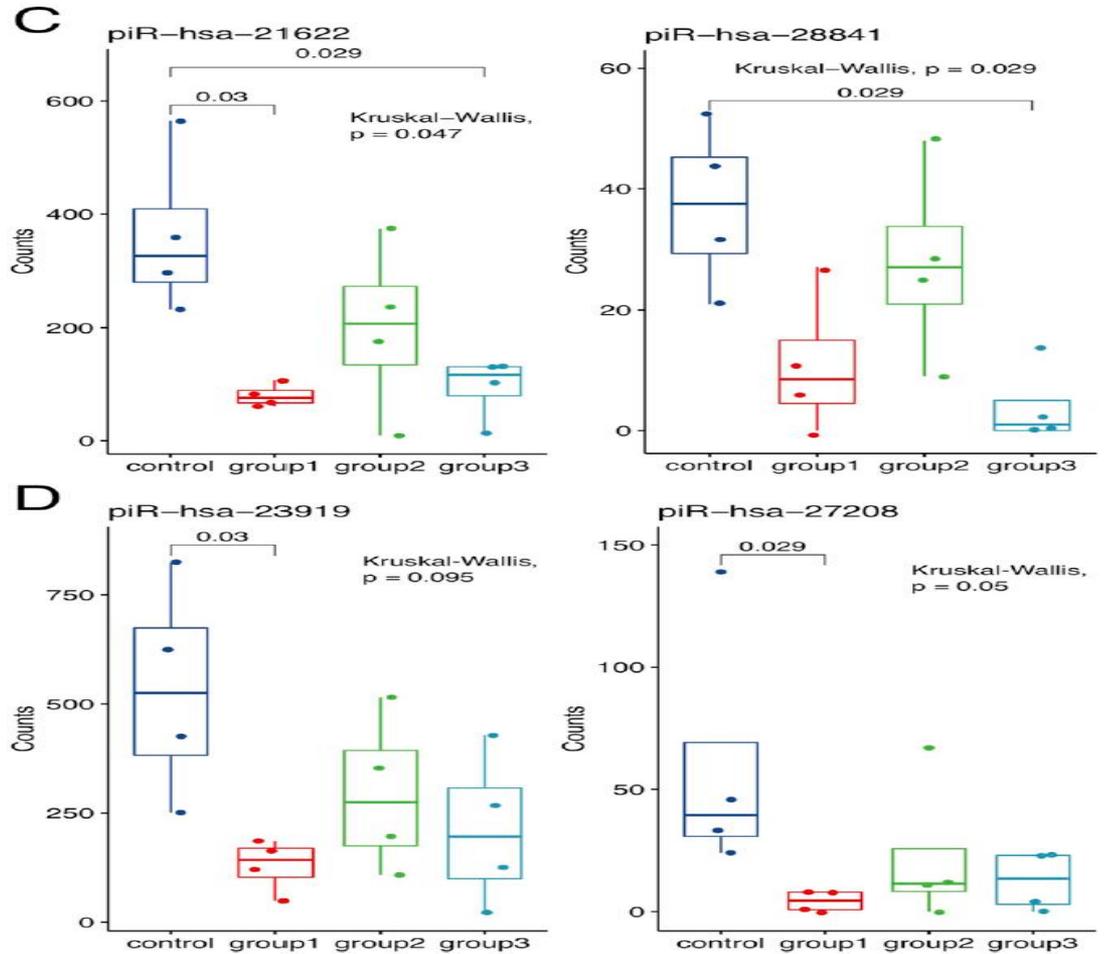




piRNA profile in the serum significantly differs between Subfertile and Control men



piRNAs differentially expressed in different groups of subfertile men





Conclusions

- ncRNA profiling technologies allow identification of functional biomarkers
- ncRNA profiling may prove more useful as a diagnostic tool from individual ncRNA or protein detection since it will provide a comprehensive view of the molecular events altered in cells or biological fluids- **low cost high throughput technologies required coupled with bioinformatics**
- miRNA profile is altered by hemodialysis contributing to the pathogenetic mechanisms associated with co-morbidities
- Serum miRNA and piRNA profile is altered in male subfertility providing novel biomarkers



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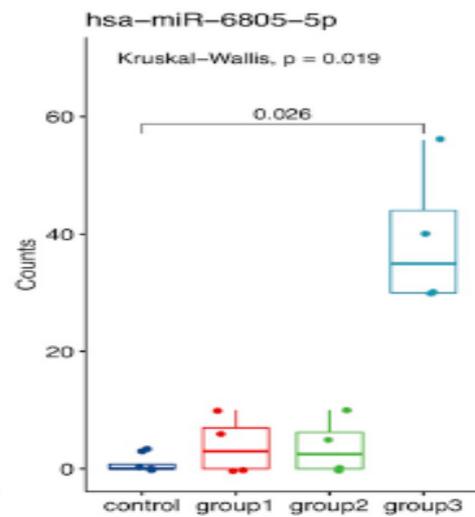
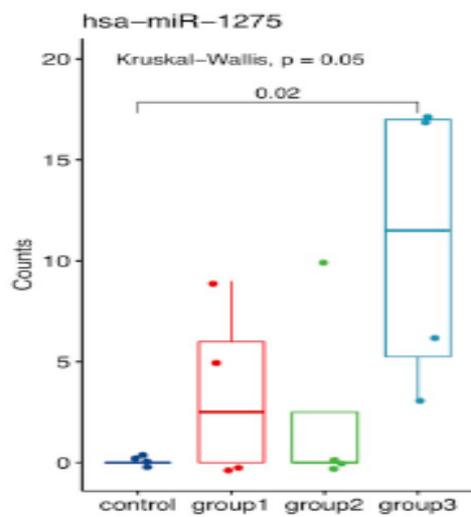
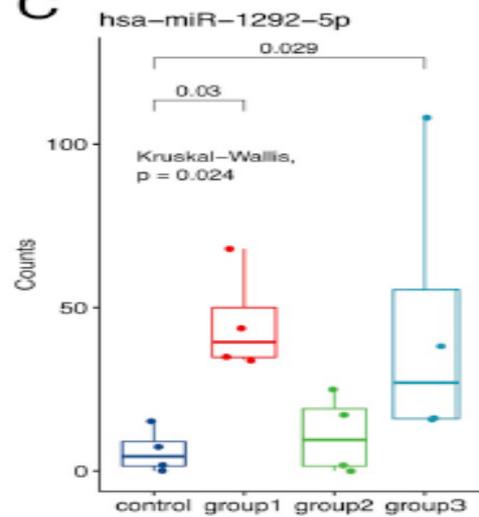
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C**D**