epigene

Sk Resident

GLAD-PCR assay of R(5mC)GY sites in aberrantly methylated regulation regions of tumor-suppression genes in lung cancer

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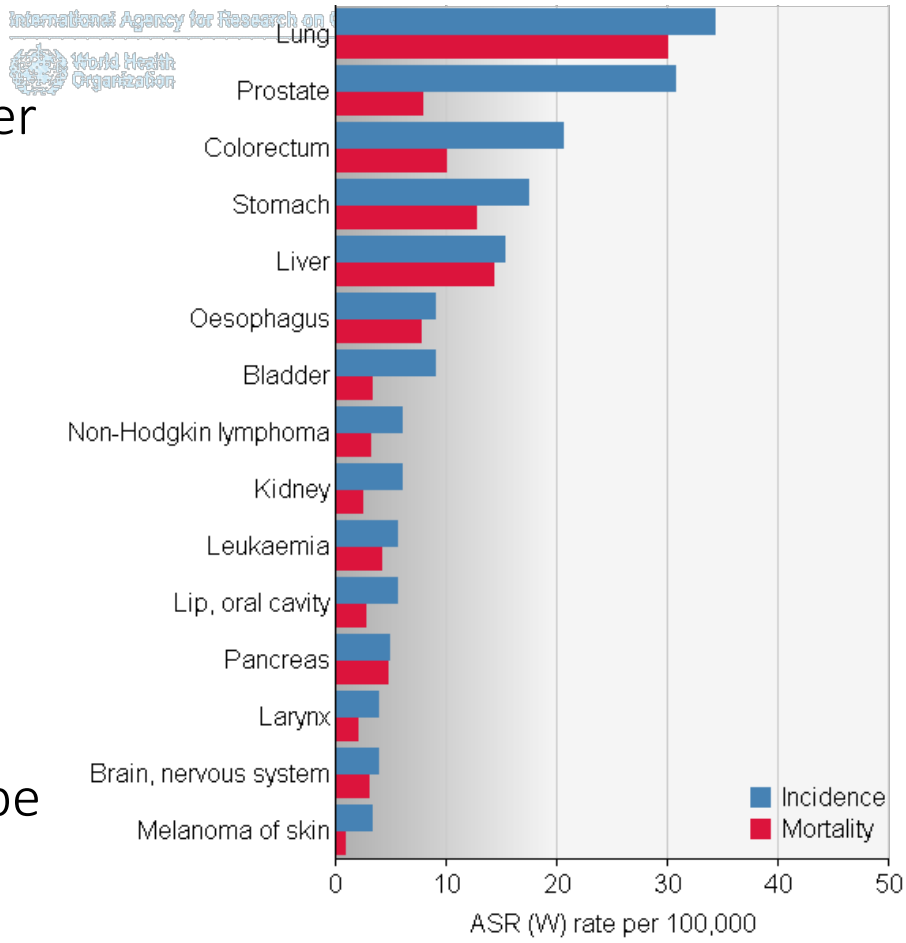
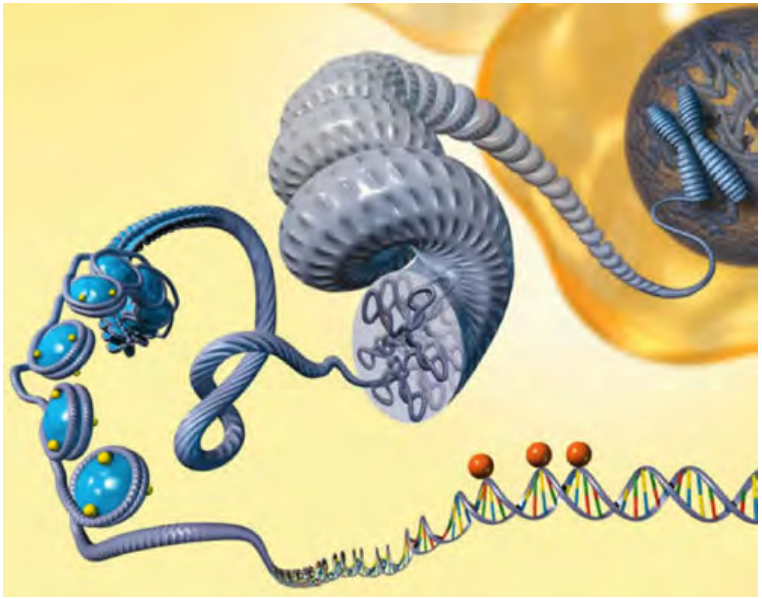
Early cancer detection

- Nowadays about 50% of all patients are diagnosed with cancer at stage III or IV.
- It's difficult to reach a positive result in the cancer treatment at these stages.
- At the same time early cancer detection significantly improves a treatment of disease and the patient cure
- Lung cancer - the most deadly type
- Incidence/Mortality – 1,8M/1,6M



DNA methylation in cancer

- DNA hypermethylation results in genes silencing
- Such methylation of tumor-suppressor genes (TSGs) shown for the most types of cancer[1]
- Occurs at the early stages[1] when there are still no clinical indications of disease
- Destructed methylated DNA gets into the blood stream where it can be detected
- Different types of cancer – different patterns of methylation[2].



Determination of TSGs methylation status allows to distinguish cancer types and detect it at the most early stages.

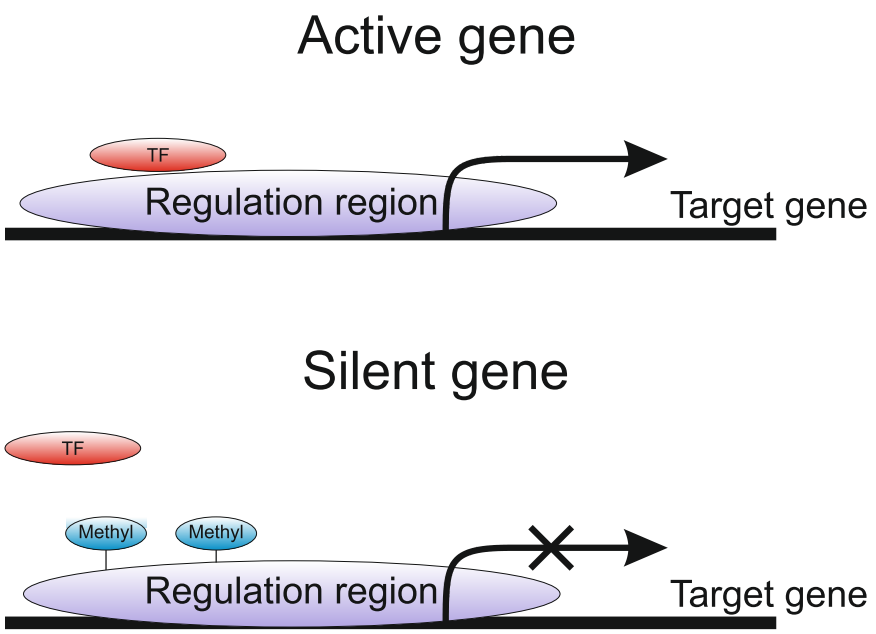
Thus epigenetic diagnostics seems to be very perspective for early cancer detection

[1] Jeronimo C., Henrique R. Epigenetic biomarkers in urological tumors: A systematic review // Cancer Lett. 2014; 342(2):264-274

[2] Xueguang Sun, Jill E. Petrisko, Lam K. Nguyen, Marc Van Eden & Xi-Yu Jia Epigenetic biomarker discovery, validation for diagnosis, and therapeutic intervention for Hepatocellular Carcinoma

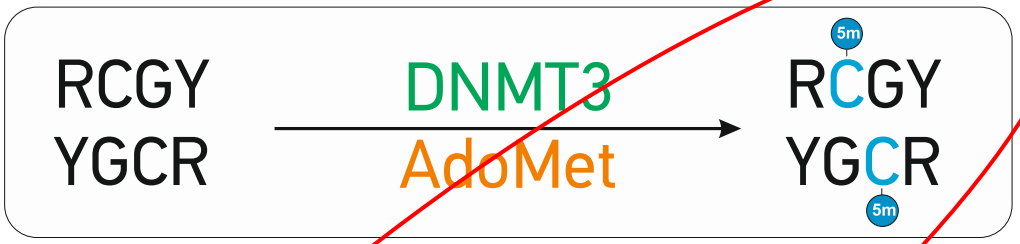
DNA methylation

- DNA methylation in mammalians genomes is mostly DNA methylation of CG dinucleotides with formation of 5-methylcytosine (5mC) in both DNA strands.
- Mammalian DNA-methyltransferases DNMT1, DNMT3a and DNMT3b catalyze a reaction of DNA methylation.
  - DNMT1 maintains DNA methylation pattern *in vivo* modifying a new strand after replication.
  - DNMT3a and DNMT3b are responsible for DNA methylation *de novo*.
    This modification in regulation region (promotor and first exon) of gene results in this gene silencing.

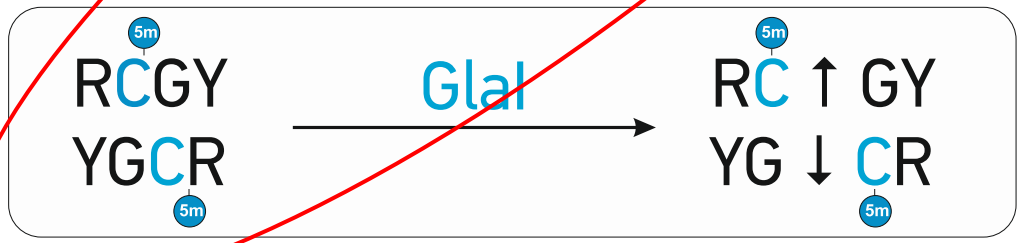


Substrate specificity of DNMT3a, DNMT3b and GlaI

Study of DNMT3a and DNMT3b substrate specificity has shown that both enzymes methylate CG-dinucleotide mostly in DNA sequence RCGY.



One of new enzymes GlaI recognizes and cleaves site R(5mC)GY, which is product of *de novo* methylation.

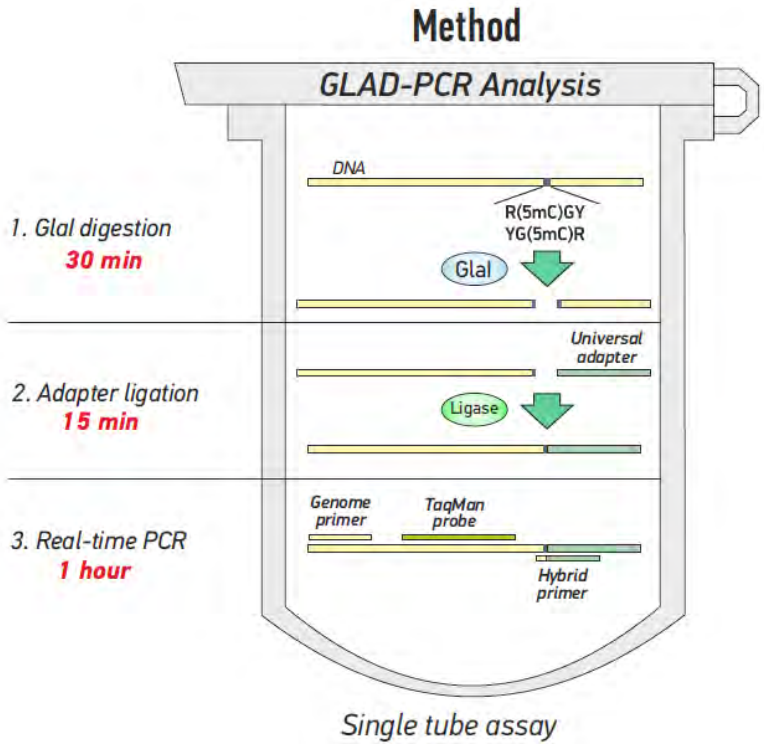


GLAD-PCR assay - effective alternative of bisulfite methods

GLAD-PCR Assay is the novel methylation detection method developed by SibEnzyme

- Simple
- Quick
- Sensitive
- Requires only standard real time PCR machine
- Cheap

Method works with any source of DNA like sputum, urine, smear, tissue samp but most versatile and convenient is blood

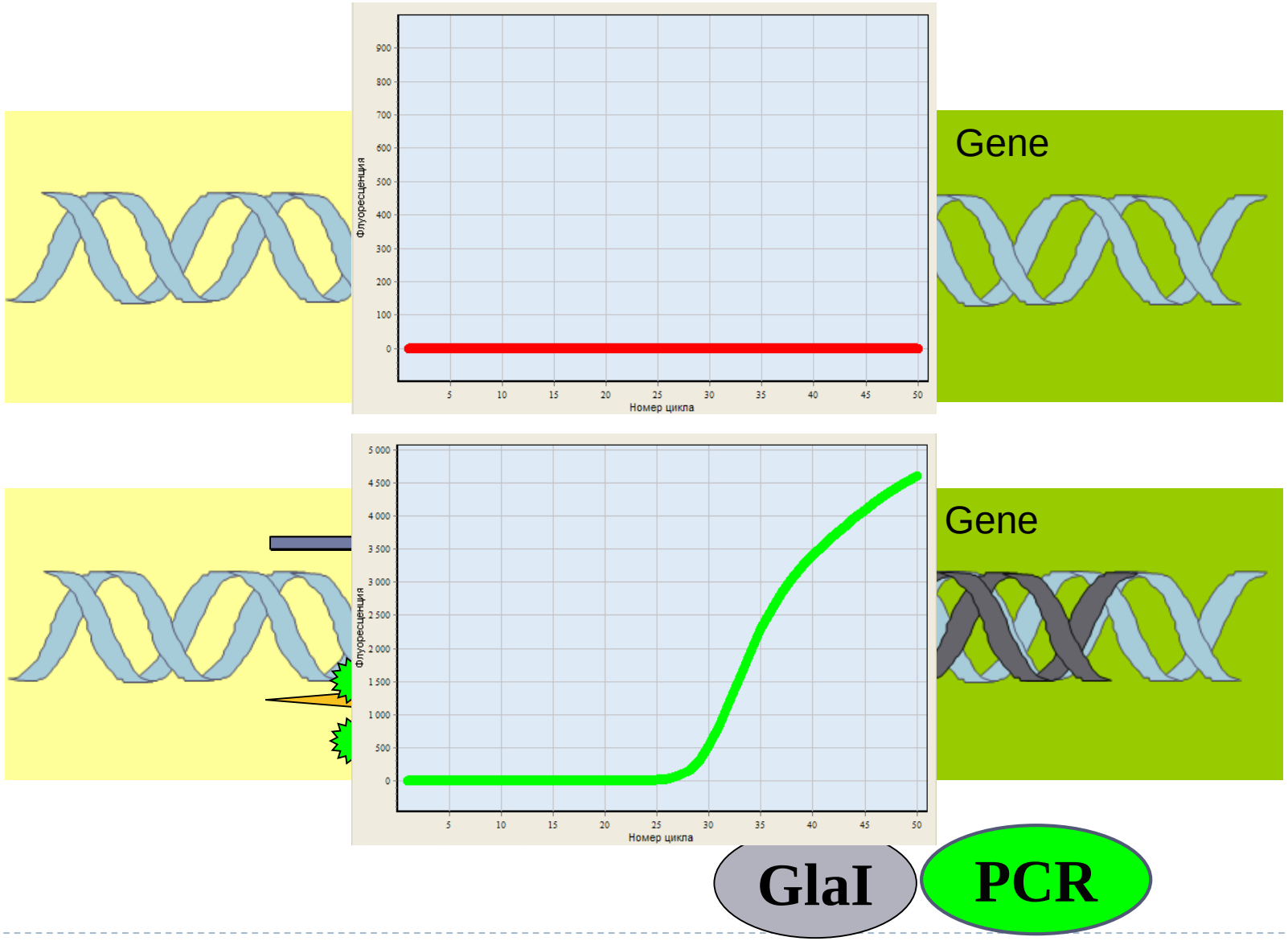


Single tube assay

For details see http://md.sibenzyme.com/2GLAD-PCR%20assay.pdf

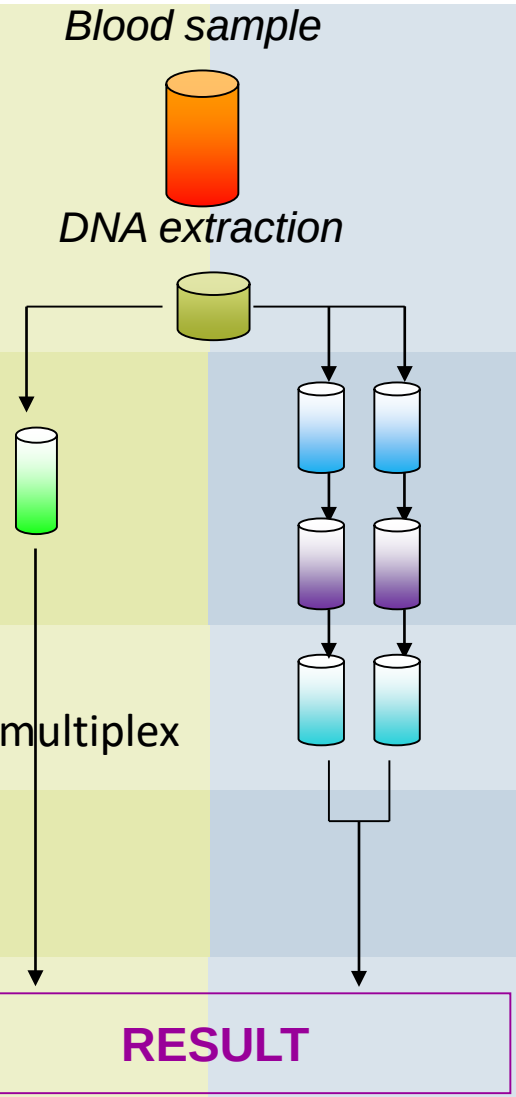
Method demonstration: http://sibenzyme.com/info7820.php
or our site http://www.epigene.ru/glad-pcr-assay/

GLAD-PCR-assay



GLAD-PCR-assay Vs Bisulfite conversion

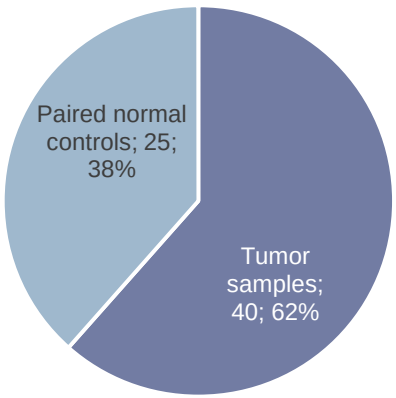
	GLAD-PCR-assay	Bisulfite conversion
Sensitivity	> 20 pg of DNA	> 500 pg of DNA
Simplicity	3 stages In 1 tube	> 5 stages
Usage	RCGY site of interest within genome, up to 4 in multiplex	DNA region excessive
Duration	4-6 hours	> 12 hours
Mistakes	Less than 1%	15-20%

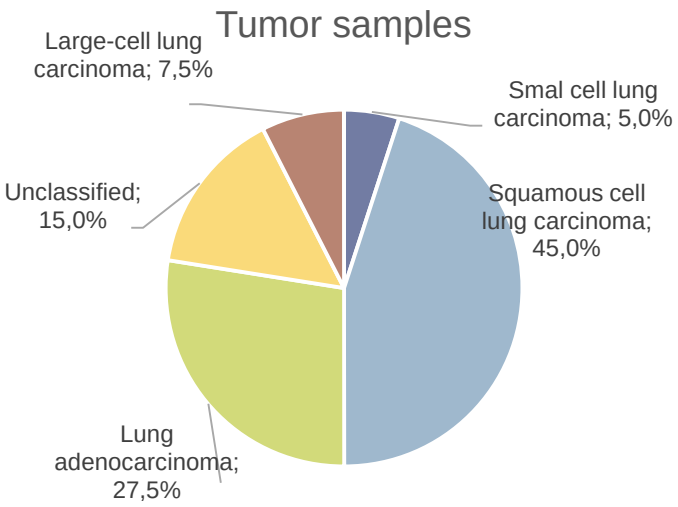


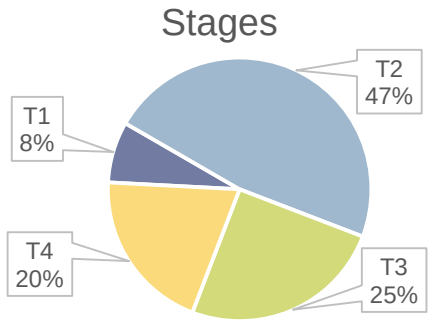
GLAD-PCR assay of RCGY sites in regulation regions of TSGs in lung cancer

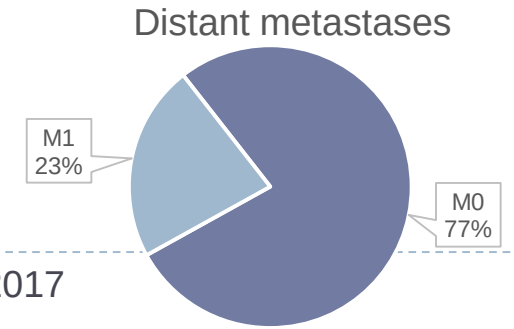
In this work we applied GLAD-PCR assay for identification of the methylated RCGY sites in the regulatory regions of some downregulated TSGs associated with lung cancer.

The study group included 40 lung cancer patients (36 male and 4 female). 31 patient had no distant metastases. A total of 65 freshfrozen surgical resection samples were studied, including 40 tumor samples and 25 paired normal controls. The samples were collected at the Seversk Biophysical Research Centre (Seversk, Russia)









Studied RCGY sites

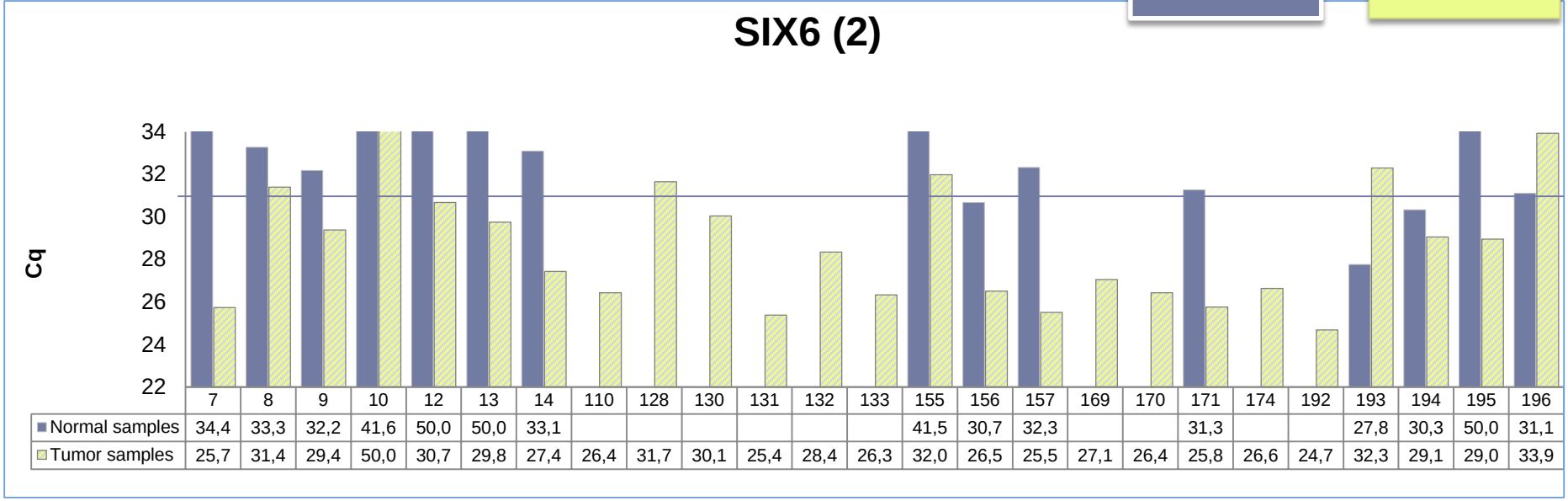
From the list of 34 candidate TSGs on the first stage we selected 11 for further testing: LHX6, MYF6, NID2, OTX1, RASSF1, RARB, RXRG, RYR2, SIX6, SKOR1 and TERT. At this moment 13 RCGY sites from regulation regions of 11 TSGs were studied.

Gene (region)	TARGET SITE	Site location[1]	Gene (region)	TARGET SITE	Site location[1]
LHX6 (1)	GCGC	chr9: 122219596–122219599	RXRG	GCGC	chr1: 165445276–165445279
LHX6 (2)	GCGT	chr9: 122219886–122219889	RYR2	GCGC	chr1: 237043053–237043056
MYF6	GCGC	chr12: 80708733–80708736	SIX6 (1)	GCGC	chr14: 60509991–60509994
NID2	GCGC	chr14: 52069060–52069063	SIX6 (2)	GCGT	chr14: 60509827–60509830
OTX1	GCGC	chr2: 63057659–63057662	SKOR1	ACGC	chr15: 67821744–67821747
RASSF1	GCGC	chr3: 50340715–50340718	TERT	GCGT	chr5: 1295645–1295648
RARB	ACGC	chr3: 25428374–25428377			

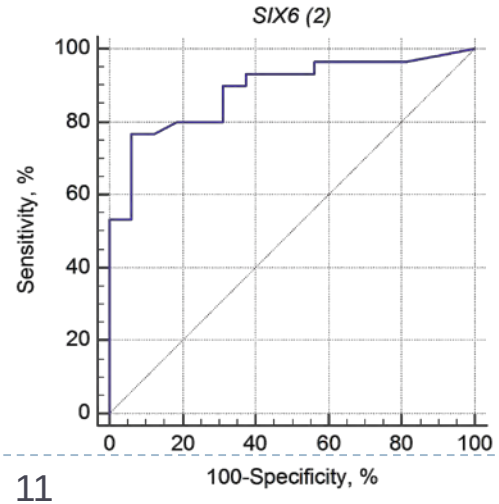
[1] Site location are given in accordance with the recent human genome assembly GRCh38/hg38

Top-five studied RCGY sites

Normal Tumor



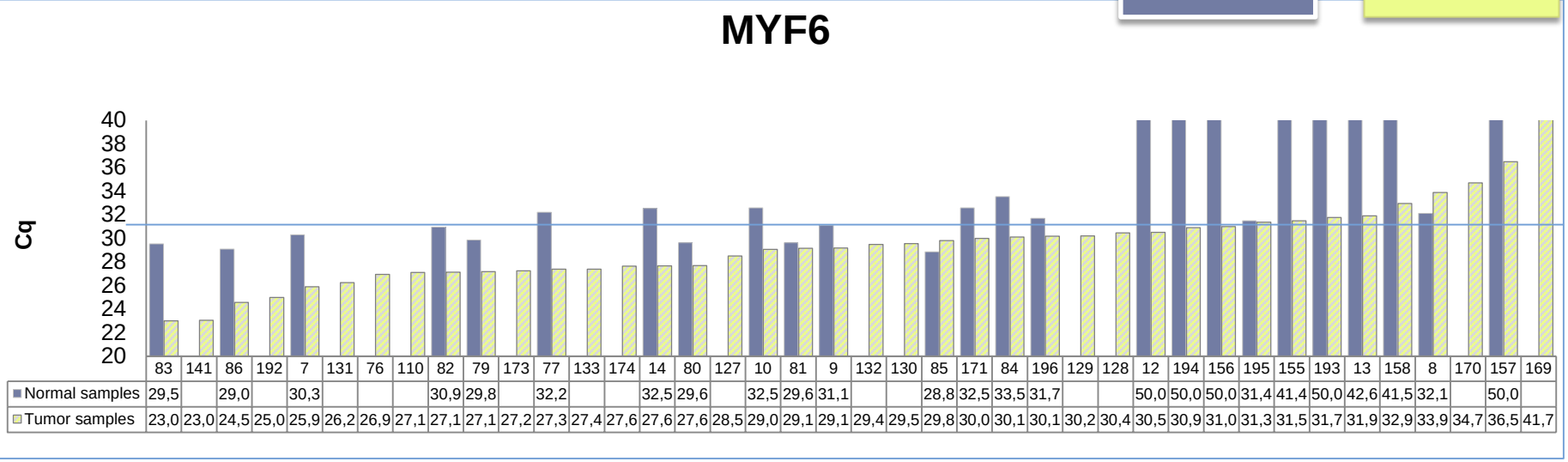
	7	8	9	10	12	13	14	110	128	130	131	132	133	155	156	157	169	170	171	174	192	193	194	195	196
Normal samples	34,4	33,3	32,2	41,6	50,0	50,0	33,1							41,5	30,7	32,3			31,3			27,8	30,3	50,0	31,1
Tumor samples	25,7	31,4	29,4	50,0	30,7	29,8	27,4	26,4	31,7	30,1	25,4	28,4	26,3	32,0	26,5	25,5	27,1	26,4	25,8	26,6	24,7	32,3	29,1	29,0	33,9



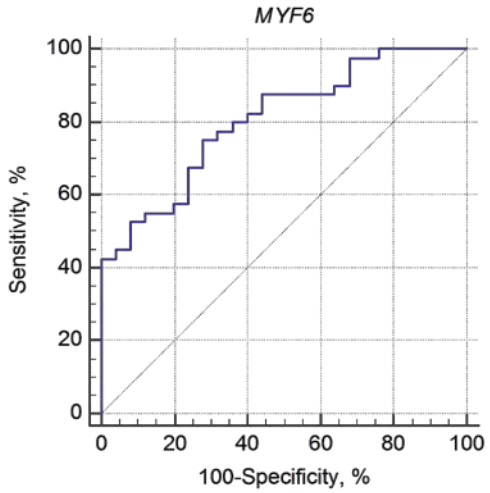
Gene (region)	Number of detected LC samples /total number of LC samples	Sensitivity, %	Number of negative controls /total number of normal tissue controls	Specificity, %	AUC (standard error)	95% CI
SIX6 (2)	23/30	76.7	15/16	93.8	0.888 (0.049)	0.760–0.962

Top-five studied RCGY sites

Normal Tumor



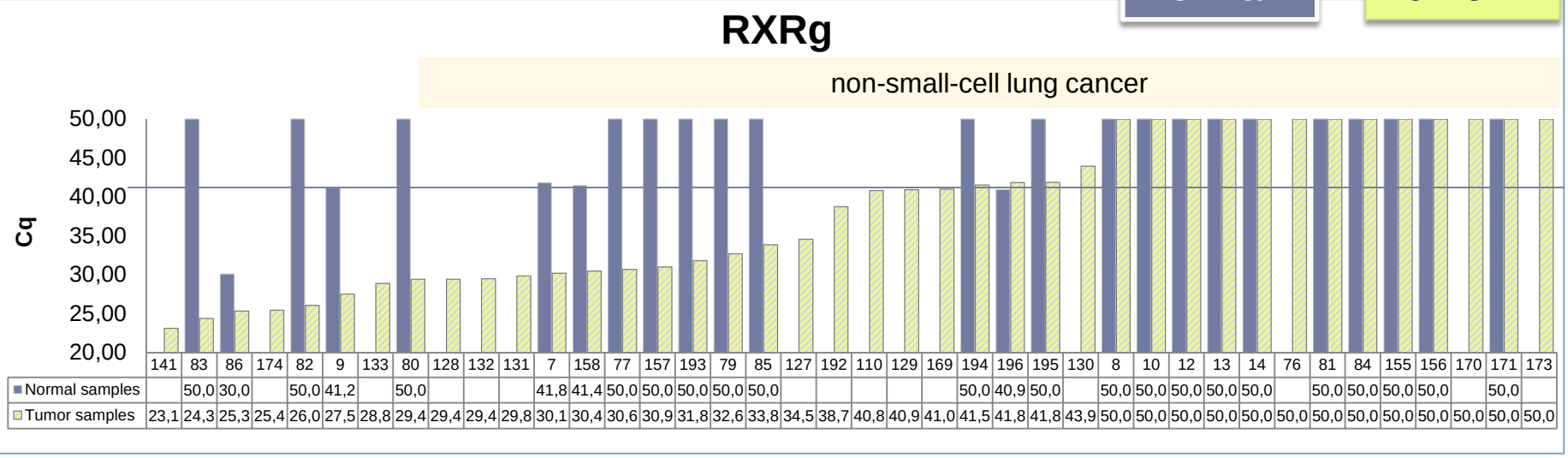
	83	141	86	192	7	131	76	110	82	79	173	77	133	174	14	80	127	10	81	9	132	130	85	171	84	196	129	128	12	194	156	195	155	193	13	158	8	170	157	169
Normal samples	29,5		29,0		30,3				30,9	29,8		32,2			32,5	29,6		32,5	29,6	31,1			28,8	32,5	33,5	31,7			50,0	50,0	50,0	31,4	41,4	50,0	42,6	41,5	32,1		50,0	
Tumor samples	23,0	23,0	24,5	25,0	25,9	26,2	26,9	27,1	27,1	27,1	27,2	27,3	27,4	27,6	27,6	27,6	28,5	29,0	29,1	29,1	29,4	29,5	29,8	30,0	30,1	30,1	30,2	30,4	30,5	30,9	31,0	31,3	31,5	31,7	31,9	32,9	33,9	34,7	36,5	41,7



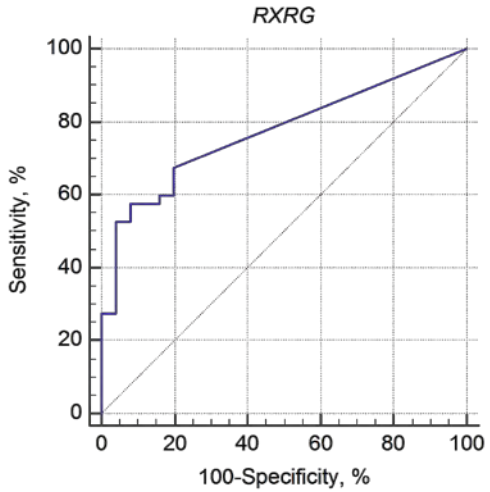
Gene (region)	Number of detected LC samples /total number of LC samples	Sensitivity, %	Number of negative controls /total number of normal tissue controls	Specificity, %	AUC (standard error)	95% CI
MYF6	30/40	75.0	18/25	72.0	0.805 (0.053)	0.688–0.893

Top-five studied RCGY sites

Normal | Tumor



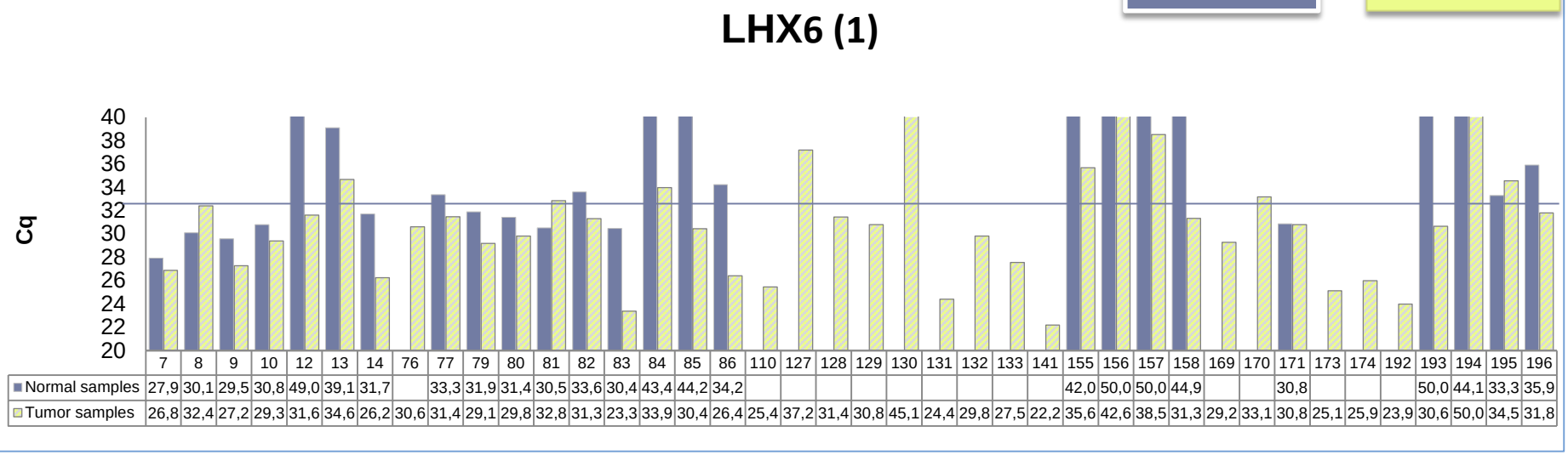
	141	83	86	174	82	9	133	80	128	132	131	7	158	77	157	193	79	85	127	192	110	129	169	194	196	195	130	8	10	12	13	14	76	81	84	155	156	170	171	173
Normal samples		50,0	30,0		50,0	41,2		50,0				41,8	41,4	50,0	50,0	50,0	50,0	50,0						50,0	40,9	50,0		50,0	50,0	50,0	50,0	50,0		50,0	50,0	50,0	50,0		50,0	
Tumor samples	23,1	24,3	25,3	25,4	26,0	27,5	28,8	29,4	29,4	29,4	29,8	30,1	30,4	30,6	30,9	31,8	32,6	33,8	34,5	38,7	40,8	40,9	41,0	41,5	41,8	41,8	43,9	50,0	50,0	50,0	50,0	50,0	50,0	50,0	50,0	50,0	50,0	50,0	50,0	50,0



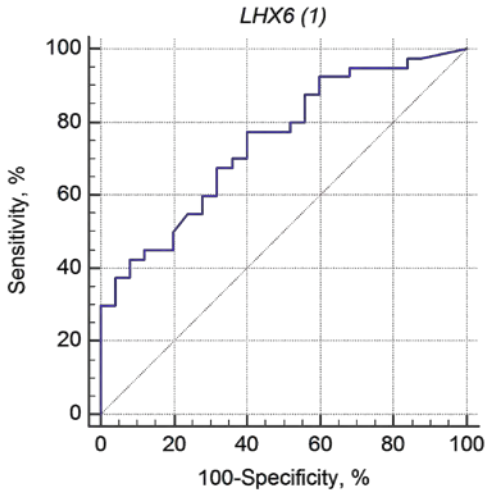
Gene (region)	Number of detected LC samples /total number of LC samples	Sensitivity, %	Number of negative controls /total number of normal tissue controls	Specificity, %	AUC (standard error)	95% CI
RXRg	23/40	57.5	23/25	92.2	0.772 (0.051)	0.651–0.867

Top-five studied RCGY sites

Normal | Tumor



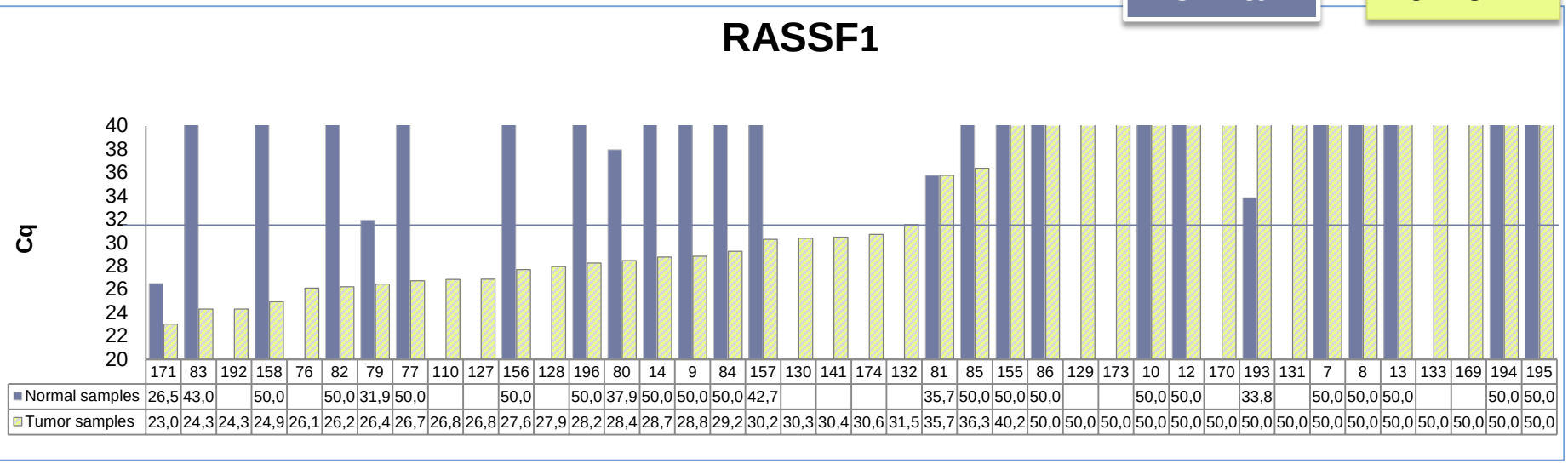
	7	8	9	10	12	13	14	76	77	79	80	81	82	83	84	85	86	110	127	128	129	130	131	132	133	141	155	156	157	158	169	170	171	173	174	192	193	194	195	196
Normal samples	27,9	30,1	29,5	30,8	49,0	39,1	31,7		33,3	31,9	31,4	30,5	33,6	30,4	43,4	44,2	34,2										42,0	50,0	50,0	44,9			30,8				50,0	44,1	33,3	35,9
Tumor samples	26,8	32,4	27,2	29,3	31,6	34,6	26,2	30,6	31,4	29,1	29,8	32,8	31,3	23,3	33,9	30,4	26,4	25,4	37,2	31,4	30,8	45,1	24,4	29,8	27,5	22,2	35,6	42,6	38,5	31,3	29,2	33,1	30,8	25,1	25,9	23,9	30,6	50,0	34,5	31,8



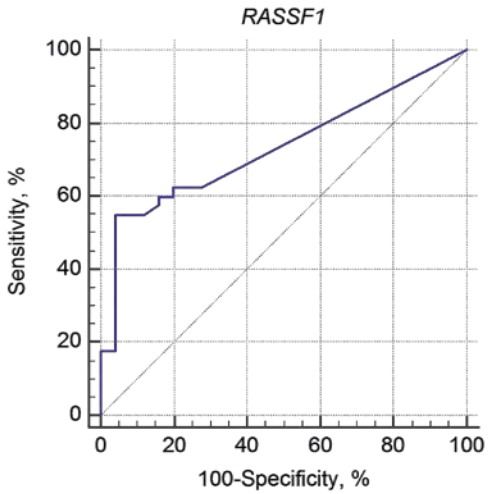
Gene (region)	Number of detected LC samples /total number of LC samples	Sensitivity, %	Number of negative controls /total number of normal tissue controls	Specificity, %	AUC (standard error)	95% CI
LHX6 (1)	31/40	77.5	15/25	60.0	0.746 (0.061)	0.622–0.845

Top-five studied RCGY sites

Normal | Tumor



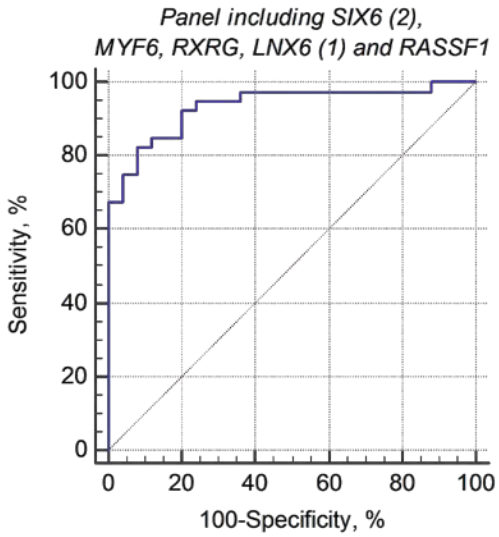
	171	83	192	158	76	82	79	77	110	127	156	128	196	80	14	9	84	157	130	141	174	132	81	85	155	86	129	173	10	12	170	193	131	7	8	13	133	169	194	195
Normal samples	26,5	43,0		50,0		50,0	31,9	50,0			50,0		50,0	37,9	50,0	50,0	50,0	42,7					35,7	50,0	50,0	50,0			50,0	50,0		33,8		50,0	50,0	50,0			50,0	50,0
Tumor samples	23,0	24,3	24,3	24,9	26,1	26,2	26,4	26,7	26,8	26,8	27,6	27,9	28,2	28,4	28,7	28,8	29,2	30,2	30,3	30,4	30,6	31,5	35,7	36,3	40,2	50,0	50,0	50,0	50,0	50,0	50,0	50,0	50,0	50,0	50,0	50,0	50,0	50,0	50,0	50,0



Gene (region)	Number of detected LC samples /total number of LC samples	Sensitivity, %	Number of negative controls /total number of normal tissue controls	Specificity, %	AUC (standard error)	95% CI
RASSF1	22/40	55.0	24/25	96.0	0.733 (0.056)	0.608–0.835

Results

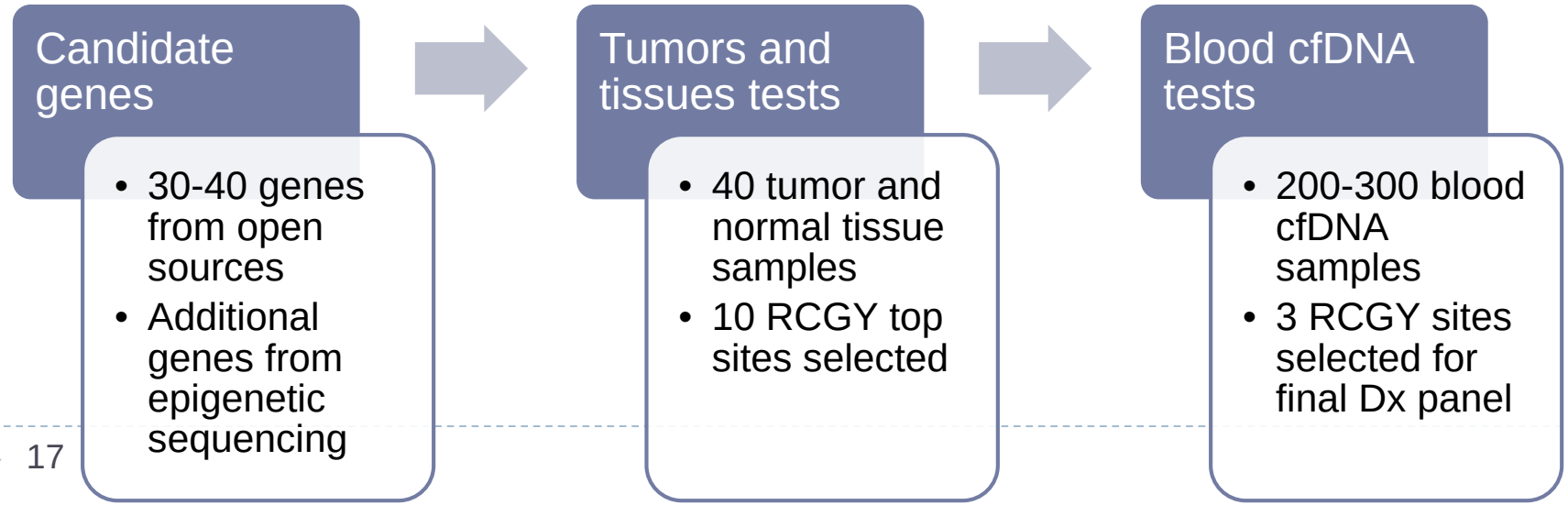
- Based on our results five sites in the regulatory regions of SIX6, MYF6, RXRG, LHX6 and RASSF1 genes seem to be perspective for diagnostic use. **The panel** including these five top sites results in AUC = 0,936
- Thus, these sites may be considered as preliminary candidate sites in GLAD PCR assay for lung cancer diagnostics



Gene (region)	Number of detected LC samples /total number of LC samples	Sensitivity, %	Number of negative controls /total number of normal tissue controls	Specificity, %	AUC (standard error)	95% CI
SIX6 (2)	23/30	76.7	15/16	93.8	0.888 (0.049)	0.760–0.962
MYF6	30/40	75.0	18/25	72.0	0.805 (0.053)	0.688–0.893
RXRG	23/40	57.5	23/25	92.2	0.772 (0.051)	0.651–0.867
LHX6 (1)	31/40	77.5	15/25	60.0	0.746 (0.061)	0.622–0.845
RASSF1	22/40	55.0	24/25	96.0	0.733 (0.056)	0.608–0.835
Panel of five top genes	**33/40**	**82.5**	**23/25**	**92.0**	**0.936 (0.030)**	**0.847–0.982**

Perspectives

- We are planning to widen the list of candidate TSGs which methylation correlates with formation of lung cancer.
- We will continue a study of methylation of RCGY sites in regulation regions of other genes to obtain the better panel. Such panel will include TSGs which are methylated in the most of tumor DNA samples and nonmethylated (or minimally methylated) in controls.
- At the next step the obtained panel of RCGY sites will be tested in GLAD-PCR assay of the blood cell-free DNA samples in order to develop the simple and cheap PCR test for lung cancer detection similar to the previously developed colorectal cancer detection test which was presented at CGS-2015.



Colorectal cancer detection kit

- GLAD-PCR assay was recently used for development of epigenetic test for colorectal cancer screening and early detection
- Colorectal cancer test is ready for usage and it is on its way to Russia market

Government Support

This work was supported by **Skolkovo Foundation** under agreement № Г102/16 from 2016.12.06

«Development of epigenetic tests for lung, breast and stomach cancer detection»

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Thank You!