Study of circulating genomic microRNA expression profiling in obese and type 2 diabetes subjects

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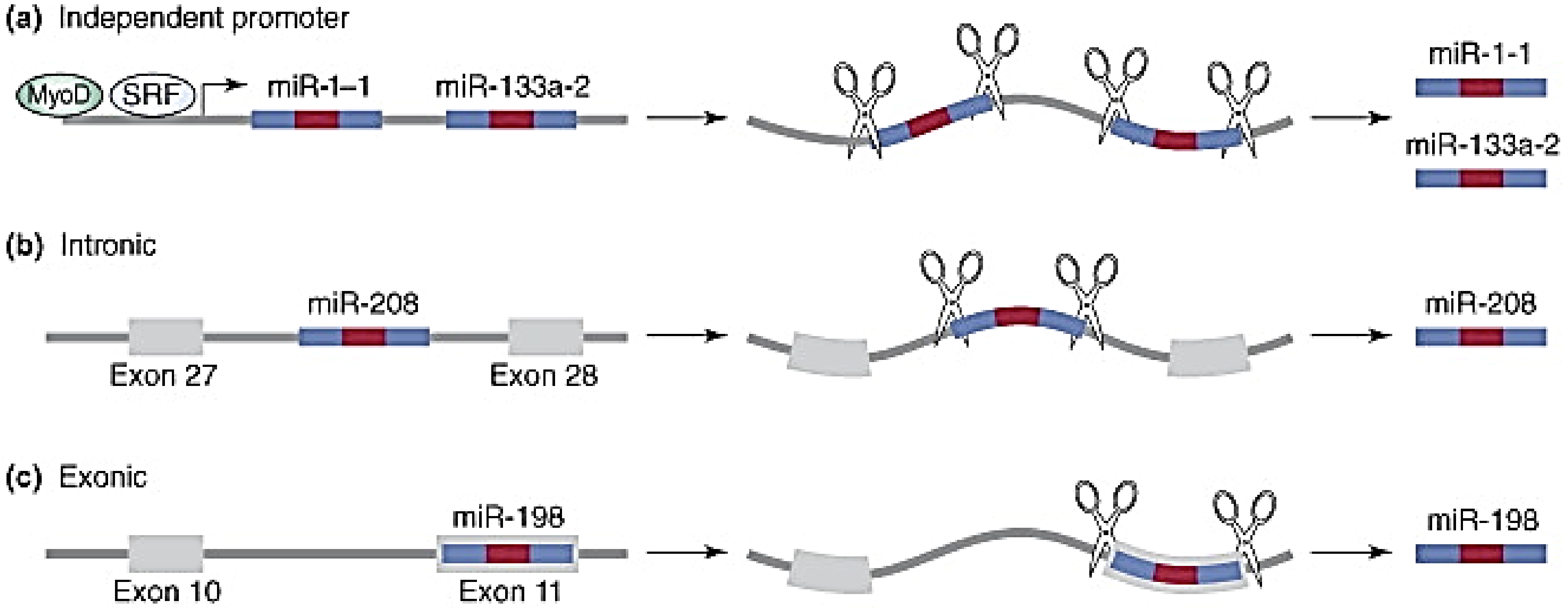
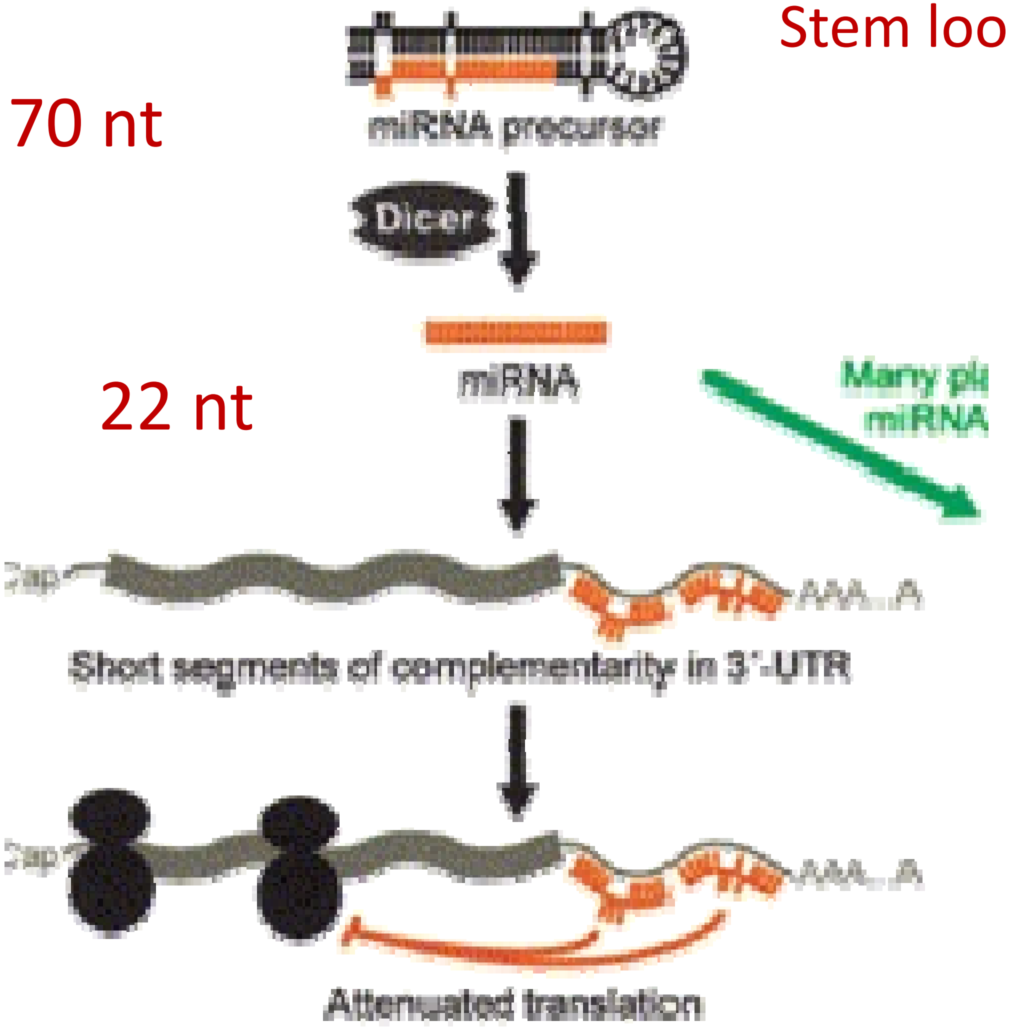
What is microRNA (miRNA)?

- A family of small RNAs, containing non-coding 21-22 nt
- locate in intronic, exonic, or promoter part of genomic regions



Zhao Y, Srivastava D, TIBS 32:189,2007

Roles of microRNA (miRNA)



Stem loops

70 nt

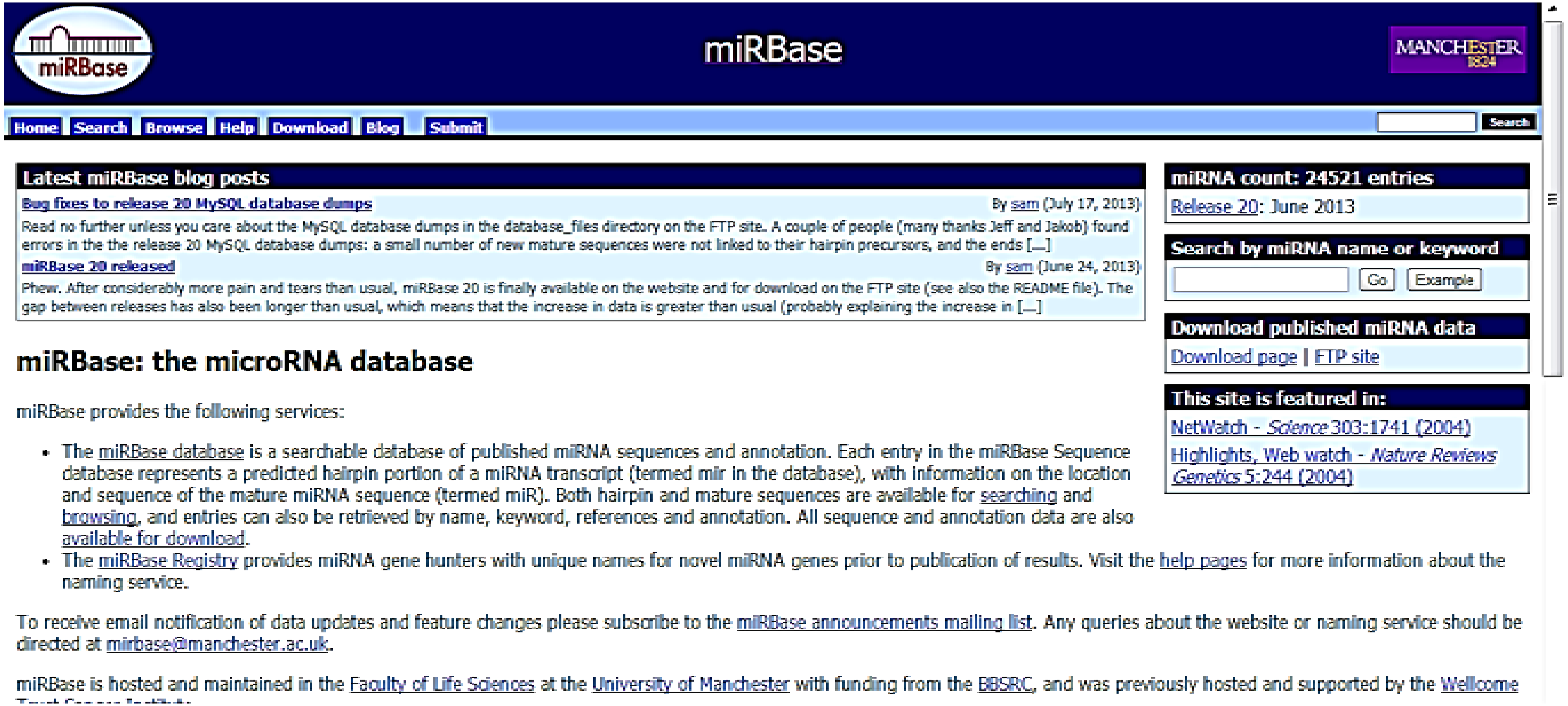
22 nt

- Regulate gene expression by inhibit mRNA translation

Annu. Rev. Cell Dev. Biol. 2007.23:175-205

miRBase

http://www.mirbase.org/



Latest miRBase blog posts

Bug fixes to release 20 MySQL database dumps By sam (July 17, 2013)

Read no further unless you care about the MySQL database dumps in the database_files directory on the FTP site. A couple of people (many thanks Jeff and Jakob) found errors in the the release 20 MySQL database dumps: a small number of new mature sequences were not linked to their hairpin precursors, and the ends […]

miRBase 20 released By sam (June 24, 2013)

Phew. After considerably more pain and tears than usual, miRBase 20 is finally available on the website and for download on the FTP site (see also the README file). The gap between releases has also been longer than usual, which means that the increase in data is greater than usual (probably explaining the increase in […]

miRNA count: 24521 entries

Release 20: June 2013

Search by miRNA name or keyword

Download published miRNA data

Download page | FTP site

This site is featured in:

NetWatch - *Science* 303:1741 (2004)

Highlights, Web watch - *Nature Reviews Genetics* 5:244 (2004)

miRBase: the microRNA database

miRBase provides the following services:

- The miRBase database is a searchable database of published miRNA sequences and annotation. Each entry in the miRBase Sequence database represents a predicted hairpin portion of a miRNA transcript (termed mir in the database), with information on the location and sequence of the mature miRNA sequence (termed miR). Both hairpin and mature sequences are available for searching and browsing, and entries can also be retrieved by name, keyword, references and annotation. All sequence and annotation data are also available for download.
- The miRBase Registry provides miRNA gene hunters with unique names for novel miRNA genes prior to publication of results. Visit the help pages for more information about the naming service.

To receive email notification of data updates and feature changes please subscribe to the miRBase announcements mailing list. Any queries about the website or naming service should be directed at mirbase@manchester.ac.uk.

miRBase is hosted and maintained in the Faculty of Life Sciences at the University of Manchester with funding from the BBSRC, and was previously hosted and supported by the Wellcome

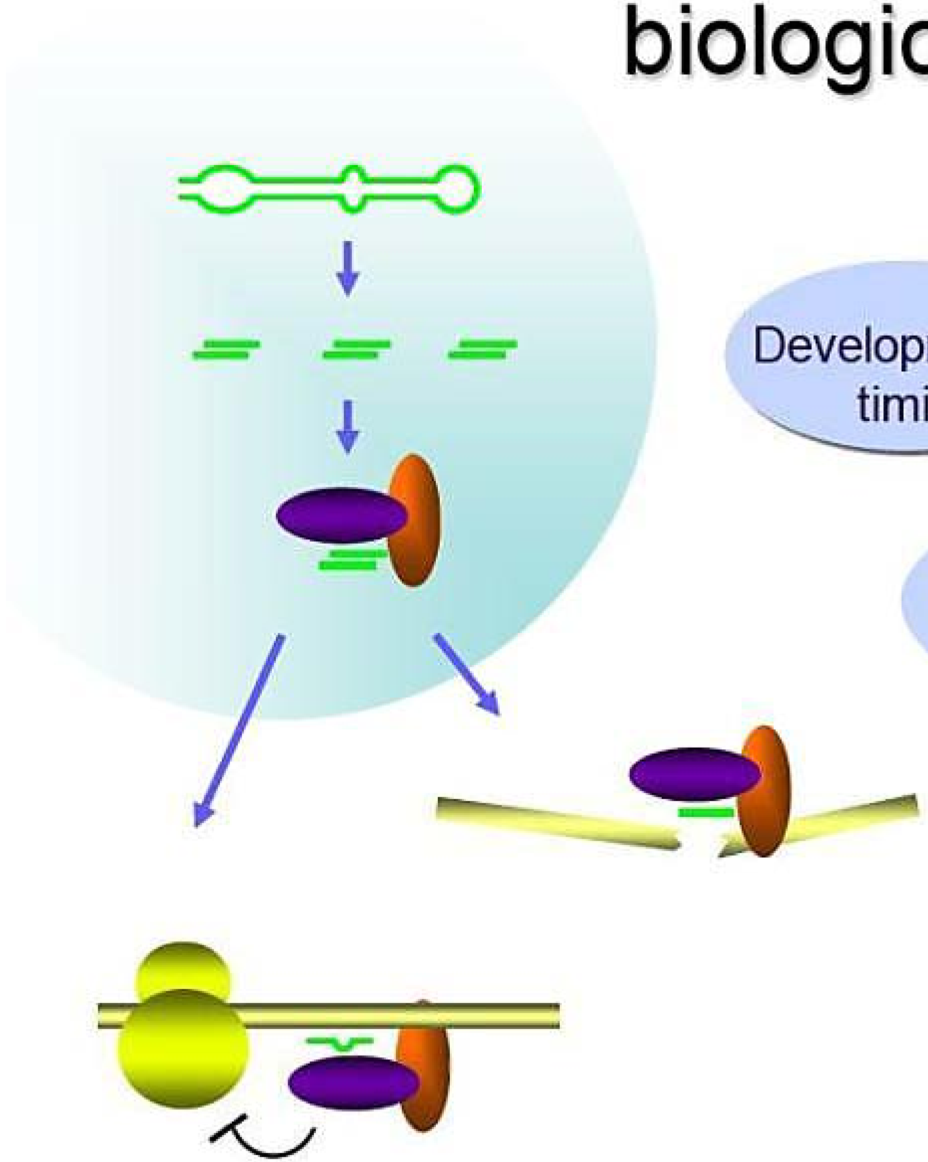
Human miRNAs (June, 2013)

Total miRNA genes in 115 species	10,882
Total number of miRNAs known	1,872
Number of human mRNA targets	34,788

- miRNAs can have multiple targets
- Target mRNAs can have multiple miRNA binding sites

miRBase @ http://www.mirbase.org/
MicroCosm @ http://www.ebi.ac.uk/enright-srv/microcosm/

Thousands of microRNAs act in multiple biological events



Developmental timing

Differentiation

Aging

Apoptosis

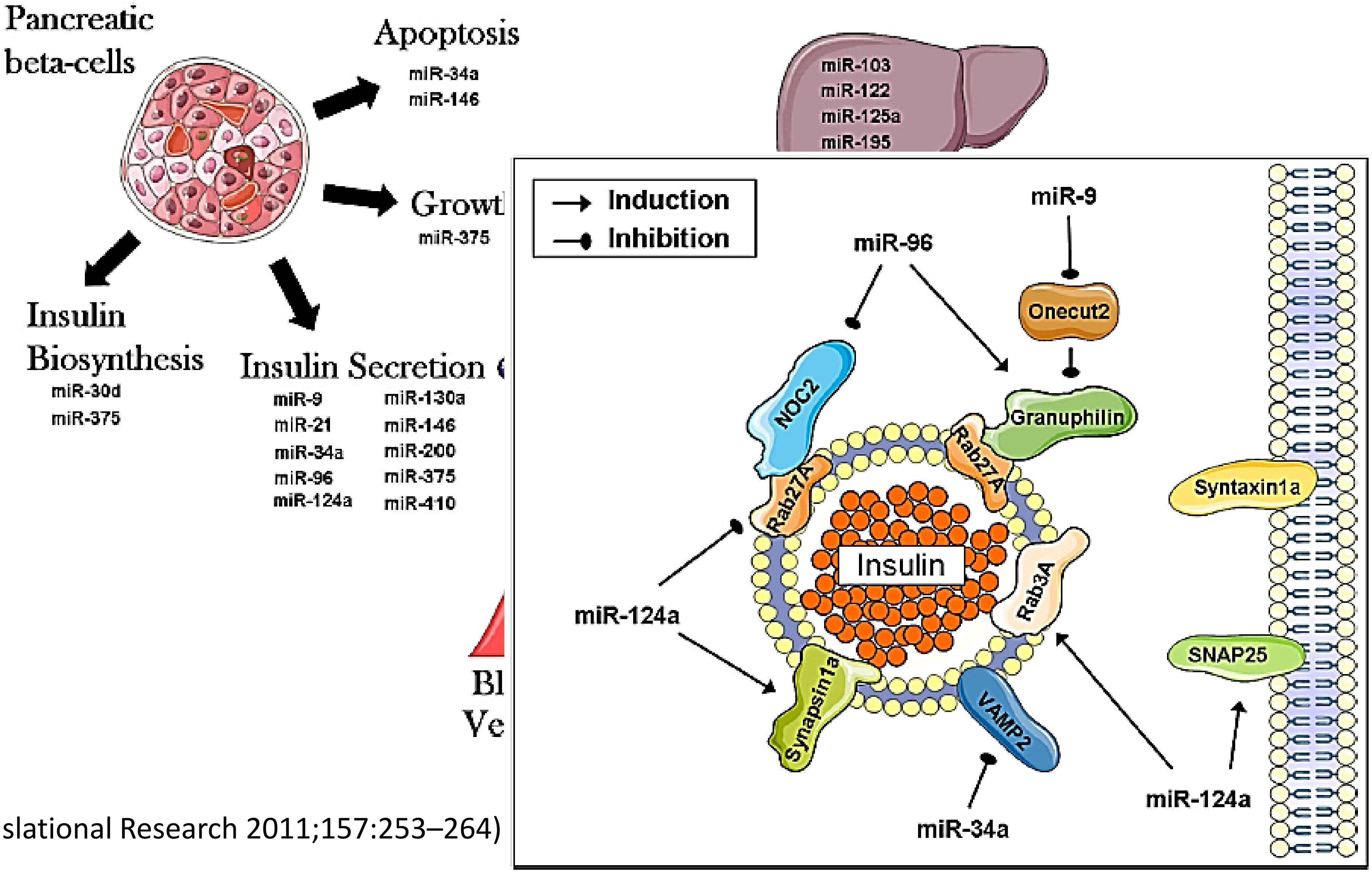
Metabolism

Cancer

…

Cell fate/differentiation, Cell cycle…

Reported miRNA that associated with T2D



(Translational Research 2011;157:253–264)

miRNAs As Potential Biomarkers For Diabetes

- Recent studies reported the detection of miRNAs in blood and other body fluids.
- Levels of miRNAs in serum samples were stable, reproducible, and consistent among individuals.
- The circulating miRNAs originate from WBCs and cells located outside of blood vessels.

Objectives

- **To identify circulating microRNA as biomarkers associated with type 2 DM**
- **To assess microRNA biomarkers for prediction of type 2 diabetes in EGAT prospectively**

Materials and Methods

Study work flow

Verification method of extracting adequate miRNA from serum for whole genome study

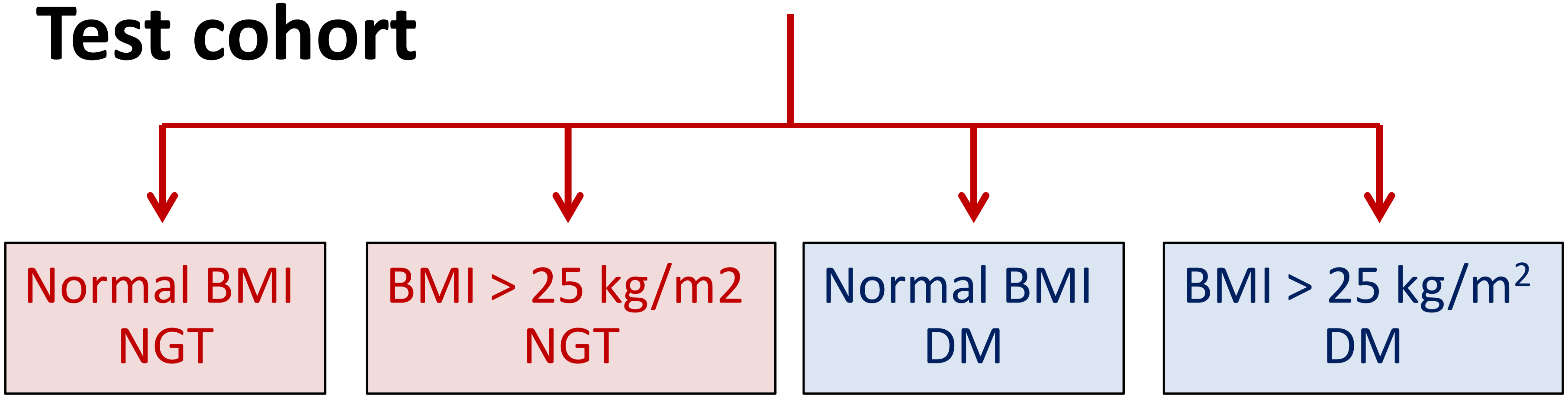
Collect serum and whole blood from test subjects

Whole genome miRNA expression study

Select miRNA that associated with T2D from test cohort to validate in prospective EGAT cohort

Subjects

Test cohort



Normal BMI
NGT

BMI > 25 kg/m2
NGT

Normal BMI
DM

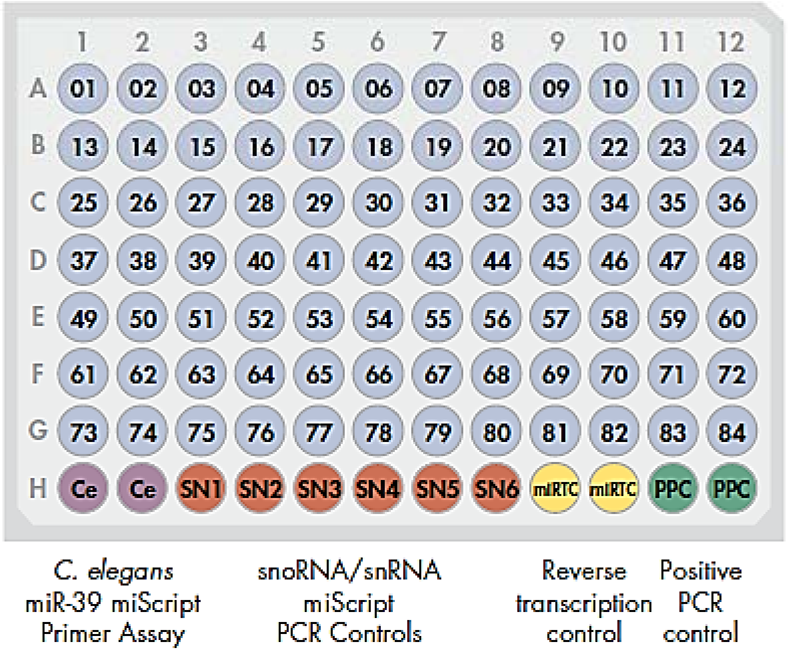
BMI > 25 kg/m^2
DM

5	5	5	5

Validation in EGAT prospective cohort

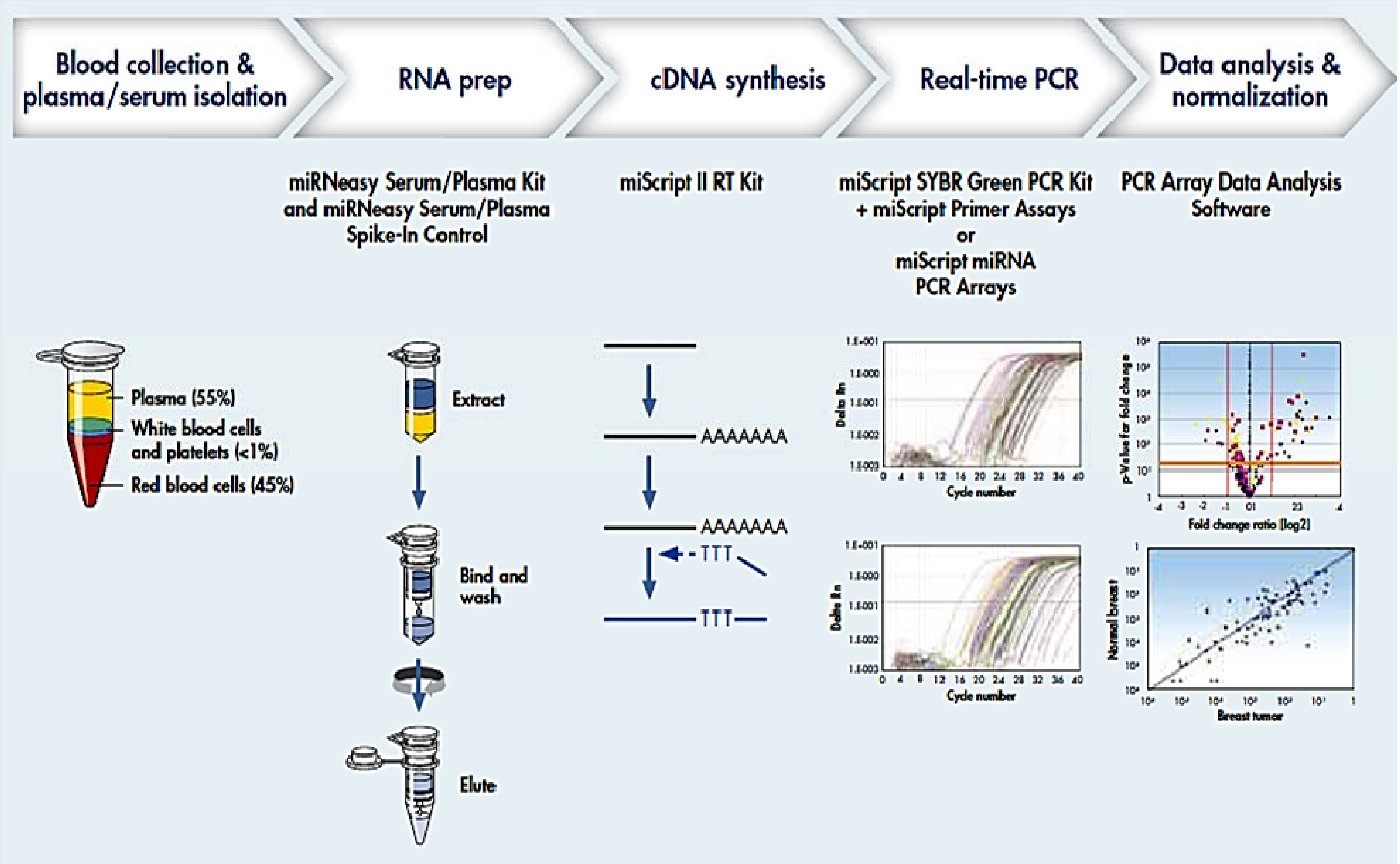
Whole genome miRNA expression study

- The miScript miRNA PCR Array Human miRNome® (384 well-plate)
  - miRNA quantification and profiling using SYBR Green real-time PCR technique.
  - Contain PCR primer pairs for 1152 miRNAs



	1	2	3	4	5	6	7	8	9	10	11	12
A	01	02	03	04	05	06	07	08	09	10	11	12
B	13	14	15	16	17	18	19	20	21	22	23	24
C	25	26	27	28	29	30	31	32	33	34	35	36
D	37	38	39	40	41	42	43	44	45	46	47	48
E	49	50	51	52	53	54	55	56	57	58	59	60
F	61	62	63	64	65	66	67	68	69	70	71	72
G	73	74	75	76	77	78	79	80	81	82	83	84
H	Ce	Ce	SN1	SN2	SN3	SN4	SN5	SN6	miRTC	miRTC	PPC	PPC

Methods



Blood collection & plasma/serum isolation

RNA prep

cDNA synthesis

Real-time PCR

Data analysis & normalization

miRNeasy Serum/Plasma Kit and miRNeasy Serum/Plasma Spike-In Control

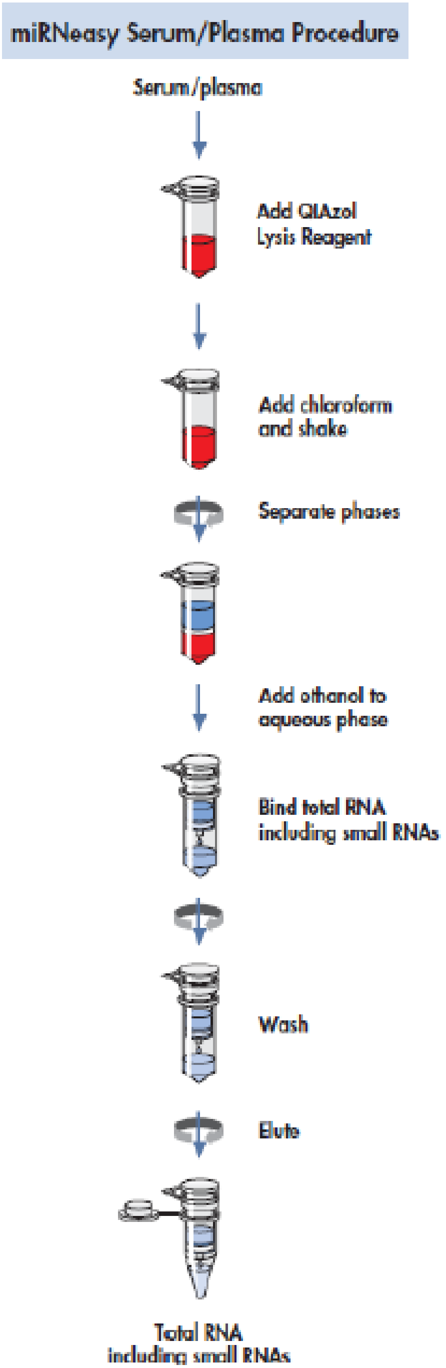
miScript II RT Kit

miScript SYBR Green PCR Kit + miScript Primer Assays
or
miScript miRNA PCR Arrays

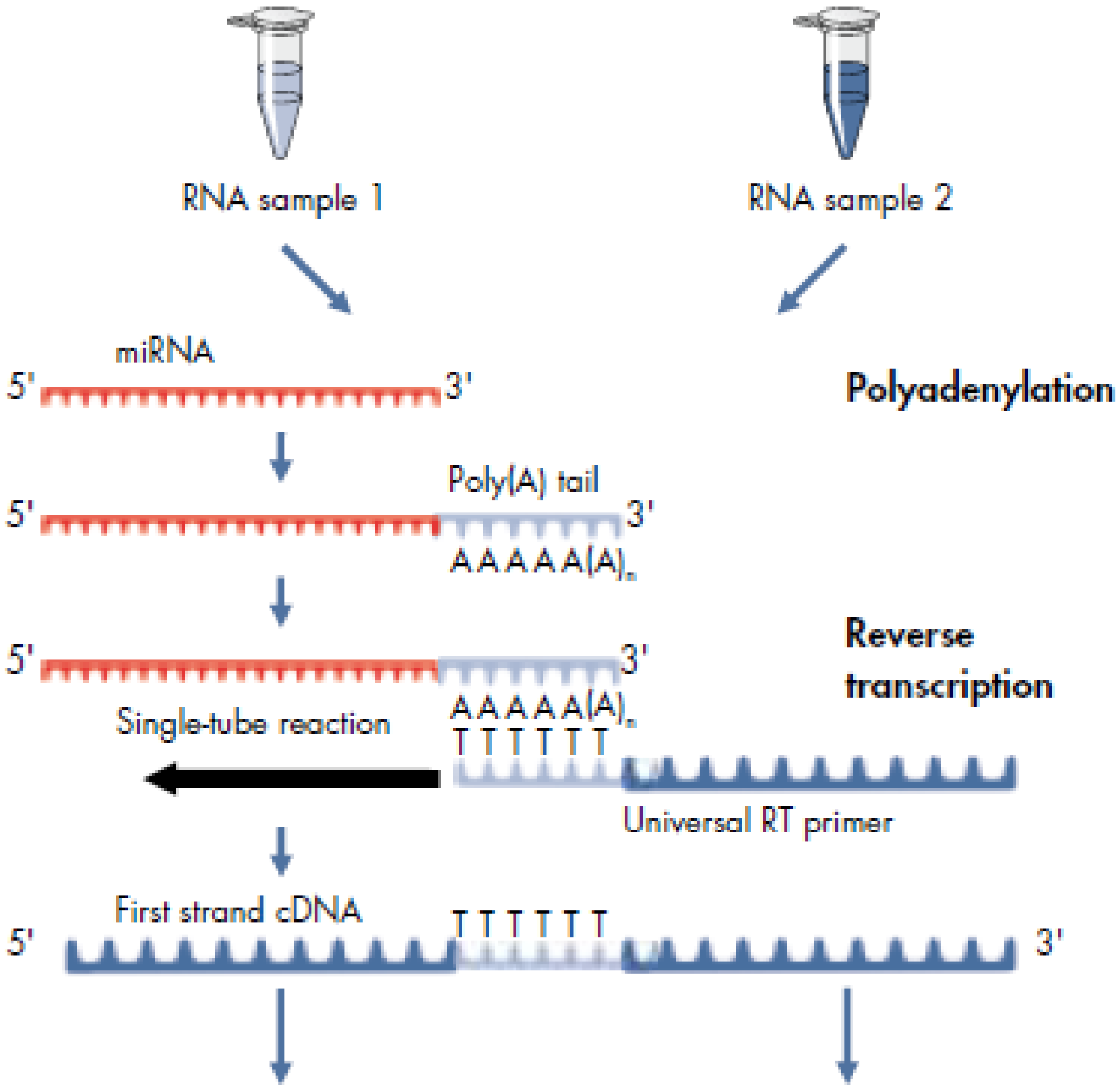
PCR Array Data Analysis Software

RNA extraction

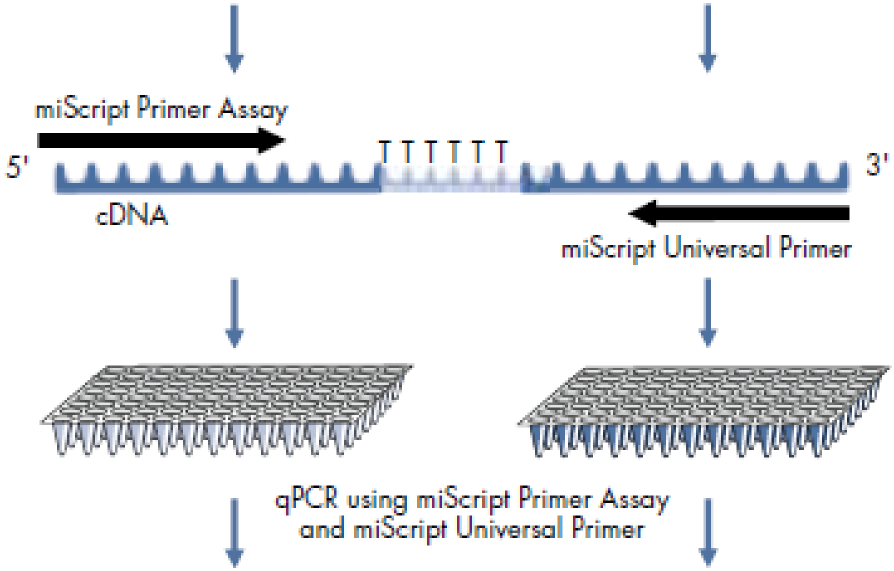
miRNeasy® for serum/plasma Kits



1. Convert miRNA to cDNA in a one-step, single-tube reverse transcription reaction.

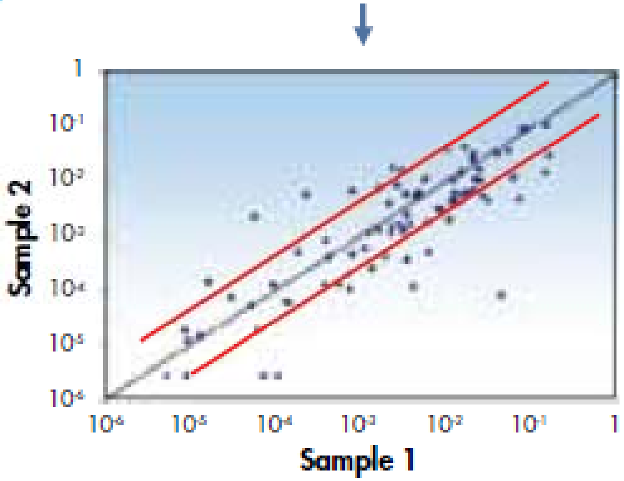


2. Combine cDNA with QuantiTect SYBR Green PCR Mastermix, miScript Universal Primer, and water.
Aliquot mixture across miScript miRNA PCR Array.



3. Run in real-time PCR cycler.

4. Analyze data.



Study Analyses

- Check reverse transcription control (miRTC) and PCR control (PPC) Ct values for reverse transcription and PCR efficiency
- Determine a prep free of cellular contamination by observing housekeeping assays Ct values (>35)
- Data normalization using cel-miRNA-39 expression
- Calculating ∆Ct ,fold change, scattergram using web-based data analysis and comparing between test group and control
- Fold change > 2.5 were selected for further validation test

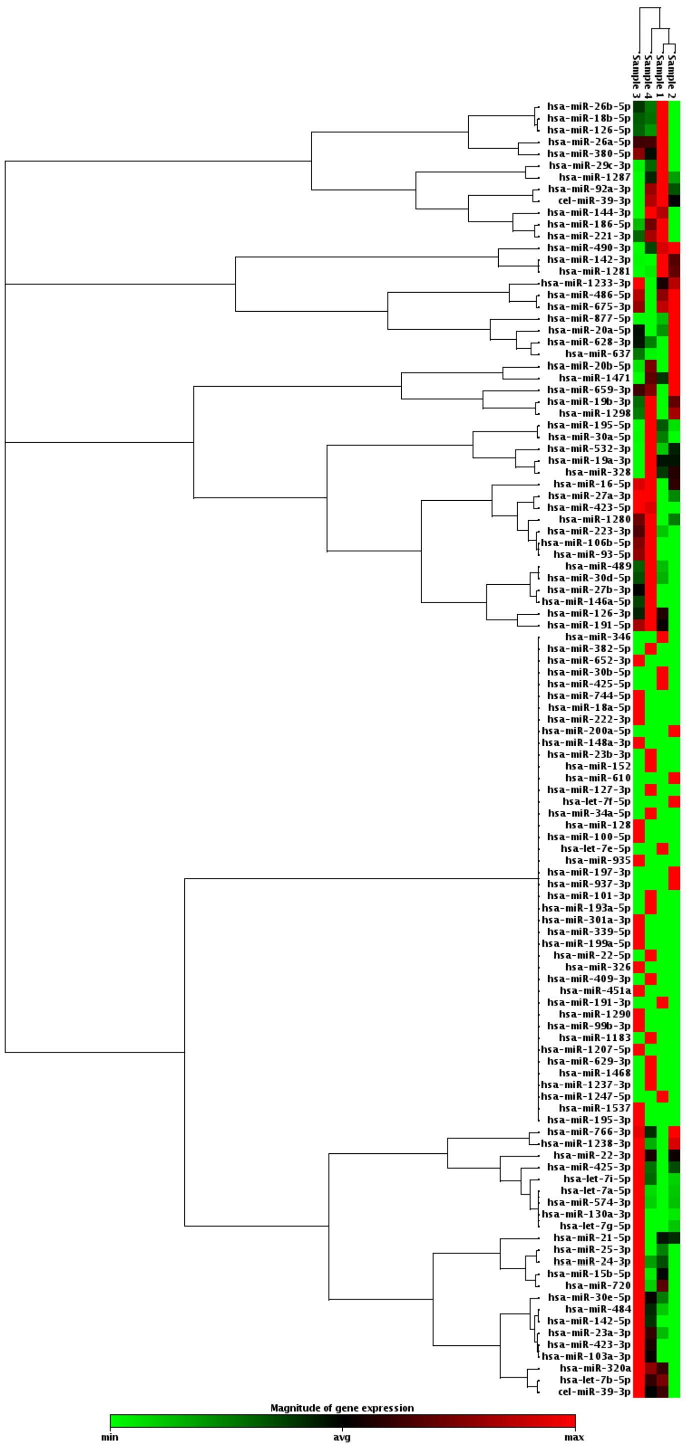
Results

Baseline Characteristics

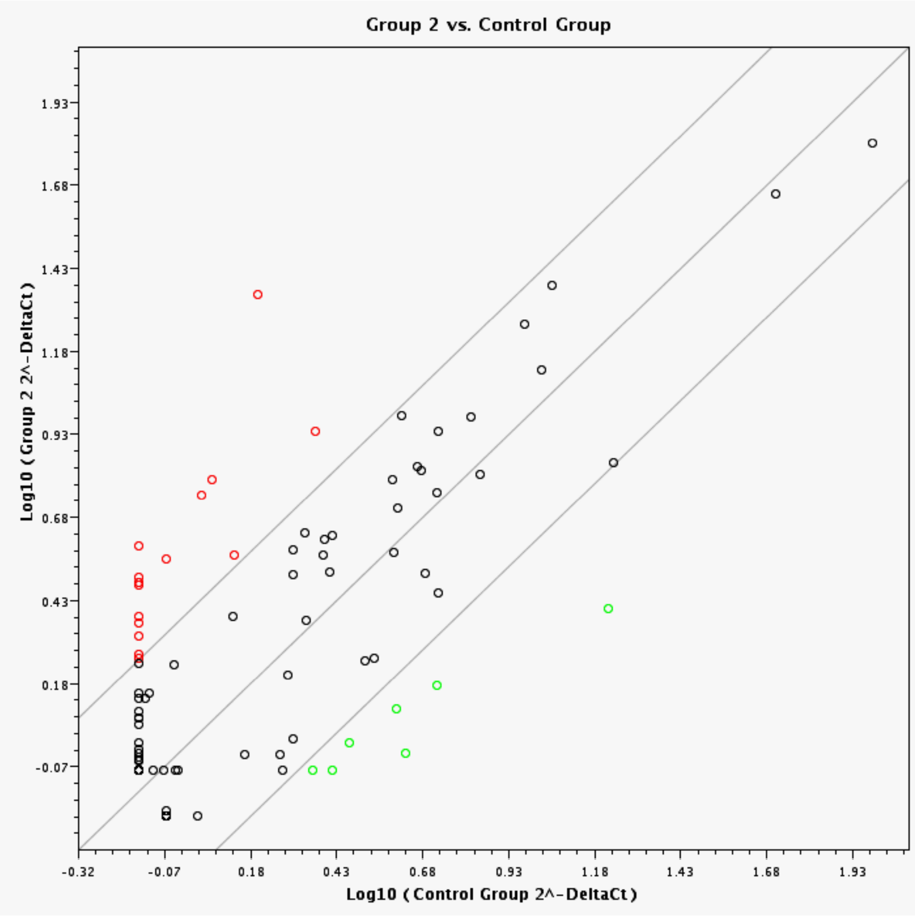
	Normal-Lean (n=5)	Normal-Obese (n=5)	DM-Lean (n=4)	DM-Obese (n=5)
Age (yr)	56.40 ± 12.40 (41-71)	62.80 ± 7.33 (52-70)	61.75 ± 7.50 (54-70)	61.60 ± 9.13 (53-75)
Fasting plasma glucose (mg/dl)	89.80 ± 9.78 (73-97)	100.2 ± 7.56 (89-109)	106.50 ± 8.39 (98-118)	106.40 ± 5.03 (102-114)
2-hr plasma glucose (mg/dl)	119.00 ± 28.04 (70-137)	108.2 ± 20.87 (80-135)	**219.0 ± 19.15** **(204-244)**	**249.60 ± 24.83** **(223-279)**
BW (kg)	51.81 ± 4.33 (45.8-57.4)	**63.85 ± 6.34** **(54.9-72.2)**	53.85 ± 4.76 (46.9-57.6)	**65.33 ± 7.31** **(57.2-74.2)**
HT (cm)	153.24 ± 5.40 (147.0-161.0)	151.16 ± 7.47 (139.8-160.0)	151.7 ± 7.44 (141.4-158.6)	148.58 ± 4.02 (143.3-153.6)
BMI (kg/m^2)	22.09 ± 1.92 (19.91-24.52)	**27.89 ± 0.87** **(26.94-29.11)**	23.37 ± 0.44 (22.93-23.93)	**29.59 ± 3.17** **(27.21-34.79**)

- Of the 1152 miRNAs profiled by The miScript miRNA PCR Array Human miRNome®,

  91 miRNAs were detected in all serum pools with Ct values <35



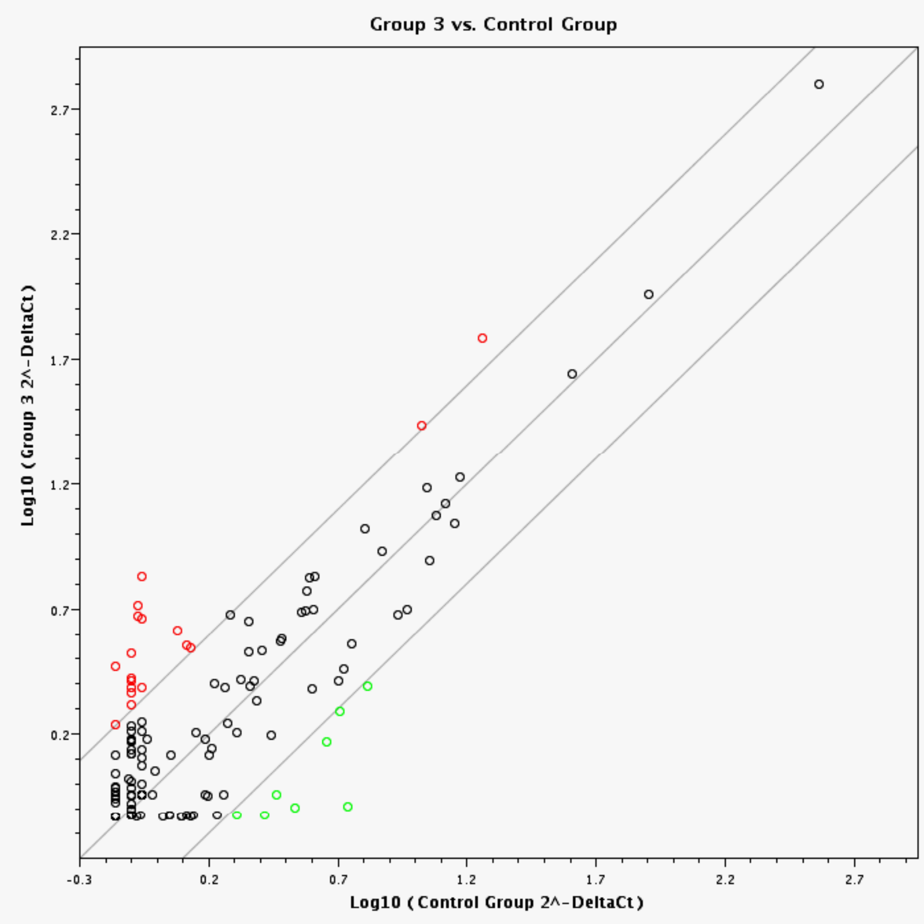
Lean NGT VS lean DM



21 miRNA

Mature ID	Fold Regulation
miR-130a-3p	4.2428
let-7i-5p	5.3332
miR-93-5p	3.2154
let-7a-5p	5.1515
let-7g-5p	5.4831
miR-744-5p	3.3519
miR-222-3p	4.4229
miR-22-3p	14.2709
miR-19b-3p	2.5053
miR-103a-3p	2.9383
miR-766-3p	3.7451
miR-142-5p	4.1554
miR-27b-3p	2.5758
miR-30e-5p	2.7226
miR-425-3p	4.1771
miR-29c-3p	-2.918
miR-144-3p	-2.7416
miR-142-3p	-4.5789
miR-221-3p	-3.1547
miR-1260a	-3.5247
miR-3173-3p	-6.4699

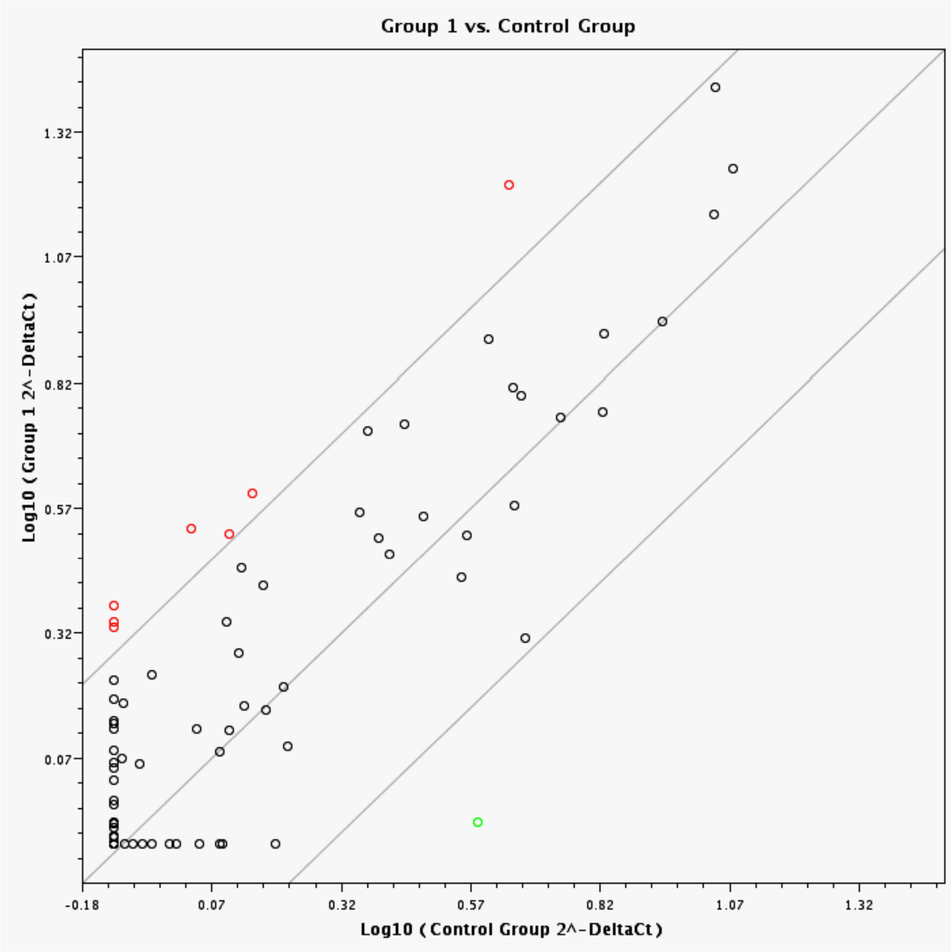
obese NGT VS obese DM



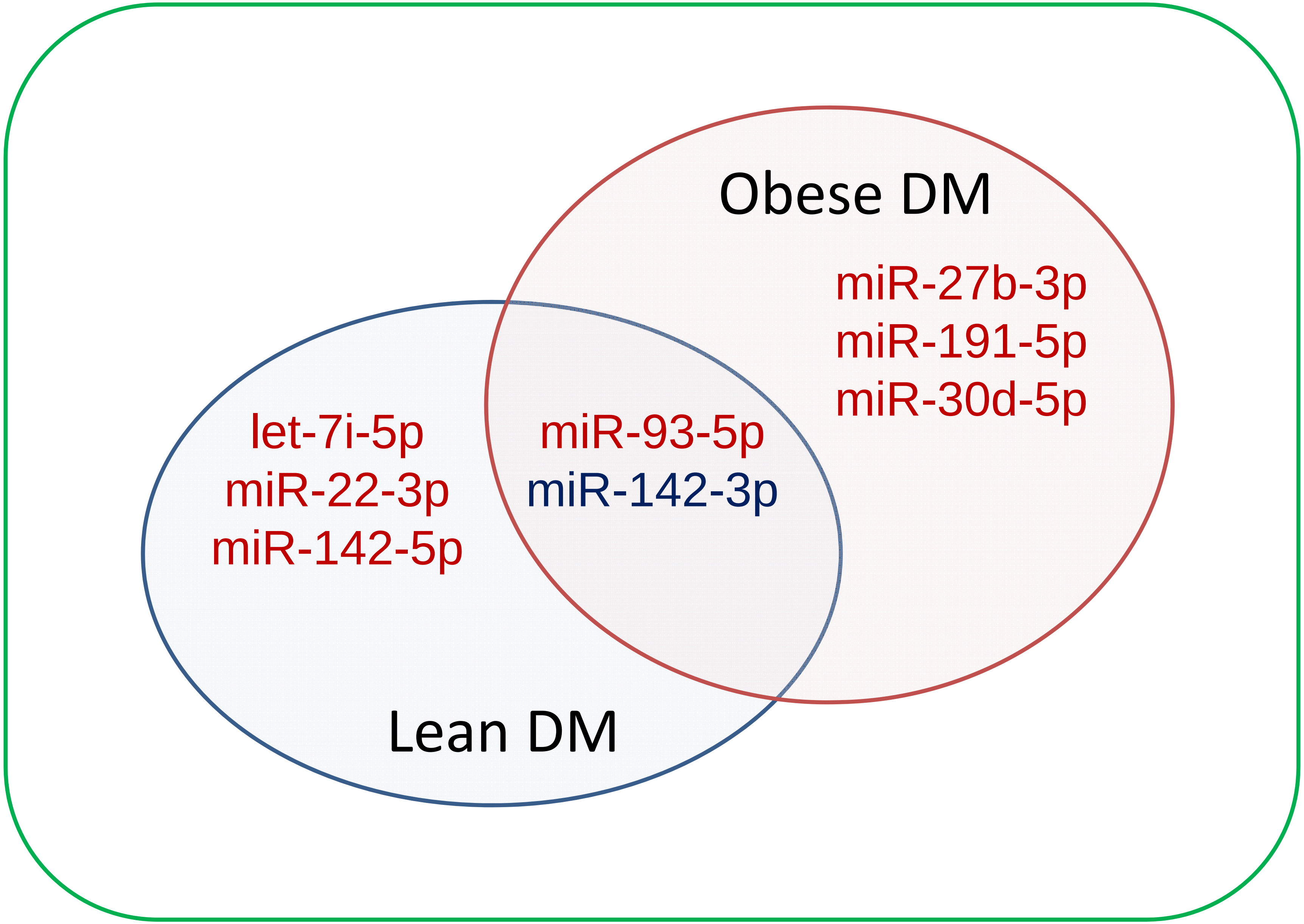
25 miRNA

Mature ID	Fold Regulation
miR-223-3p	2.5892
miR-382-5p	2.9332
miR-93-5p	3.0578
miR-144-3p	3.3461
miR-27b-3p	3.2546
let-7b-5p	3.4402
miR-191-5p	4.2061
miR-30d-5p	5.5887
miR-30a-5p	6.1582
miR-30e-5p	2.6253
miR-221-3p	4.3357
miR-423-5p	2.5249
miR-1280	2.614
miR-625-3p	2.763
miR-1260b	5.2461
miR-4301	3.3432
miR-181c-3p	2.7919
miR-676-5p	7.788
miR-486-5p	-6.7155
miR-142-3p	-4.2208
miR-610	-2.7274
miR-877-5p	-3.5004
miR-20a-5p	-2.6162
miR-4274	-2.6459
miR-17-5p	-3.1794

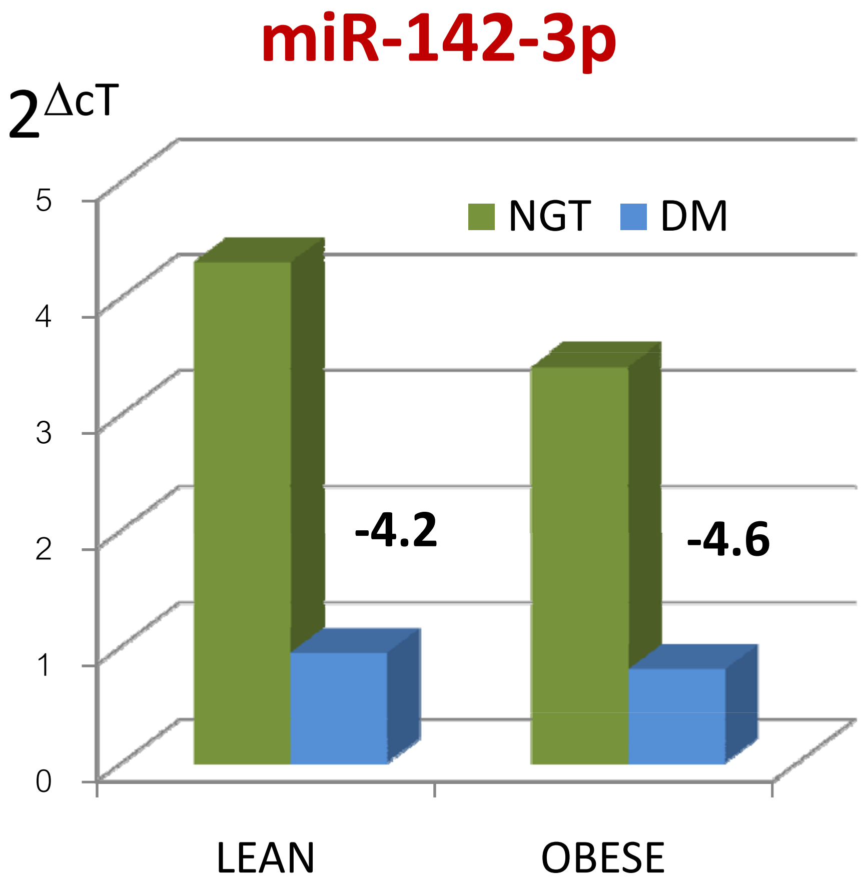
Differential expression of miRNA between NGT (lean+obese) VS DM (lean+obese)

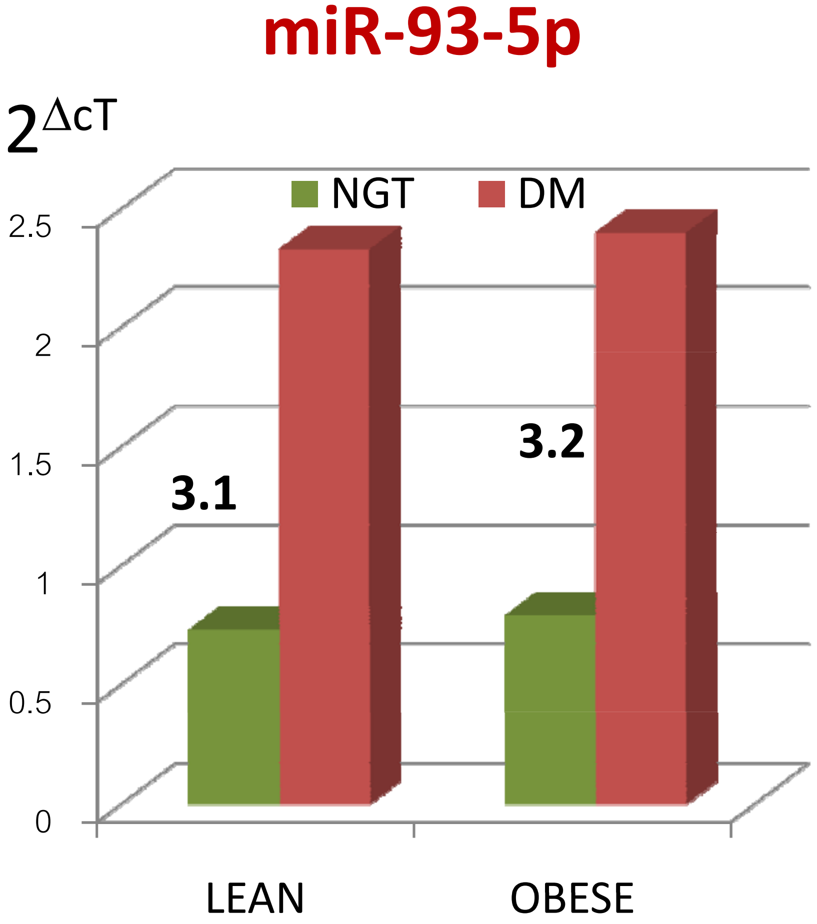


Mature ID	Fold Regulation
let-7i-5p	2.8358
miR-93-5p	3.1356
miR-22-3p	3.7418
miR-142-5p	2.826
miR-27b-3p	2.8954
miR-191-5p	2.6004
miR-30d-5p	3.1684
miR-142-3p	-4.3962


Obese DM
miR-27b-3p
miR-191-5p
miR-30d-5p
let-7i-5p
miR-22-3p
miR-142-5p
miR-93-5p
miR-142-3p
Lean DM

miRNAs that differentially expressed in both lean and obese T2D





Conclusions

- This is the first study of whole genome miRNA expression profiling in T2D patients.
- There are 7 miRNA up-regulated and 1 miRNA down-regulated (> 2.5 fold change) in T2D patients comparing with NGT subjects.
- miR-142-3p was down-regulated and miT-93-5p was up-regulated in both lean and obese T2D, therefore these 2 miRNA are circulating biomarker for T2D prediction

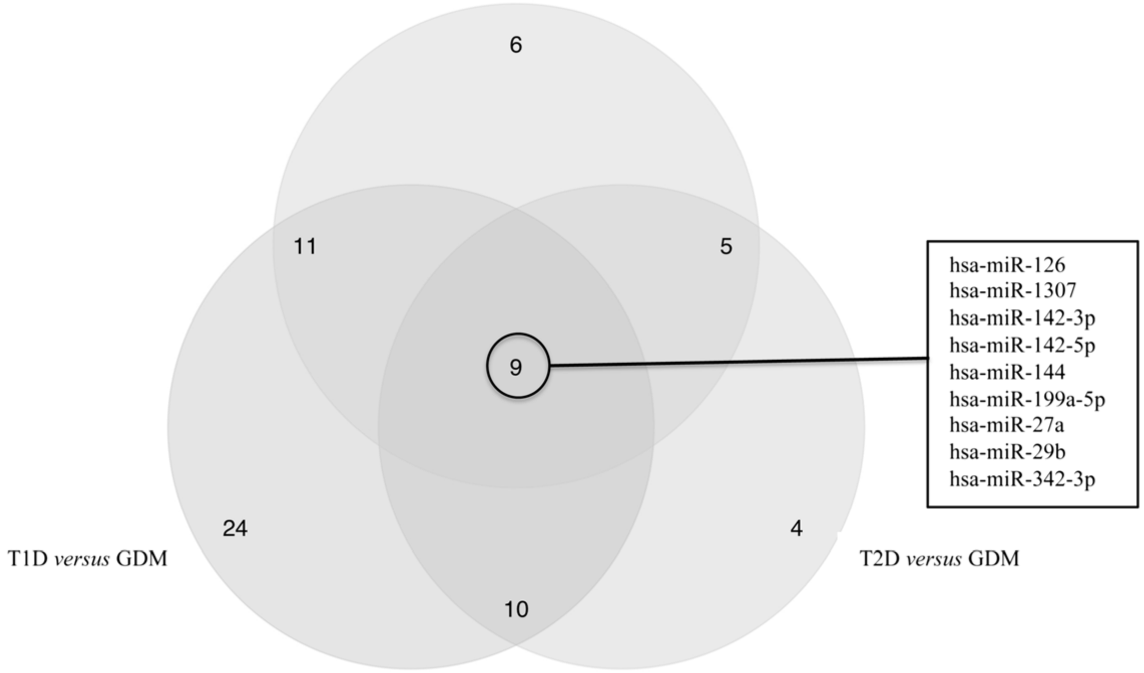
Collares *et al. BMC Research Notes* 2013, **6**:491
http://www.biomedcentral.com/1756-0500/6/491

BMC
Research Notes

RESEARCH ARTICLE **Open Access**

Identifying common and specific microRNAs expressed in peripheral blood mononuclear cell of type 1, type 2, and gestational diabetes mellitus patients

Cristhianna VA Collares[1,2†], Adriane F Evangelista[2†], Danilo J Xavier[2], Diane M Rassi[1], Thais Arns[2], Maria C Foss-Freitas[3], Milton C Foss[3], Denis Puthier[4], Elza T Sakamoto-Hojo[2,5], Geraldo A Passos[2] and Eduardo A Donadi[1,2*]



hsa-miR-126
hsa-miR-1307
hsa-miR-142-3p
hsa-miR-142-5p
hsa-miR-144
hsa-miR-199a-5p
hsa-miR-27a
hsa-miR-29b
hsa-miR-342-3p

Further study

- Individual genotyping by real-time PCR in larger T2D cohort vs control group.
- Assess these circulating biomarkers as predictors for T2D in EGAT cohort.
- Functional assay to find target gene regulation.

Acknowledgement

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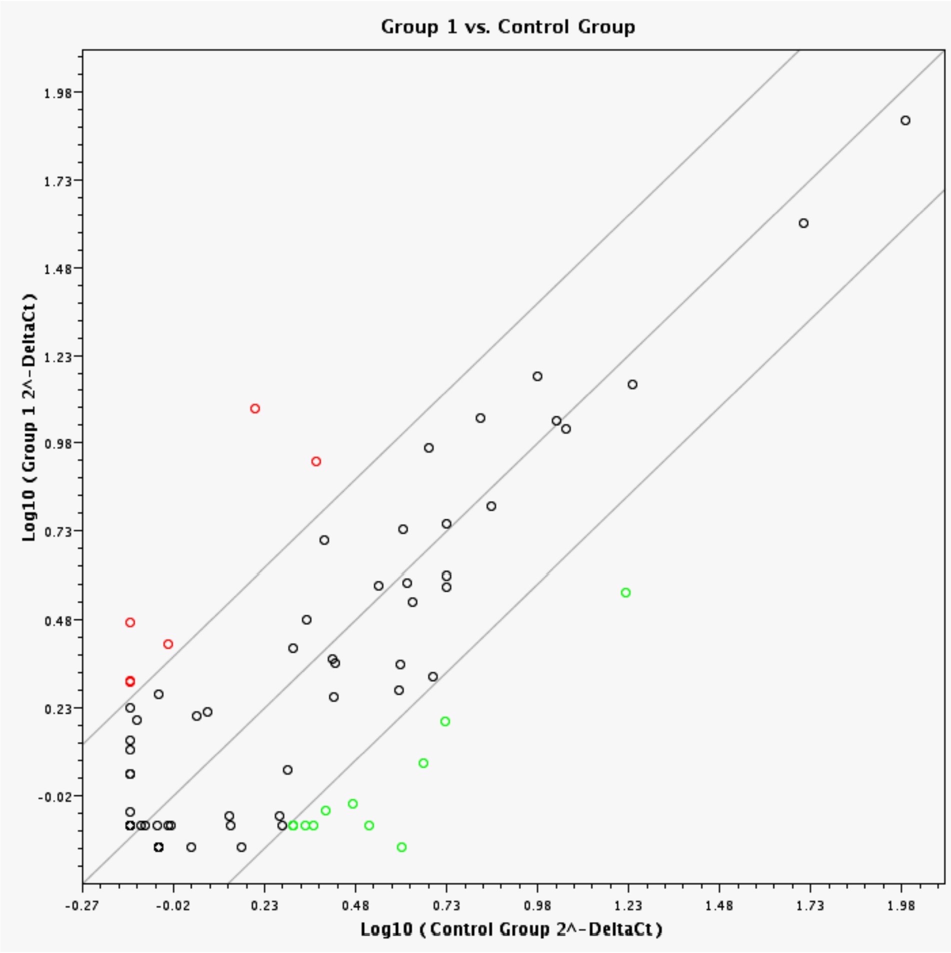
Differential expression of miRNA between lean NGT VS obese NGT



Mature ID	Fold Regulation
hsa-miR-20b-5p	2.7967
hsa-miR-22-3p	7.4837
hsa-miR-19b-3p	4.0947
hsa-miR-610	2.7774
hsa-miR-766-3p	3.5894
hsa-miR-877-5p	2.7967
hsa-miR-29c-3p	-3.2858
hsa-miR-15b-5p	-2.8016
hsa-miR-18b-5p	-4.188
hsa-miR-144-3p	-2.9409
hsa-miR-186-5p	-2.5959
hsa-let-7b-5p	-3.9347
hsa-miR-191-5p	-2.578
hsa-miR-221-3p	-5.9432
hsa-miR-1260a	-3.4135
hsa-miR-3173-3p	-4.6268