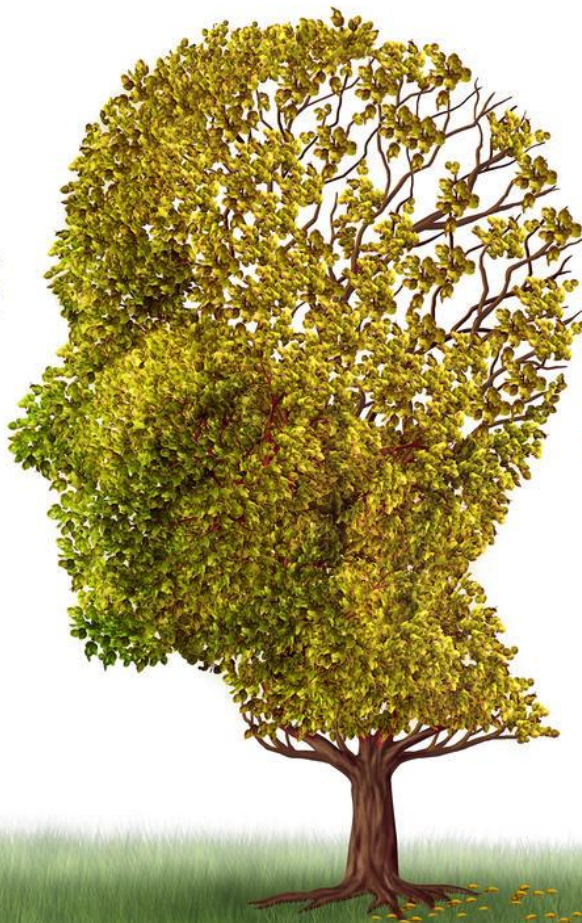


Genetic and epigenetic analysis of neuronal responses to external risk factors (Jacco Briedé)



Maastricht University

Toxicogenomics



28-11-2018

“Herinneringen” is gefinancierd binnen het Interreg V programma Vlaanderen-Nederland, het grensoverschrijdend samenwerkingsprogramma met financiële steun van het Europees Fonds voor Regionale Ontwikkeling. Meer info: www.grensregio.eu

Overview of “Memories”

Aim: To develop a **data based test strategy** for refining **early biomarker** discovery in Alzheimer's disease

In vitro data: iPSC cells

AD Patients: brain regions, blood CSF

Mouse models: blood, CSF

miRNA,
mRNA
sequencing

Data
analysis and
integration

Alzheimer's
disease
model

1. Biomarker
2. Prevention
3. Drug discovery



Content

- What are (circulating) miRNAs
- Why relevant biomarkers?
- Relevance for neurodegenerative diseases
- Detection by sequencing
- Data analysis



Central Dogma

TRANSCRIPTION

TRANSLATION

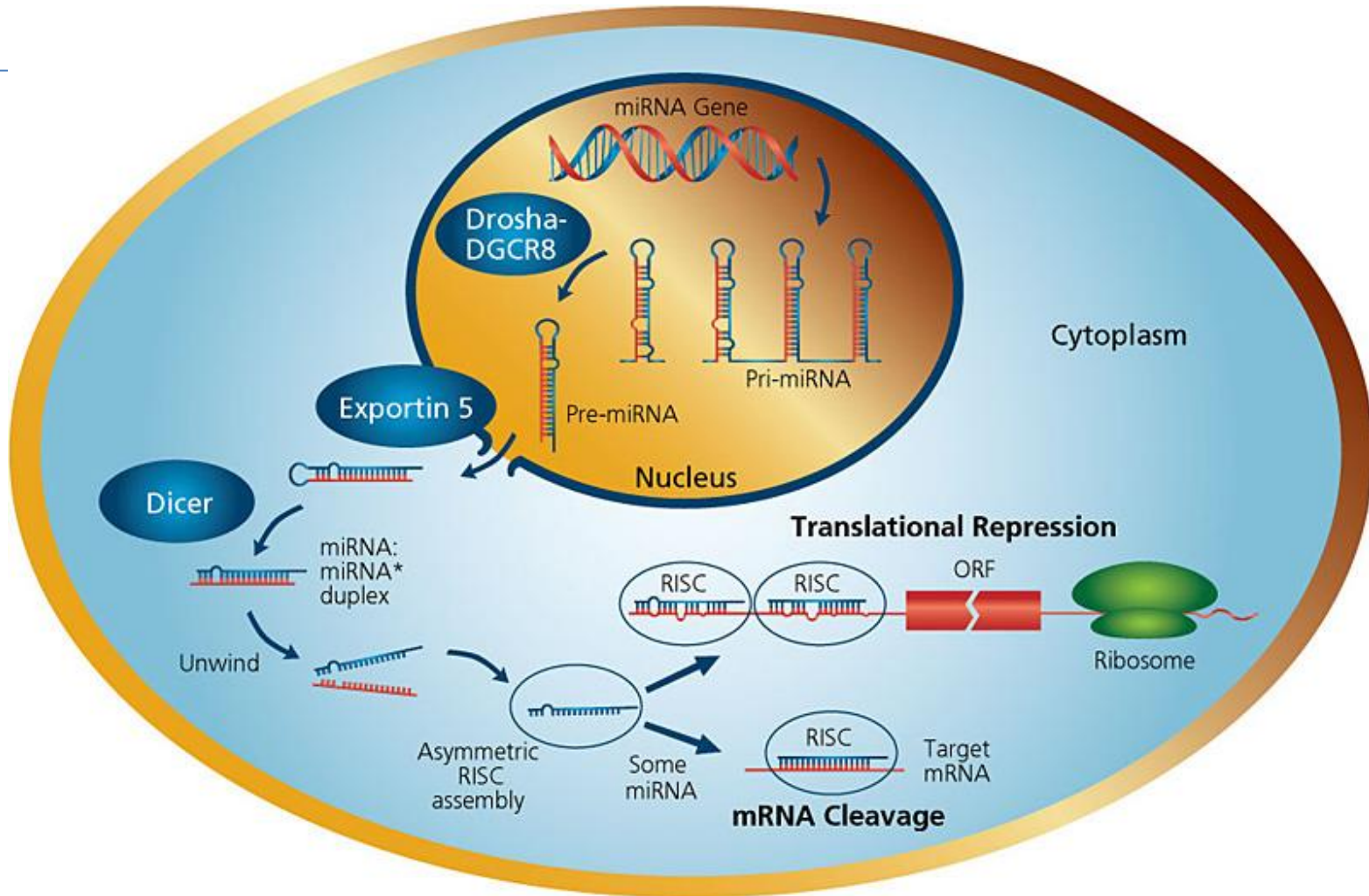


19-23 bp
microRNA – mRNA interaction
RNA silencing
Post transcriptional regulation

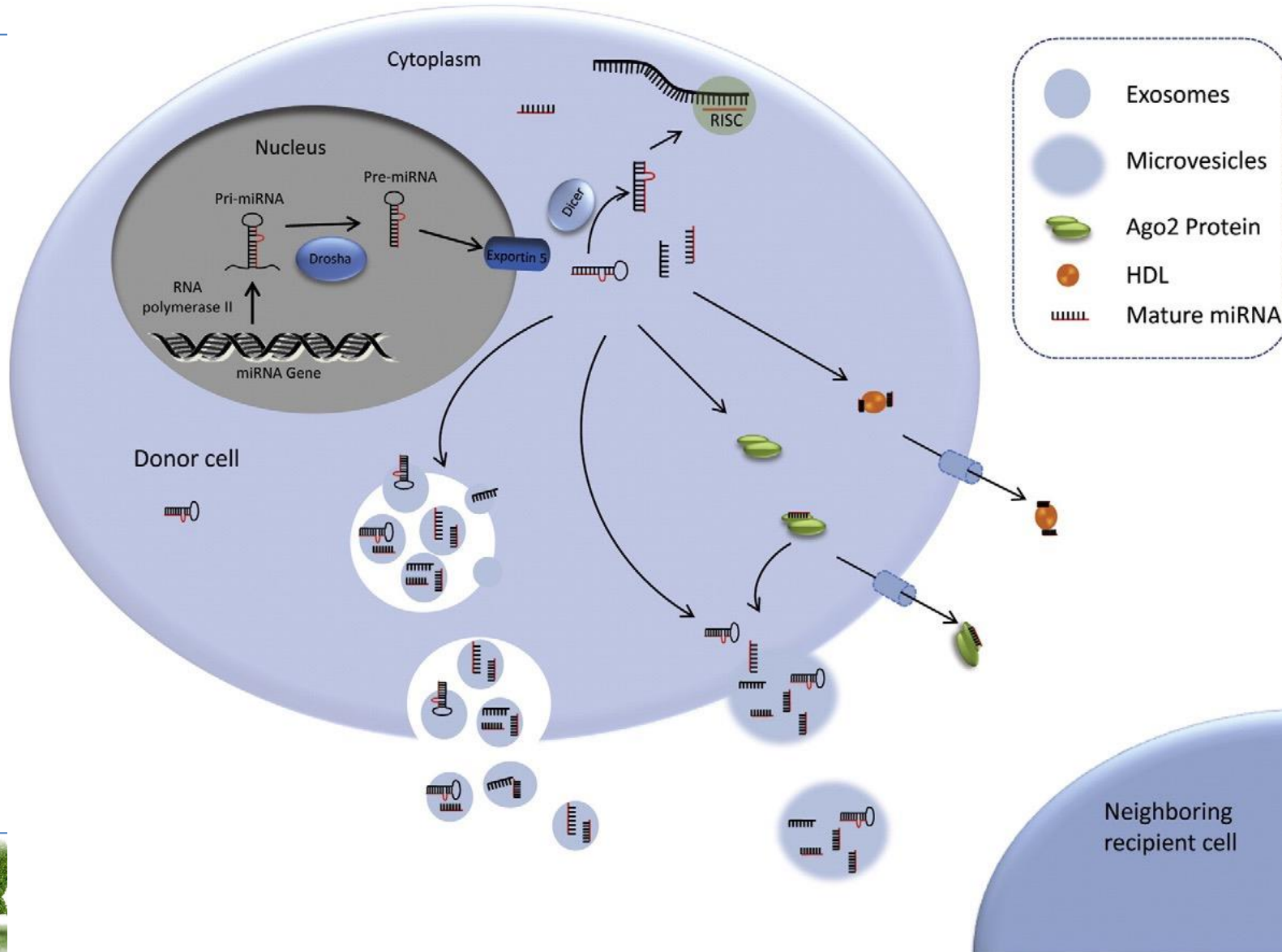
2693 distinct miRNAs (miRBase release March 2018)



Production of miRNAs



Secretion of circulating miRNAs



Stability of cmiRNAs



Long term
storage



Freeze thaw
cycles



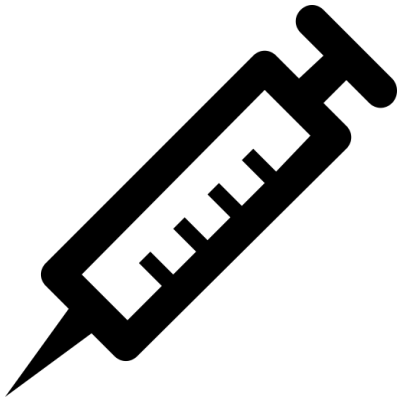
High/low
pH



Boiling



miRNAs as potential biomarkers



Plasma

Serum

Cerebrospinal Fluid



Feces

Urine

Seminal fluid



Tears

saliva



Good biomarkers?

- Stability
- Sequence of miRNAs conserved among various of species; translational
- Detectable in different bodyfluids
- Actively secreted: Changes in cmiRNAs reflect changes in diseased tissue
- Tissue specific and enriched

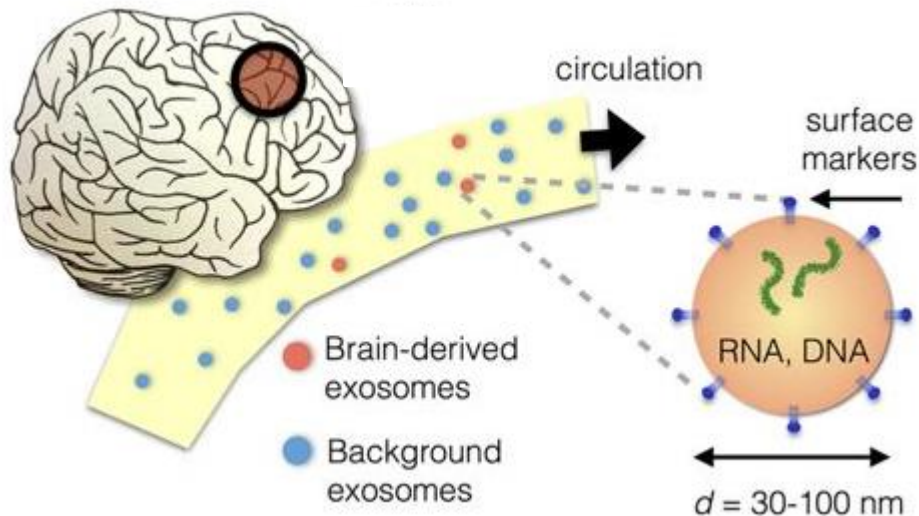
Limitations

- Complex relationship between single miRNA and protein/pathways
- Low miRNA yield in body fluids
- No “Golden standard” for analysis (sequencing, qRT-PCR, microarray)



miRNAs and the blood brain barrier

Exosomes, small (30–100 nm diameter) membrane-bound vesicles carrying proteins, RNA, and DNA from their mother cells have been found to pass through the BBB



J. Ko et al. Scientific Reports volume 6, 31215 (2016)



Received: 2017.12.30
Accepted: 2018.01.12
Published: 2018.08.13

Micro-RNA-137 Inhibits Tau Hyperphosphorylation in Alzheimer's Disease and Targets the *CACNA1C* Gene in Transgenic Mice and Human Neuroblastoma SH-SY5Y Cells

Authors' Contribution:
Study Design: A
ABCE 1,2 Yang Jiang
BDE 2 Bing Xu

1 Department of Neurology, The Fourth Affiliated Hospital of China Medical University, Shenyang, Liaoning, P.R. China



RESEARCH ARTICLE

Distinct expression of the neurotoxic microRNA family *let-7* in the cerebrospinal fluid of patients with Alzheimer's disease

Katja Derkow¹*, Rosa Rössling¹*, Carola Schipke², Christina Krüger¹, Jakob Bauer¹, Michael Fähring³, Andrea Stroux⁴, Eckart Schott⁵, Klemens Rupprecht⁶, Oliver Peters^{7,8†}, Seija Lehnardt^{1,6,9†‡}



RESEARCH ARTICLE

Specific serum and CSF microRNA profiles distinguish sporadic behavioural variant of frontotemporal dementia compared with Alzheimer patients and cognitively healthy controls

Johannes Denk¹, Felix Oberhauser¹, Johannes Kornhuber², Jens Wiltfang³, Klaus Fassbender⁴, Matthias L. Schroeter⁵, Alexander E. Volk⁶, Janine Diehl-Schr Johannes Prudio⁸, Adrian Danek⁹, Bernhard Landwehrmeyer¹⁰, Martin Lauer¹¹, Markus Otto¹⁰, Holger Jahn^{1,12*}, for the FTLDc study group¹¹



Sierksma et al. *Molecular Neurodegeneration* (2018) 13:54
<https://doi.org/10.1186/s13024-018-0285-1>

Molecular Neurodegeneration

RESEARCH ARTICLE

Open Access

Deregulation of neuronal miRNAs induced by amyloid- β or TAU pathology

Annerieke Sierksma^{1,2}, Ashley Lu^{1,2}, Evgenia Salta^{1,2}, Elke Vanden Eynden^{1,2}, Zsuzsanna Callaerts-Vegh³, Rudi D'Hooge³, David Blum⁴, Luc Buée⁴, Mark Fiers^{1,2†} and Bart De Strooper^{1,2,5†}



Interactions between miRNAs and genes involved in the pathogenesis of AD in mouse models, cell-lines and postmortem AD brains

Table 1
miRNAs involved in the pathogenesis of AD.

miRNAs	Target	Cell line/tissue	Effect
miR-613 miR-431	Brain-derived neurotrophic factor (BDNF) Kremen1 (Krm1)	Hippocampus of APP/PSEN1 mice Cortico-hippocampal cultures isolated from triple transgenic 3xTg-AD mice	Decreases neuronal survival and proliferation Prevents amyloid- β -mediated synapse
miR-146	IRAK1	Murine PSEN2 knockout (KO) and wild-type microglia	PSEN2 participates in maintaining the basal and cytokine induced expression of the innate immunity regulating miR-146
miR-146a miR-330	NF κ B p65 VAV1	ApoE3 and apoE4 targeted replacement mice C57 mice randomized into AD and control groups	Regulates the inflammatory response reduces A β production and alleviates mitochondrial dysfunction in AD
miR-124-3p	Tau protein	N2a/APP695swe cells	Plays a neuroprotective role in AD by inhibiting abnormal hyperphosphorylation of Tau
miR-98-5p	Sorting nexin 6 (SNX6)	SK-N-SH and HEK293 cells	Inhibits A β accumulation
miR-146a	Low-density lipoprotein receptor-related protein-2 (Lrp2)	SH-SY5Y cells	Increases cell apoptosis
miR-21 miR-124 and miR-15b	Programmed cell death protein 4 (PDCD4) BACE1	SH-SY5Y cells SH-SY5Y cells	Attenuates apoptosis Inhibit BACE1 expression
miR-221	A Disintegrin and Metalloproteinase 10 (ADAM10)	SH-SY5Y cells	Decreases the cleavage of APP
miR-219 miR-140-5p	Tau ADAM10	Mammalian cellular models AD postmortem brain hippocampus	Represses tau synthesis at the post-transcriptional level AD-related neurotoxic effects exacerbate downregulation of ADAM10 by acting on miR-140-5p
miR-34a	Triggering receptor expressed in myeloid cells 2 (TREM2)	Sporadic Alzheimer hippocampal CA1	Deficiencies in phagocytosis and the innate immune system in AD

Zendjabil M, Clin Chim Acta. 2018 484: 99-104.



Brain-born biomarkers?

Review cmiRNAs as biomarkers for neuronal disorders



Major Depression Disorder



Parkinson Disease



Alzheimer's Disease



Bipolar Disorder

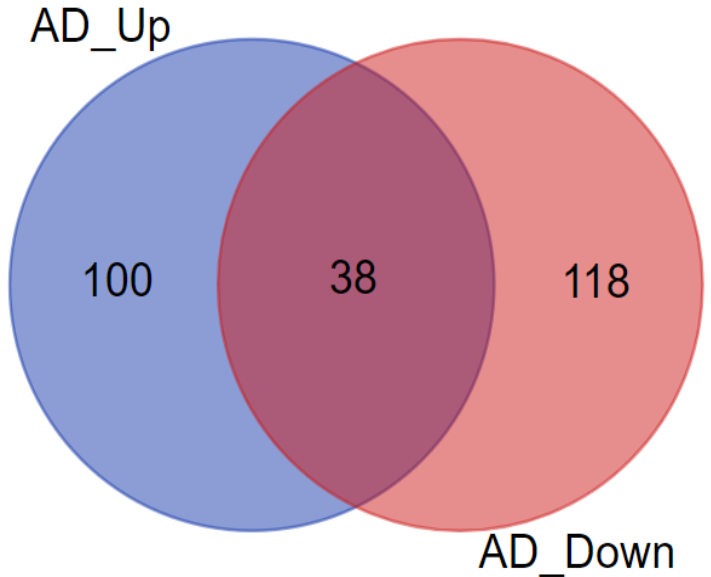


Schizophrenia



Review cmiRNAs as biomarkers for neuronal disorders

Names	total	elements
AD_Down AD_Up	38	let-7f-5p miR-132-3p miR-23a-3p miR-106b-5p miR-485-5p miR-143-3p miR-181b-5p miR-29b-3p miR-141-3p miR-30d-5p miR-26a-5p miR-125b-5p miR-34a-5p miR-127-3p miR-375 miR-335-5p miR-142-5p miR-146a-5p miR-200b-3p miR-191-5p miR-135a-5p miR-30a-3p miR-3613-3p miR-138-5p miR-27a-3p miR-15a-5p miR-9-5p miR-106a-5p miR-502-3p miR-154-5p miR-3065-5p miR-29a-3p miR-660-5p miR-30b-5p miR-26b-5p miR-4674 miR-93-5p miR-30e-5p
AD_Up	100	miR-429 miR-361-5p miR-32-5p miR-3158-3p let-7f-5p miR-4741 miR-371a-3p miR-197-3p miR-185-5p miR-494 miR18b-5p miR-161 miR-378a-3p miR-339-5p miR-424-5p miR-550a-5p miR-517b-3p miR-320b miR-873-3p miR-590-5p miR-483-5p miR-517a-3p miR-455-3p miR-296-5p miR-1285-5p miR-222-3p miR15a-5p miR-219-2-3p miR-4467 miR-371 miR-548a-5p miR-30c-5p miR-5001-3p miR-26b-3p miR-28-3p miR-30a-5p miR-519 let-7d-3p miR-3622b-3p miR-659-5p miR-345-5p miR-505-3p miR-34b-3p miR-151a-3p miR-216a-5p miR-1246 miR-30c-2-3p miR-148-5p miR-6119-5p miR-106b-3p miR-671-3p miR-125a-3p miR-20a-5p miR-380-3p let-7b-5p miR-206 miR-4668-5p miR-155-5p miR-27b-3p miR-320a-5p miR-320c miR-125-1-3p miR-518f-3p miR-448 miR-520a-3p miR-132-5p miR-362-5p miR-112 miR-302b-3p miR-1291 miR-5001-5p miR-205-5p miR-34c-5p miR-184 miR-1468 miR-456-5p miR-100-5p miR-3176 miR-1285-3p miR-101-3p miR-186-5p miR-518b miR-449a miR-5010-3p miR-105-5p miR-501-5p miR-6513-3p miR-22-5p miR-425-5p miR-4781-3p miR-204-5p miR-1260a miR-1307-5p miR-597-5p miR-200a-3p miR-526a miR-766-3p miR-128 miR-151b miR-582-5p
AD_Down	118	miR-708-3p miR-151a-5p miR-137 miR-142-3p miR-181d miR-152-3p Let-7d-5p miR-760 miR-139-5p let-7a-5p miR-1274a miR-381-3p miR-342-5p miR-6722 miR-150-5p miR-15b-5p miR-497-5p miR-31-5p miR-9-3p miR-129-5p miR-17-3p miR-135b-5p miR-181a-3p miR-598 miR-22-3p miR-1331-5p miR-1306-5p miR-122-5p miR-39-5p miR-193-3p miR-410 miR-29c-3p miR-24-3p miR-384 miR-1842 miR-195-5p miR-194-5p miR-377-5p miR-18a-5p miR-639 miR-127-5p let-7e-5p let-7g-5p miR-21-5p miR-15b-3p miR-43a-5p miR-342-3p miR-301a-3p miR-769-5p miR-3200-3p miR-1294 miR-98-5p miR-953 miR-181c-3p miR-708-5p miR-148a-3p miR-10a-5p miR-455-5p miR-4331 miR-146b-5p miR-885-5p miR-338-3p miR-23b-3p miR-873-5p miR-221-3p miR-193a-5p miR-148b-5p miR-590-3p let-7d-5p miR-101-5p miR-214-3p miR-19b-3p Let7g-5p miR-532-5p miR-218-5p miR-126-5p miR-451a miR-4449 miR-223-3p miR-181c-5p miR-3293 miR-33b-5p miR-181a-5p miR-134 miR-125b miR-33a-5p miR-36 miR-99a-5p miR-210 miR-16-2 miR-199b-5p miR-326 miR-545-3p miR-4953 miR-483-3p miR-2c-3p miR-219a-5p miR-144-5p miR-136-3p miR-874 miR-3916 miR-10b-5p miR-323a-3p miR-199a-5p miR-16-5p miR-328-3p miR-126-3p miR-431-3p miR-149-5p miR-140-5p miR-488-3p miR-340-5p miR-4772-3p miR-501-3p miR-422b miR-182-5p miR-103a-3p miR-107



Detection of cMiRNAs by Next-Generation Sequencing Technology

Targeted approaches:

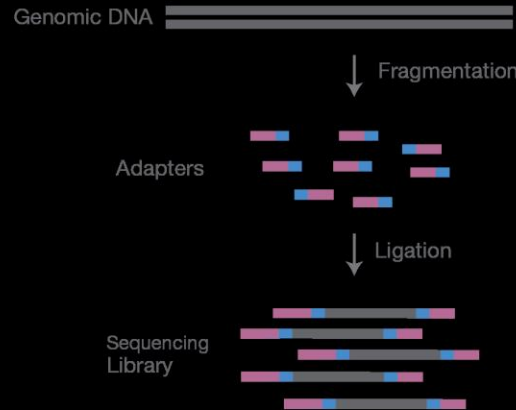
- qPCR
- Microarrays

In “Memories” Next-Generation Sequencing Technology will be applied:

- Untargeted
- miRNA profiles
- Low abundance miRNA
- Novel miRNAs

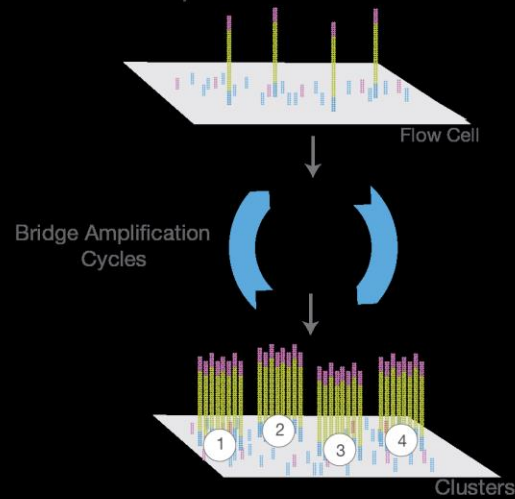


A. Library Preparation



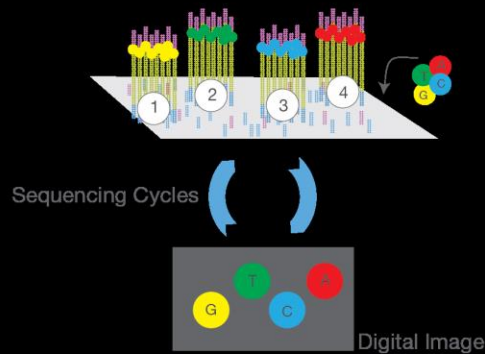
NGS library is prepared by fragmenting a gDNA sample and ligating specialized adapters to both fragment ends.

B. Cluster Amplification



Library is loaded into a flow cell and the fragments are hybridized to the flow cell surface. Each bound fragment is amplified into a clonal cluster through bridge amplification.

C. Sequencing



Data is exported to an output file

```
Cluster 1 > Read 1: GAGT...
Cluster 2 > Read 2: TTGA...
Cluster 3 > Read 3: CTAG...
Cluster 4 > Read 4: ATAC...
```

Text File

Sequencing reagents, including fluorescently labeled nucleotides, are added and the first base is incorporated. The flow cell is imaged and the emission from each cluster is recorded. The emission wavelength and intensity are used to identify the base. This cycle is repeated "n" times to create a read length of "n" bases.

D. Alignment and Data Analysis

	ATGGCATTGCAATTTGACAT
	TGGCATTGCAATTTG
Reads	AGATGGTATTG
	GATGGCATTGCAA
	GCATTGCAATTTGAC
	ATGGCATTGCAATT
	AGATGGCATTGCAATTTG
	Reference Genome

Reads are aligned to a reference sequence with bioinformatics software. After alignment, differences between the reference genome and the newly sequenced reads can be identified.

https://www.youtube.com/watch?annotation_id=annotation_228575861&feature=iv&src_vid=womKfikWlxM&v=fCd6B5HRaZ8



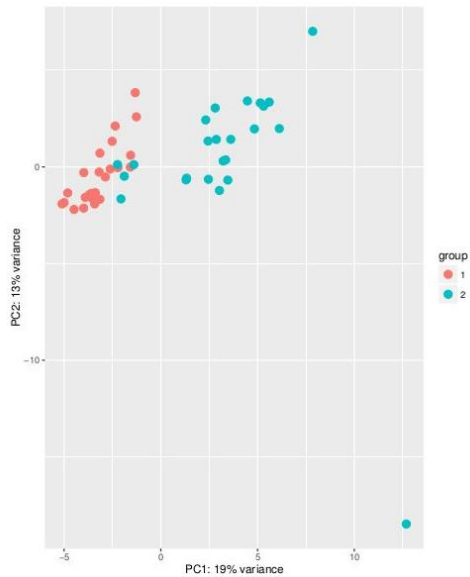
Data preprocessing

- Quality control
 - FastQC
- Extract miRNA sequences
 - Adapter removal, size and quality filter
- Quantify miRNAs
 - Align remaining reads to miRNA sequence database (miRBase.org)
 - BWA, Bowtie, Novoalign
- Normalize between samples
 - Counts per million, TMM, Quantile, DESeq etc.

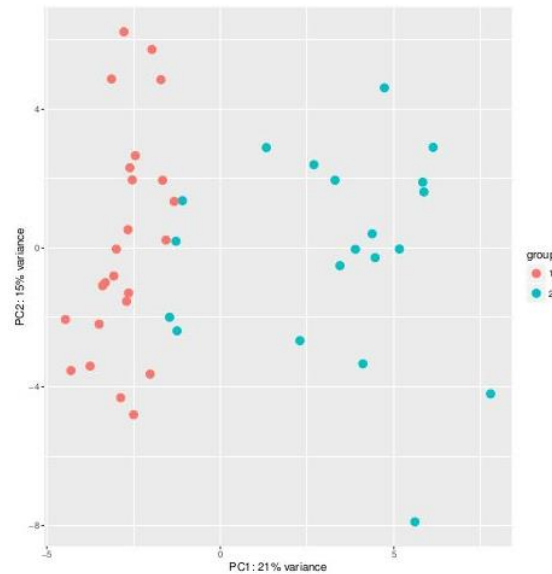


DESeq2 - PlotPCA

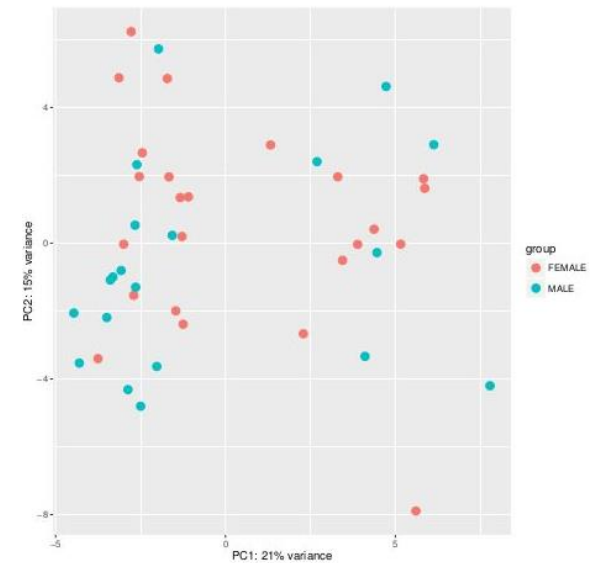
outliers



batch effect



confounders



Overview of “Memories”

Aim: To develop a **data based test strategy** for refining **early biomarker** discovery in Alzheimer's disease

In vitro data: iPSC cells

AD Patients: brain regions, blood CSF

Mouse models: blood, CSF

miRNA,
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cing

Data
analysis and
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Alzheimer's
disease
model

1. Biomarker
2. Prevention
3. Drug discovery



Acknowledgments



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Dr. Julian Krauskopf, Ph.D.

Manon van den Berg, M.Sc.



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