Project BASTION „From Basic to Translational Research in Oncology"

Krystian Jazdzewski, group-leader

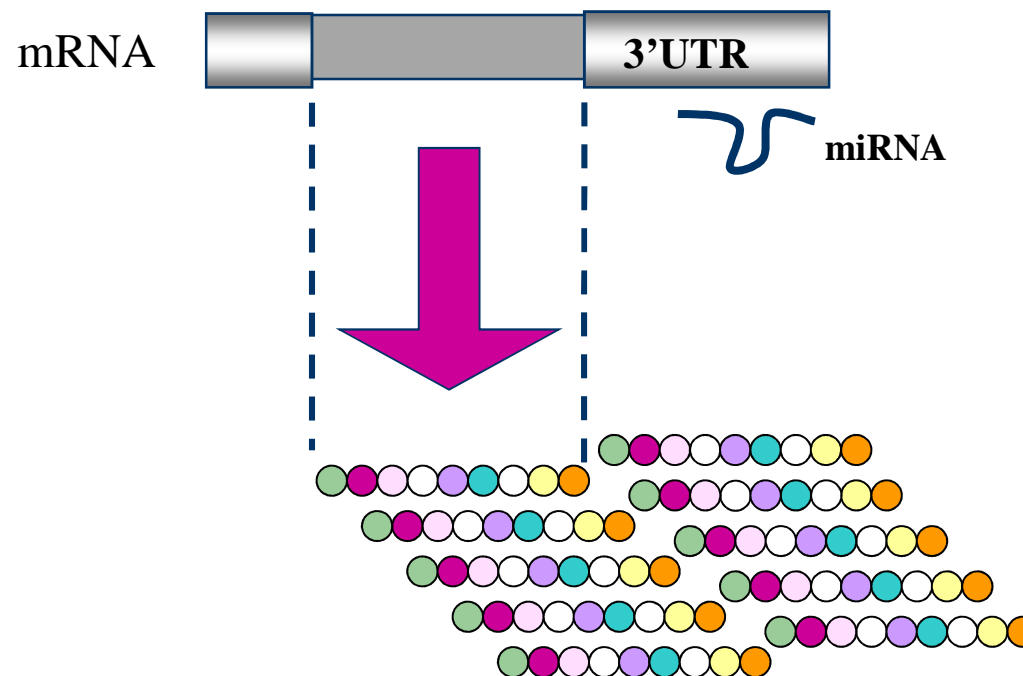### Medical University of Warsaw

Ohio State University

Warsaw

November 27th 2012

microRNAs (miRs)

- ~ 22 nt long non-coding RNAs

- bind 3' UTRs of protein-coding genes and repress their expression

microRNAs (miRs)

- tissue specific

- overexpressed in numerous cancers leading to downregulation

  of target genes

- overexpressed miRs are excreted to blood – circulating miRs can
  serve as prognostic and diagnostic tools

In search of new pathways of tumorigenesis - genome-wide functional analysis of microRNAs deregulated in human cancers.

Aims of the study:

- To discover and annotate on the genome-wide level previously unidentified RNA genes, including miRs, by the next-generation deep sequencing technology in thyroid, colon and liver cancers.

- To detect and characterize the germline and somatic sequence variations of all known and newly discovered miRs in cancer/unaffected tissue pairs of thyroid, liver or colon origin.

In search of new pathways of tumorigenesis - genome-wide functional analysis of microRNAs deregulated in human cancers.

Aims of the study:

- To identify and measure circulating microRNAs in blood of patients with benign and malignant tumors of the thyroid, liver and colon (diagnostic part)

- To determine the potential use of microRNA expression profiles in prediction of surgical outcome and tumor recurrence risk (prognostic part)
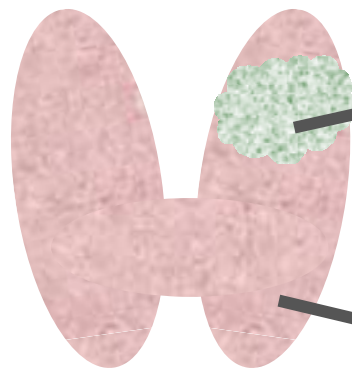
Material and Methods:

- Training set:

  28 RNA samples from 14 PTC patients *(14 pairs of tumor - unaffected tissue from the same patient),*

- Validation set:

  10 RNA samples from 5 PTC patients *(5 pairs of tumor - unaffected tissue from the same patient),*

- Control set:

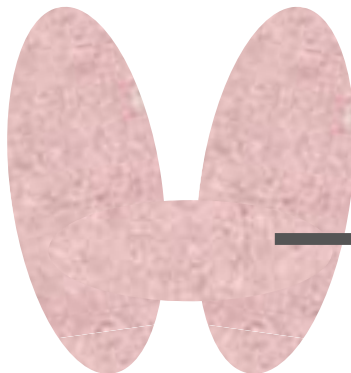 14 RNA samples from non-cancerous thyroid samples

Material and Methods:
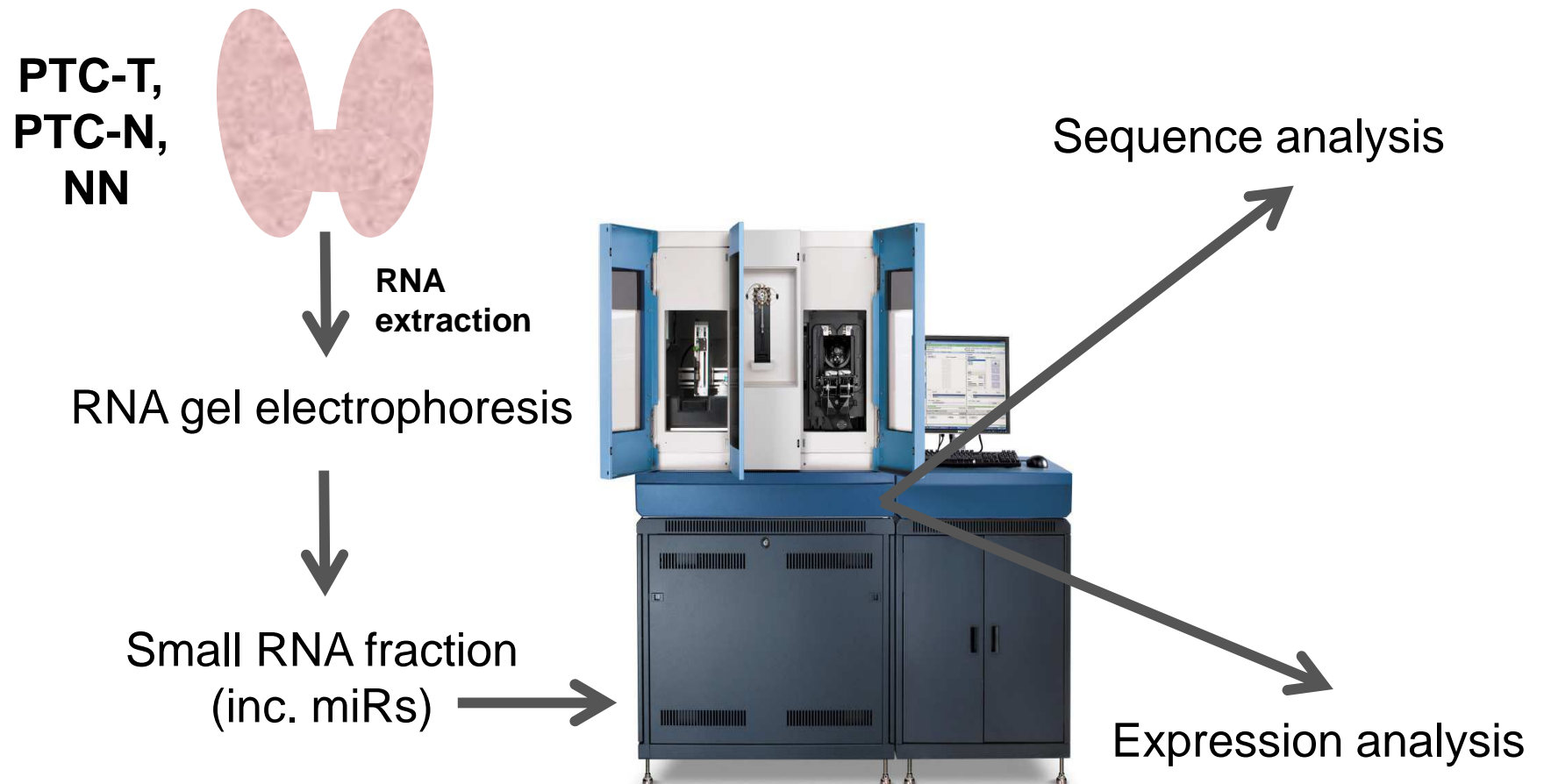
PTC Tumor, PTC-T (n=19)

Unaffected control tissue adjacent to tumor, PTC-N (n=19)
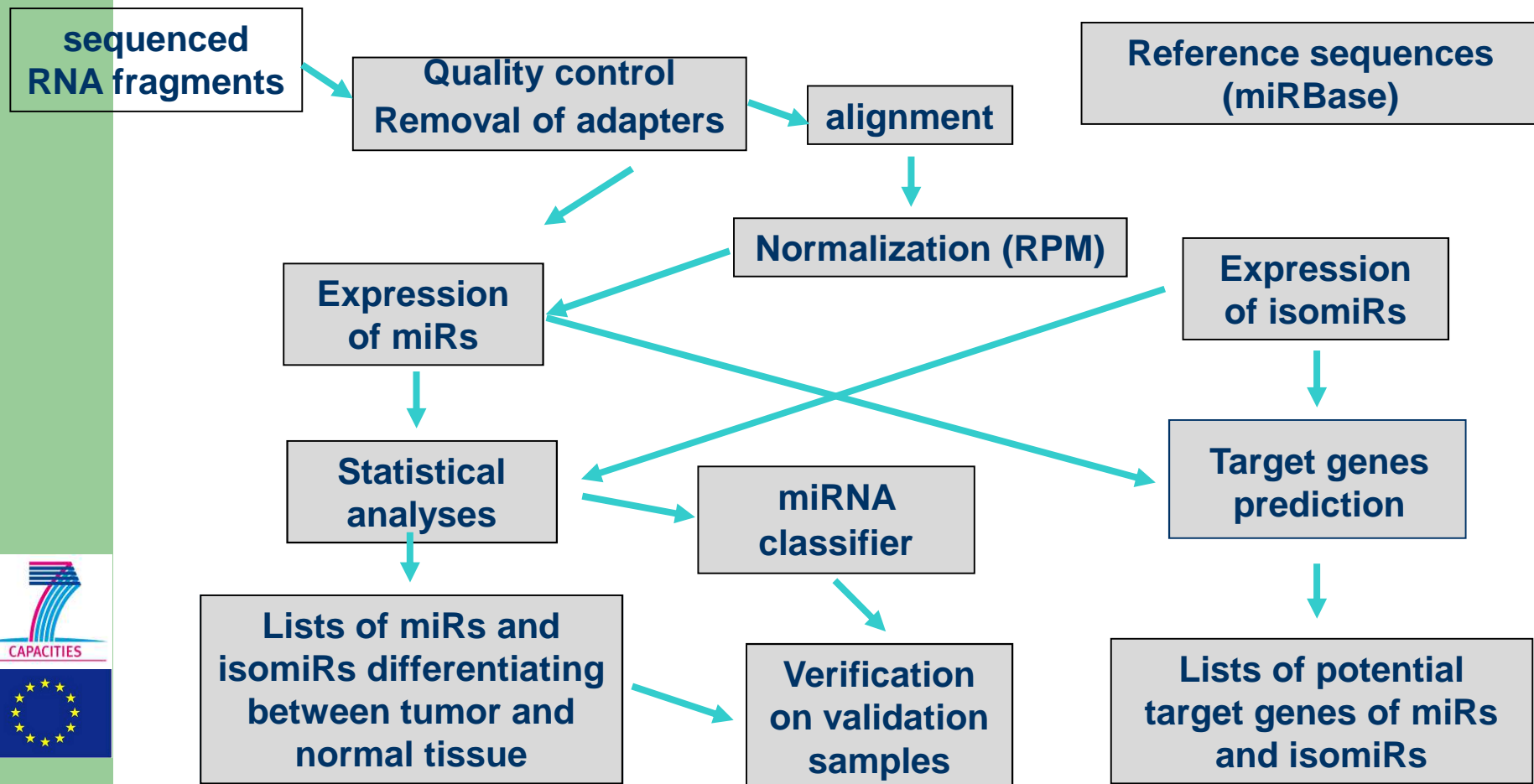
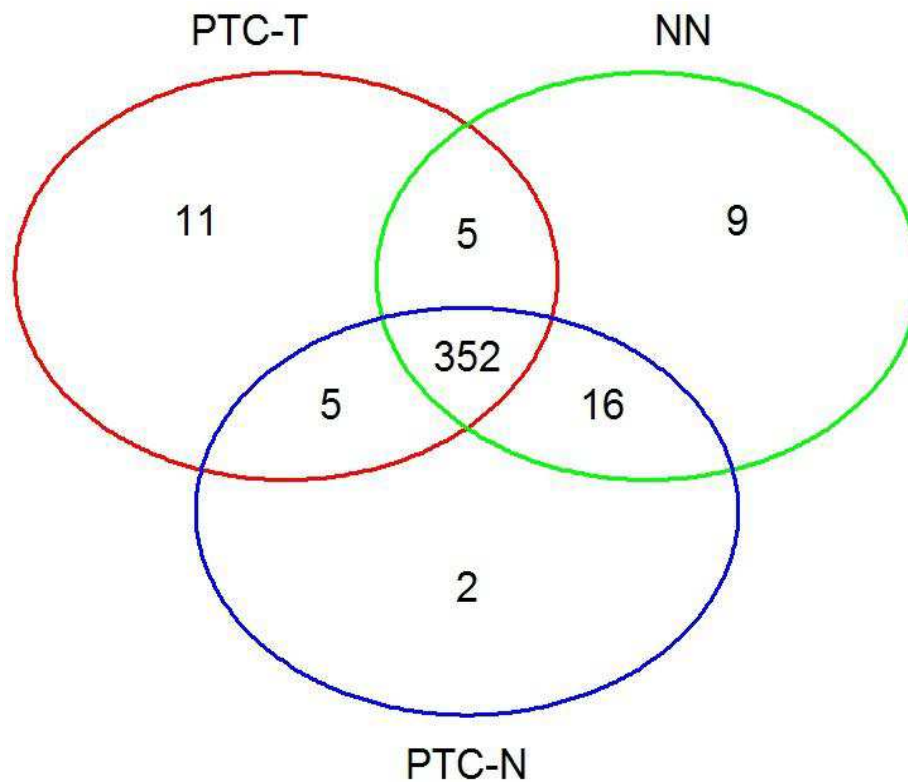Normal thyroid tissue, NN (n=14)

Material and Methods:

**PTC-T,
PTC-N,
NN**

↓ **RNA
extraction**

RNA gel electrophoresis

↓

Small RNA fraction
(inc. miRs) →

Sequence analysis

Expression analysis

Material and Methods:

| sequenced RNA fragments | | Quality control Removal of adapters | | alignment | | Reference sequences (miRBase) |

Normalization (RPM)

Expression of miRs

Expression of isomiRs

Statistical analyses

miRNA classifier

Target genes prediction

Lists of miRs and isomiRs differentiating between tumor and normal tissue

Verification on validation samples

Lists of potential target genes of miRs and isomiRs

Results: miRNome of the thyroid gland

400 microRNAs

expressed in the thyroid.

352 (88%) are expressed

in all tissue types

Results: miRNome of the thyroid gland

miRs aberrantly

expressed in PTC

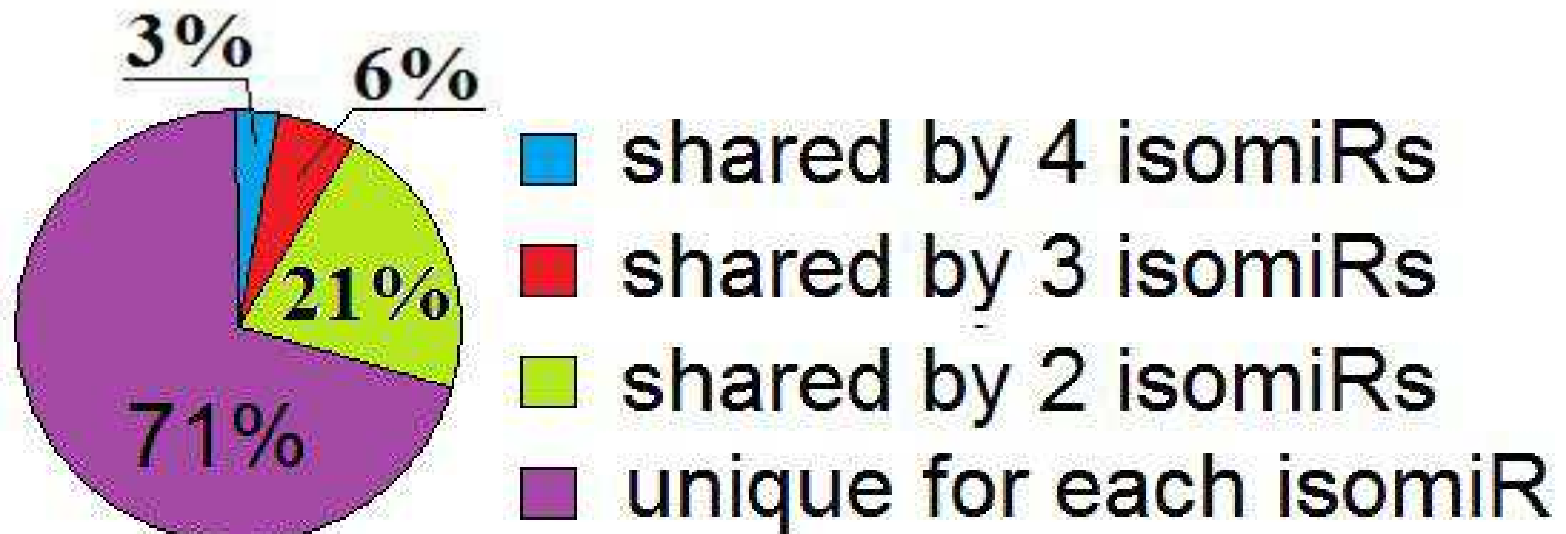| microRNA	PTC-T vs. NN		PTC-T vs. PTC-N	
	FDR	Fold change	FDR	Fold change
hsa-miR-146b-5p	0.001	51.8	0.011	21.7
hsa-miR-146b-3p	0.001	43.1	0.013	18.9
hsa-miR-551b	0.002	12.9	0.009	12.0
hsa-miR-221	0.032	9.1	0.058	8.2
hsa-miR-222	0.008	8.9	0.031	7.7
hsa-miR-187	0.003	8.3	0.013	7.6
hsa-miR-221*	0.001	7.2	0.009	6.3
hsa-miR-891a	0.029	7.0	0.013	31.2
hsa-miR-222*	0.003	4.7	0.013	4.4
hsa-miR-34a*	0.015	4.5	0.089	3.5
hsa-miR-34a	0.015	4.5	0.079	5.0
hsa-miR-182	0.002	3.9	0.013	3.6
hsa-miR-486-3p	0	0.17	0.001	0.25
hsa-miR-27a*	0.025	0.17	0.823	0.62
hsa-miR-3676	0.001	0.23	0.085	0.5
hsa-miR-1247	0.002	0.24	0.09	0.34
hsa-miR-532-3p	0.001	0.24	0.519	0.55
hsa-miR-1249	0.001	0.26	0.068	0.43
hsa-miR-125b-1*	0.002	0.27	0.092	0.52

Results: new microRNA isoforms (isomiRs)
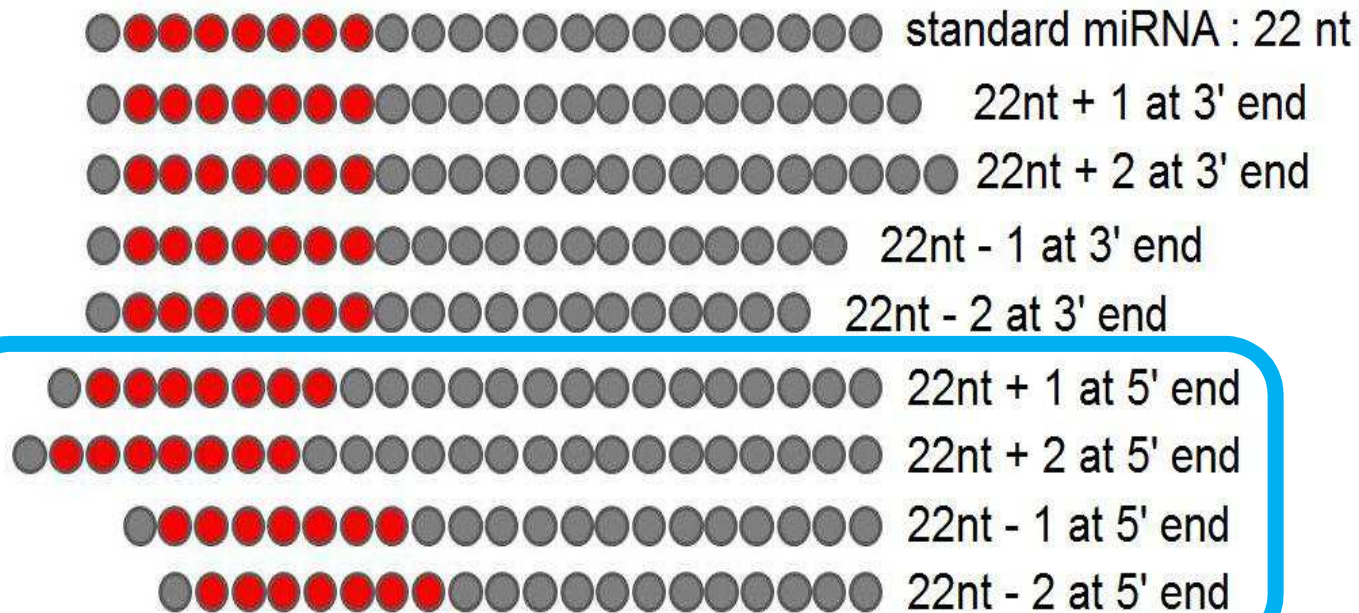
The seed sequence of isoforms differing at the 5' end is changed – they regulate distinct sets of target genes
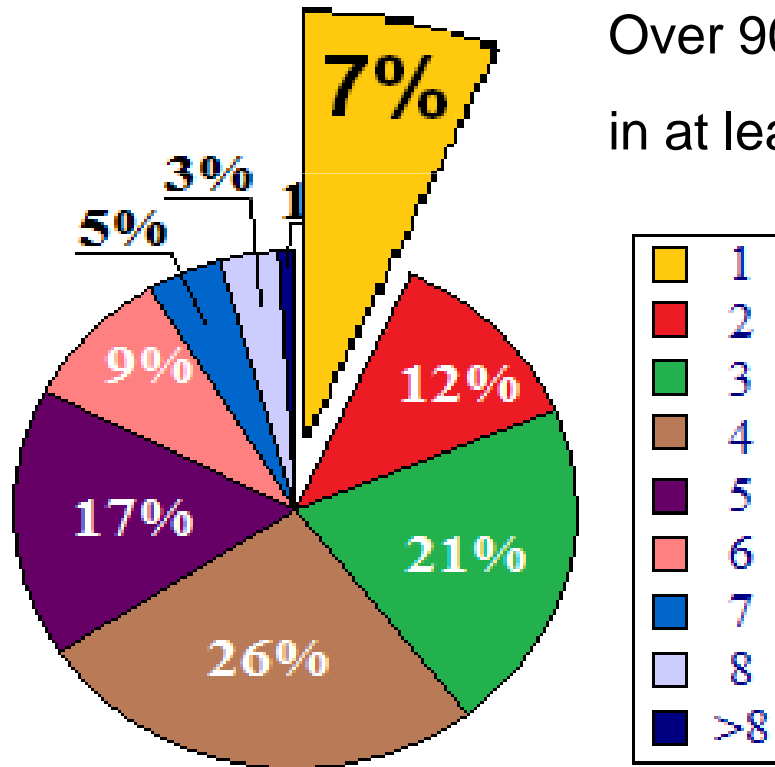
Results: target genes for newly identified isomiRs

- 3%
- 6%
- 21%
- 71%

- ☐ shared by 4 isomiRs
- ☐ shared by 3 isomiRs
- ☐ shared by 2 isomiRs
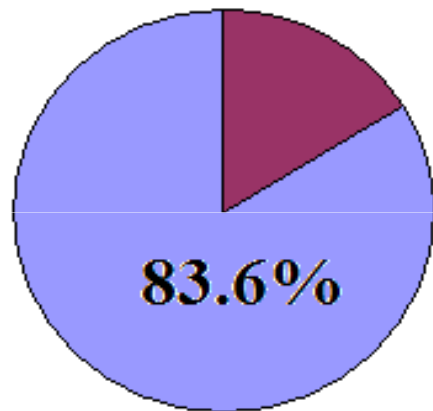- ☐ unique for each isomiR

Results: new microRNA isoforms (isomiRs)

Over 90% of microRNAs are expressed

in at least 2 different length variants.

🟨	1
🟥	2
🟩	3
🟫	4
🟪	5
🟧	6
🟦	7
⬜	8
🟦	>8

CAPACITIES

BASTION

Results: new microRNA isoforms (isomiRs)

83.6%

- miRs with the highest expression of a standard, reference isoform
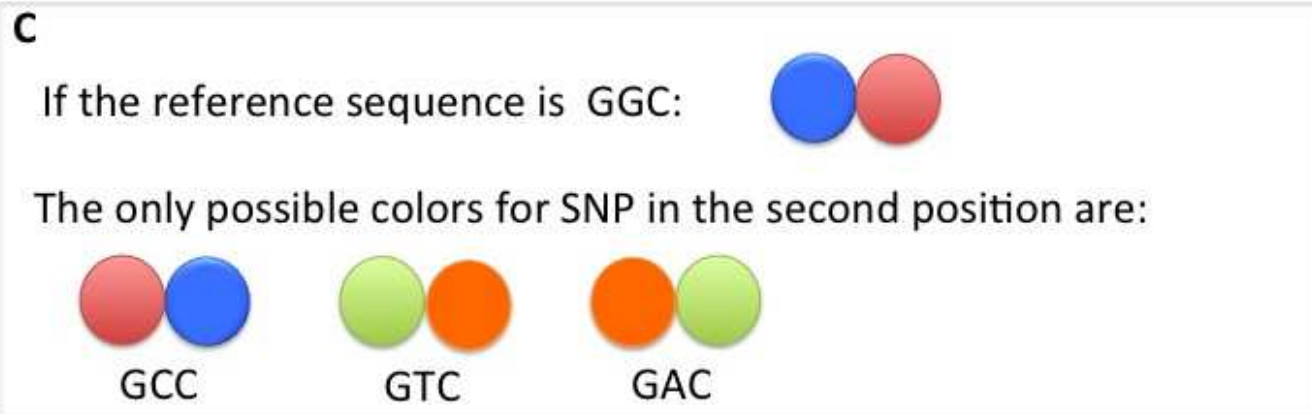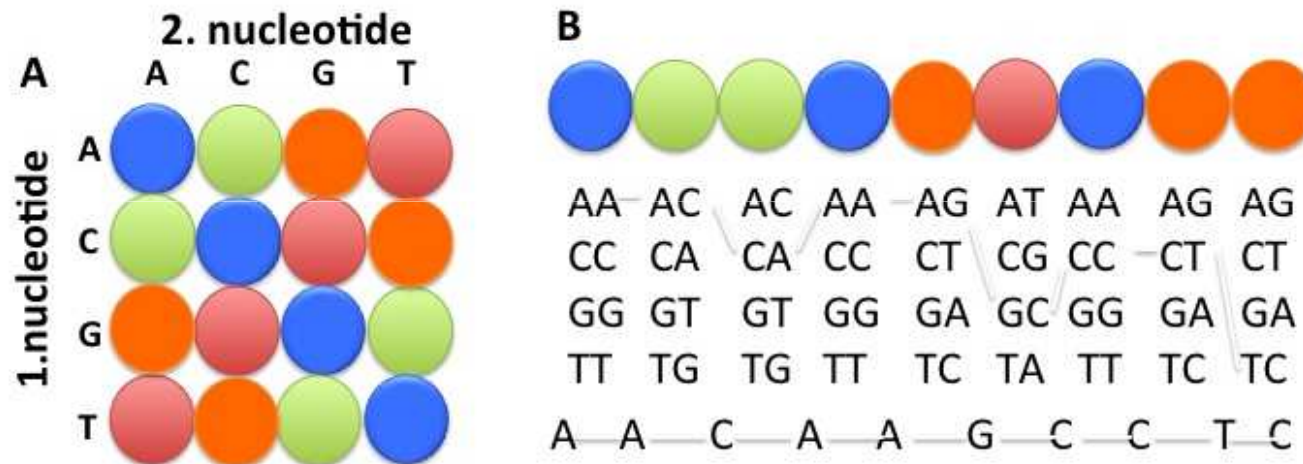- miRs with the highest expression of a newly identified isoform

34.1%
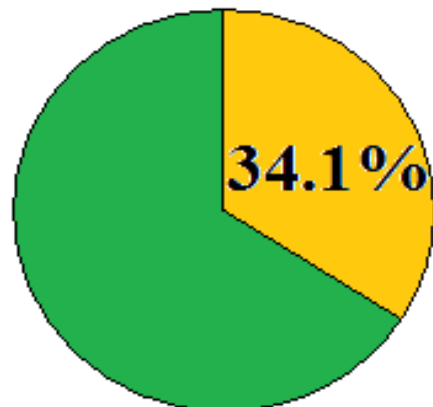
- standard isoform is not expressed in the thyroid
- standard isoform is expressed

Results: miRNA-based diagnostic panel for PTC

	Crossvalidation		Validation set
	PTC-T *vs* PTC-N	**PTC-T *vs* NN**	**PTC-T *vs* PTC-N**
classifier sample size	12	11	12
accuracy	86%	86%	80%
sensitivity	71%	71%	100%
specificity	100%	100%	60%
PPV	100%	100%	71%
NPV	78%	78%	100%

Results: Single Nucleotide Polymorphisms (SNPs) in miRs

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microRNA	no of cases	mean RPM	max RPM
hsa-miR-xxx1_T_C_20	12	17.15	57.0
hsa-miR-xxx2_C_T_15	11	10.21	27.0
hsa-miR-xxx3_G_A_14	11	9.31	22.2
hsa-miR-xxx4_A_G_3	9	7.98	48.7
hsa-miR-xxx5_G_A_17	9	6.44	32.7
hsa-miR-xxx6_T_C_16	9	5.37	17.5
hsa-miR-xxx7_A_G_16	8	6.26	37.1
hsa-miR-xxx8_A_G_18	8	6.21	32.9
hsa-miR-xxx9_C_T_15	8	5.92	32.3
hsa-miR-xxx10_T_C_8	8	5.73	38.0
hsa-miR-xxx11_C_T_6	8	4.97	17.1
hsa-miR-xxx12_A_G_10	8	4.84	23.5
hsa-miR-xxx13_T_C_12	8	4.74	26.4
hsa-miR-xxx14_A_G_5	7	7.64	57.5
hsa-miR-xxx15_A_G_6	7	6.67	46.5
hsa-miR-xxx16_T_C_4	7	5.66	23.6
hsa-miR-xxx17_A_G_4	7	4.03	10.1
hsa-miR-xxx18_T_C_7	5	4.21	19.0
hsa-miR-xxx19_A_G_11	5	3.96	24.4
hsa-miR-xxx20_T_C_17	5	3.69	21.3

CAPACITIES

Results: Single Nucleotide Polymorphisms (SNPs) in miRs

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hsa-miR-xxx1_T_C_20	12	17.15	57.0
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hsa-miR-xxx3_G_A_14	11	9.31	22.2
hsa-miR-xxx4_A_G_3	9	7.98	48.7
hsa-miR-xxx5_G_A_17	9	6.44	32.7
hsa-miR-xxx6_T_C_16	9	5.37	17.5
hsa-miR-xxx7_A_G_16	8	6.26	37.1
hsa-miR-xxx8_A_G_18	8	6.21	32.9
hsa-miR-xxx9_C_T_15	8	5.92	32.3
hsa-miR-xxx10_T_C_8	8	5.73	38.0
hsa-miR-xxx11_C_T_6	8	4.97	17.1
hsa-miR-xxx12_A_G_10	8	4.84	23.5
hsa-miR-xxx13_T_C_12	8	4.74	26.4
hsa-miR-xxx14_A_G_5	7	7.64	57.5
hsa-miR-xxx15_A_G_6	7	6.67	46.5
hsa-miR-xxx16_T_C_4	7	5.66	23.6
hsa-miR-xxx17_A_G_4	7	4.03	10.1
hsa-miR-xxx18_T_C_7	5	4.21	19.0
hsa-miR-xxx19_A_G_11	5	3.96	24.4
hsa-miR-xxx20_T_C_17	5	3.69	21.3

Confirmation:

Association study
- 3000 PTC patients
- 3000 controls

Disease allele frequency	POWER (%)		
	OR=1.2	OR=1.4	OR=1.6
0.1	45	100	100
0.2	81	100	100
0.3	92	100	100
0.4	96	100	100
0.5	96	100	100
0.6	95	100	100
0.7	90	100	100
0.8	75	100	100
0.9	37	97	100

Results: novel microRNA genes

Legend:
- No of total reads
- No of reads after adapter removal
- No of mapped reads

Results: novel microRNA genes

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Results: novel microRNA genes
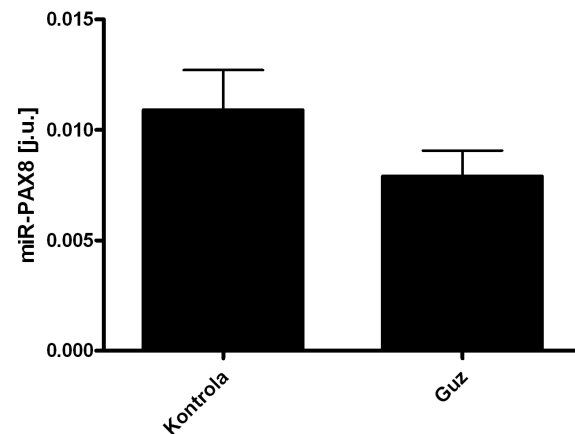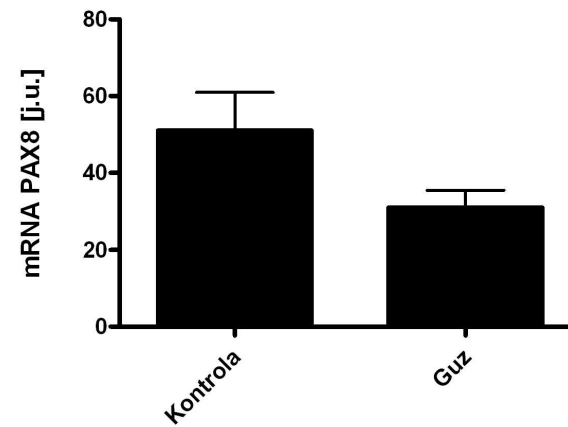
Results: novel microRNA genes

Acknowledgments

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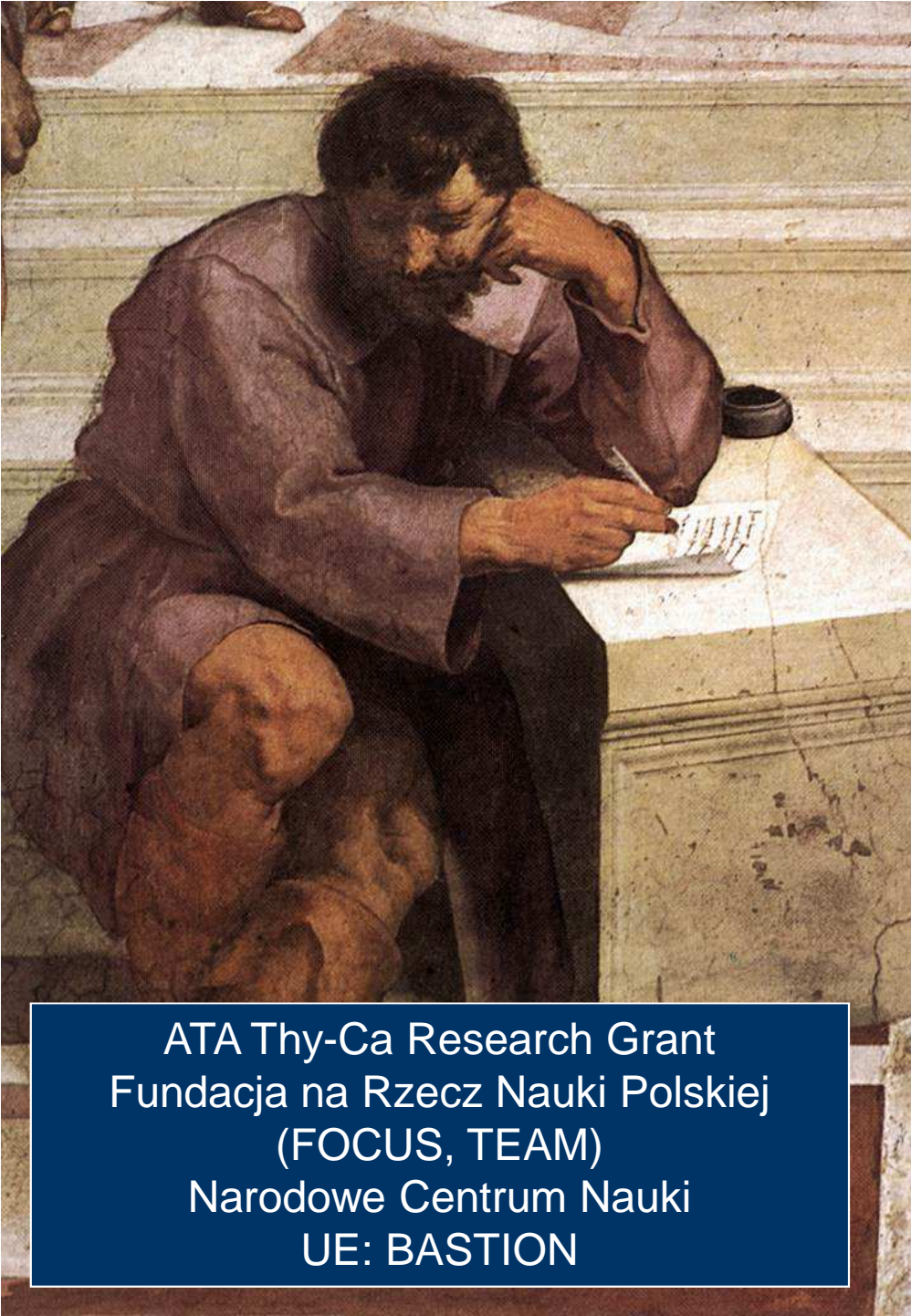 Barbara Górnicka

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  Monika Maciąg
  Anna Wójcicka

PhD students
  Monika Kolanowska
  Kinga Dymecka
  Marta Świech
  Agnieszka Czajka
  Anna Kubiak
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Students
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  Wojciech Gierlikowski