Competence Centre on Reproductive Medicine & Biology

iGenomix

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Micro-RNA signature of receptive human endometrium

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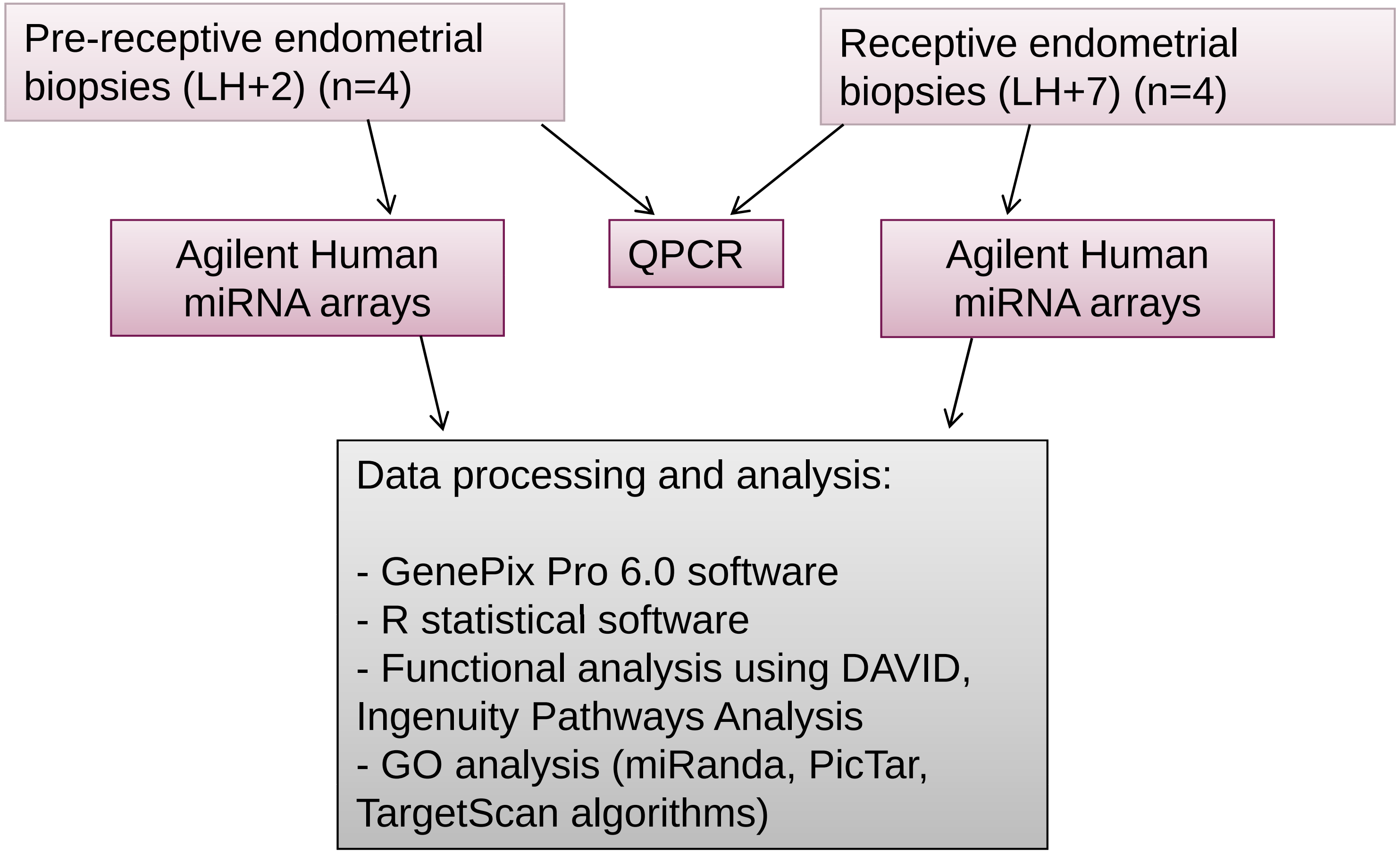
ESHRE workshop, 2. December 2010

MicroRNAs (miRNAs)

- miRNAs are small non-coding RNAs (~22 nucleotides)
- Post-transcriptional regulators that
  bind to complementary sequences on target mRNA =>
  transcriptional repression or mRNA degradation => gene silencing
- Human genome encodes >1000 miRNAs
- Target ~60% of genes
- Evolutionarily conservative
- miRNAs in human endometrium

Experimental design



Pre-receptive endometrial biopsies (LH+2) (n=4)

Receptive endometrial biopsies (LH+7) (n=4)

Agilent Human miRNA arrays

QPCR

Agilent Human miRNA arrays

Data processing and analysis:

- GenePix Pro 6.0 software
- R statistical software
- Functional analysis using DAVID, Ingenuity Pathways Analysis
- GO analysis (miRanda, PicTar, TargetScan algorithms)

Differentially expressed miRNAs (receptive *vs* pre-receptive endometrium)

- 84 differentially expressed miRNAs (41 ↑ , 43 ↓)
-

miRNA	Fold change (R/pre-R)	P-value
hsa-miR-30b	4.23	<0.001
hsa-miR-30d	3.29	0.015
hsa-miR-494	-2.15	<0.001
hsa-miR-923	-4.27	<0.001

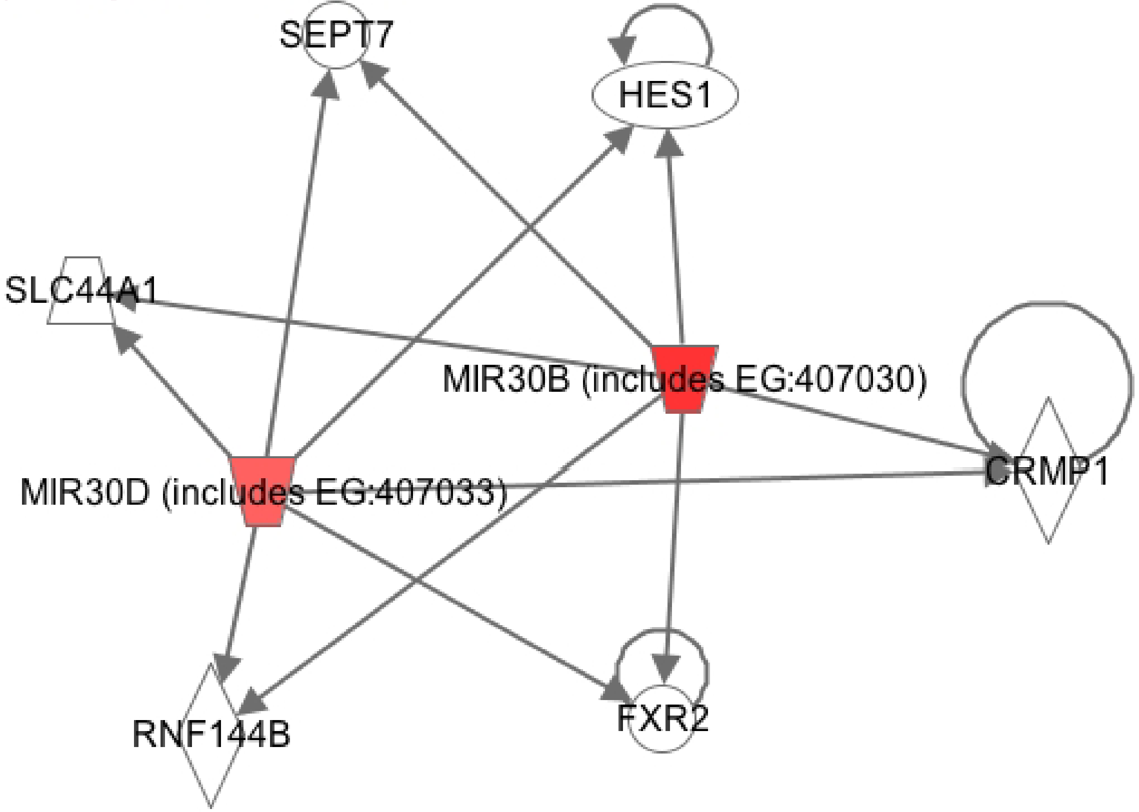
miRNAs in relation to function and disease

(IPA analysis)

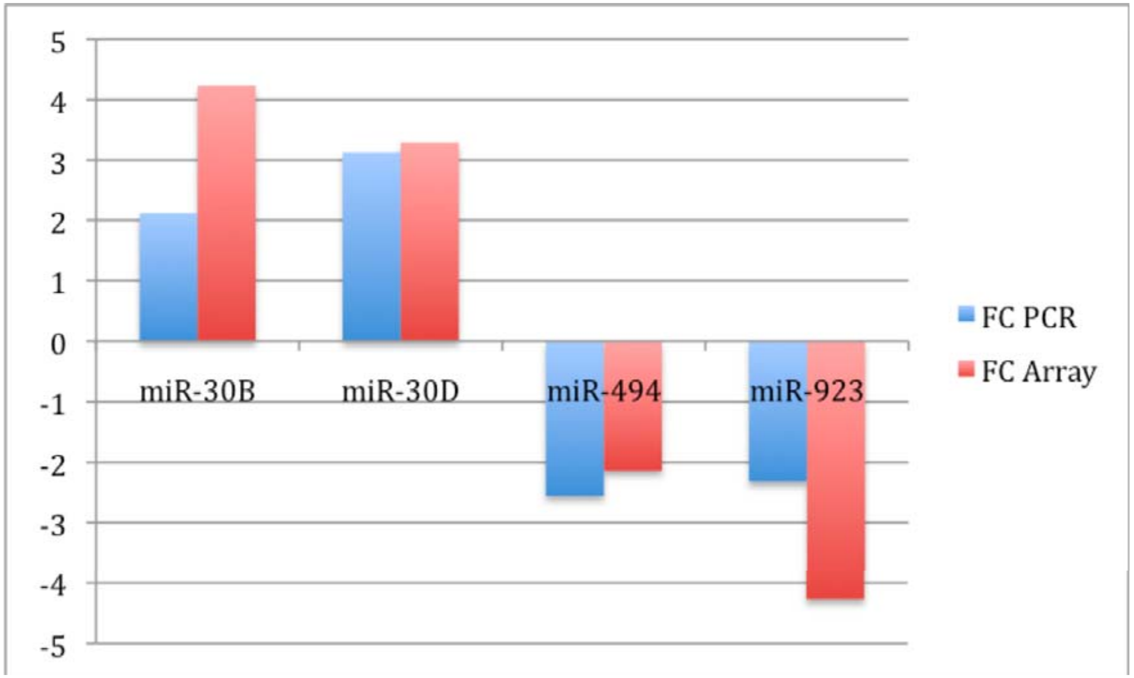
Category	Function	Function Annotation	p-value	Molecules
Cancer	carcinoma	carcinoma	9.35E-04	MIR30B, MIR30D, MIR494
Reproductive System Disease	breast carcinoma	breast carcinoma	1.62E-03	MIR30B, MIR30D
Neurological Disease	schizophrenia	schizophrenia	3.48E-03	MIR30B, MIR30D

Molecular relationship between the 4 miRNAs

(IPA analysis)



Array validation

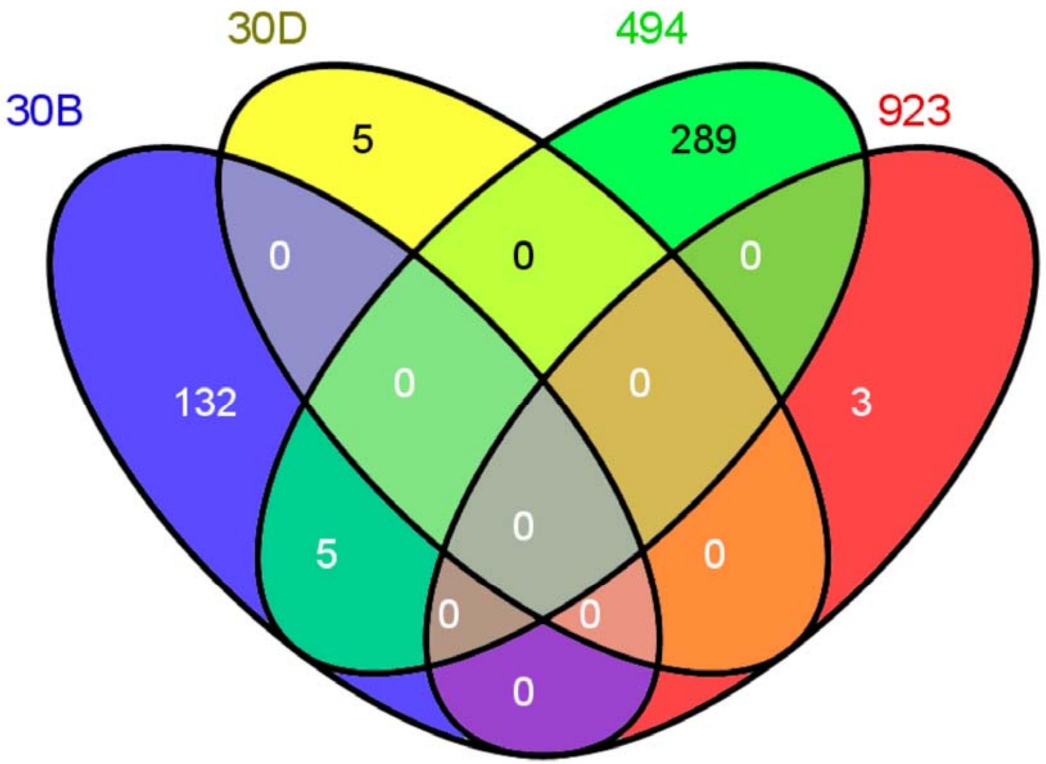


Prediction of gene targets of differentially expressed miRNAs:

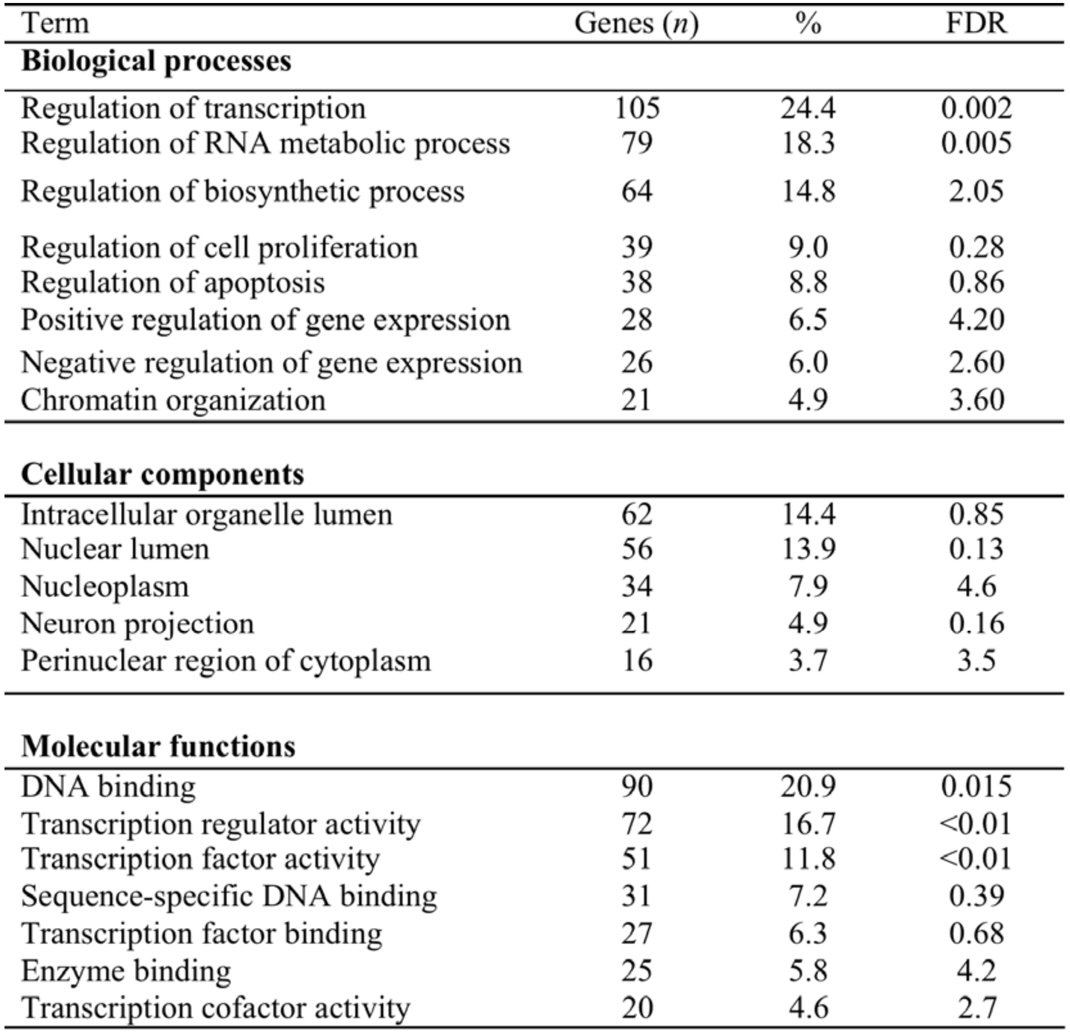
- miRanda, August 2010 release (www.microrna.org)
- PicTar (pictar.mdc-berlin.de/)
- TargetScan 5.1, April 2009 release (www.targetscan.org)

Predicted gene targets of miRNAs

miRanda, PicTar, TargetScan algorithms = 434 genes

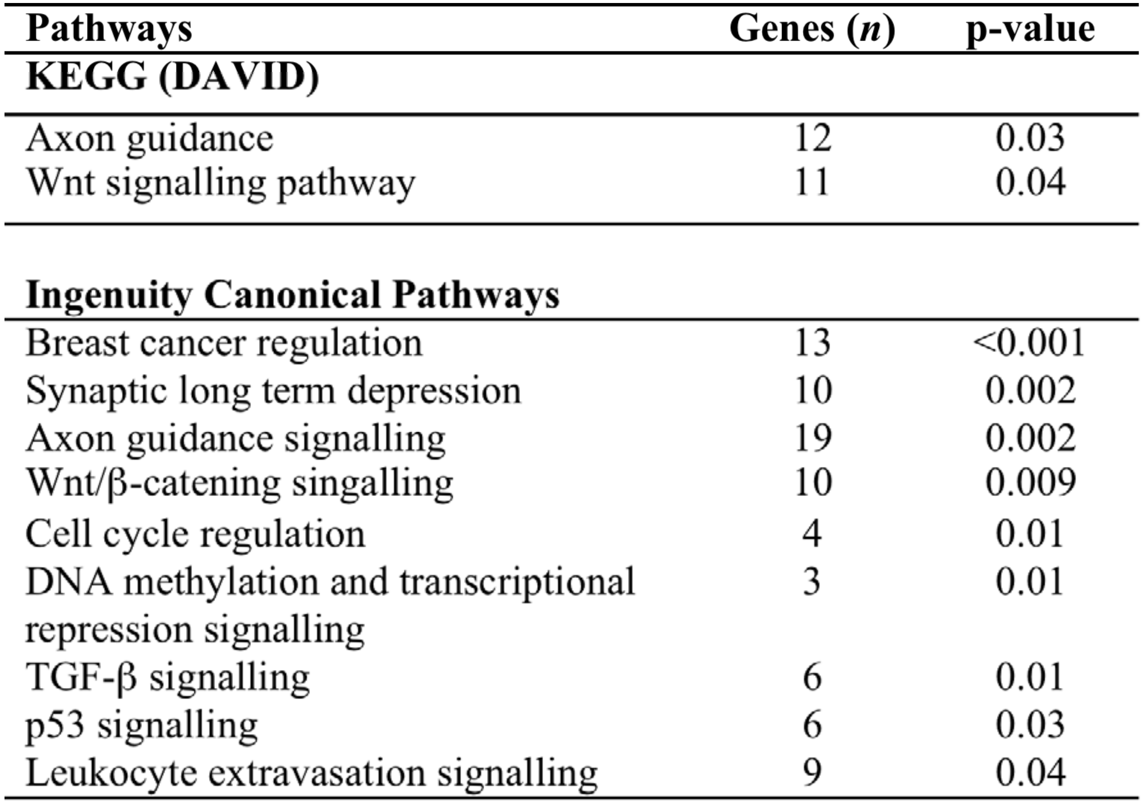


Functional annotation of predicted targets



Term	Genes (*n*)	%	FDR
Biological processes			
Regulation of transcription	105	24.4	0.002
Regulation of RNA metabolic process	79	18.3	0.005
Regulation of biosynthetic process	64	14.8	2.05
Regulation of cell proliferation	39	9.0	0.28
Regulation of apoptosis	38	8.8	0.86
Positive regulation of gene expression	28	6.5	4.20
Negative regulation of gene expression	26	6.0	2.60
Chromatin organization	21	4.9	3.60
Cellular components			
Intracellular organelle lumen	62	14.4	0.85
Nuclear lumen	56	13.9	0.13
Nucleoplasm	34	7.9	4.6
Neuron projection	21	4.9	0.16
Perinuclear region of cytoplasm	16	3.7	3.5
Molecular functions			
DNA binding	90	20.9	0.015
Transcription regulator activity	72	16.7	<0.01
Transcription factor activity	51	11.8	<0.01
Sequence-specific DNA binding	31	7.2	0.39
Transcription factor binding	27	6.3	0.68
Enzyme binding	25	5.8	4.2
Transcription cofactor activity	20	4.6	2.7

Pathways for predicted target genes



Pathways	Genes (*n*)	p-value
KEGG (DAVID)		
Axon guidance	12	0.03
Wnt signalling pathway	11	0.04
Ingenuity Canonical Pathways		
Breast cancer regulation	13	<0.001
Synaptic long term depression	10	0.002
Axon guidance signalling	19	0.002
Wnt/β-catening singalling	10	0.009
Cell cycle regulation	4	0.01
DNA methylation and transcriptional repression signalling	3	0.01
TGF-β signalling	6	0.01
p53 signalling	6	0.03
Leukocyte extravasation signalling	9	0.04

Conclusions

- New insights to the complex process of endometrial receptivity
- This knowledge could be used to understand and identify fertility complications caused by dys-regulation of endometrial receptivity

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Acknowledgements

José A. Horcajadas
Francisco J. Esteban
José A. Martínez-Conejero
María Ruiz-Alonso

Anneli Stavreus-Evers
Andres Salumets