DNA methylation in HNSCC samples

A story of needles and haystacks

Daria Meyer

TBI Winterseminar
10.02.2025

Background: Head and Neck Squamous Cell Carcinoma (HNSCC)

HNSCC specific DNA methylation (5mC) exist

Research Article

Cancer Prevention Research

New DNA Methylation Markers and Global DNA Hypomethylation Are Associated with Oral Cancer Development

Jean-Philippe Foy[1,2,3], Curtis R. Pickering[4], Vassiliki A. Papadimitrakopoulou[5], Jaroslav Jelinek[6], Steven H. Lin[7], William N. William Jr[5], Mitchell J. Frederick[4], Jing Wang[8], Wenhua Lang[5], Lei Feng[9], Li Zhang[8], Edward S. Kim[10], You H. Fan[5], Waun K. Hong[11], Adel K. El-Naggar[12], J. Jack Lee[9], Jeffrey N. Myers[4], Jean-Pierre Issa[6], Scott M. Lippman[13], Li Mao[14], and Pierre Saintigny[1,2,15,16]

International Journal of *Molecular Sciences*

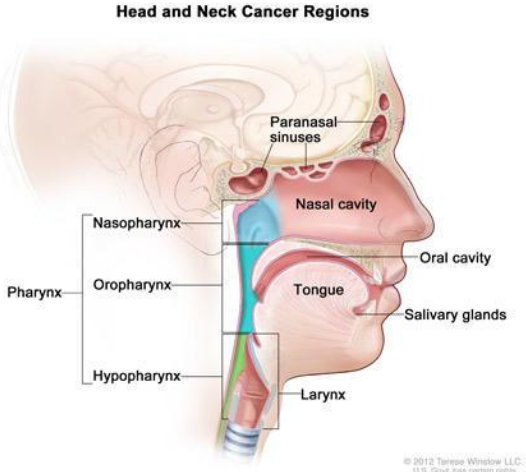
MDPI

Article

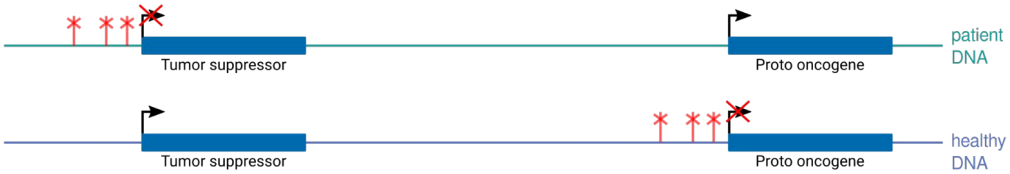
Pre-Operative Evaluation of DNA Methylation Profile in Oral Squamous Cell Carcinoma Can Predict Tumor Aggressive Potential

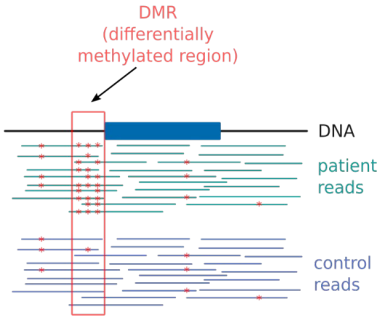
Davide B. Gissi [1,†], Viscardo P. Fabbri [2,†], Andrea Gabusi [1], Jacopo Lenzi [3], Luca Morandi [4,*], Sofia Melotti [2], Sofia Asioli [2], Achille Tarsitano [5], Tiziana Balbi [6], Claudio Marchetti [5] and Lucio Montebugnoli [1]

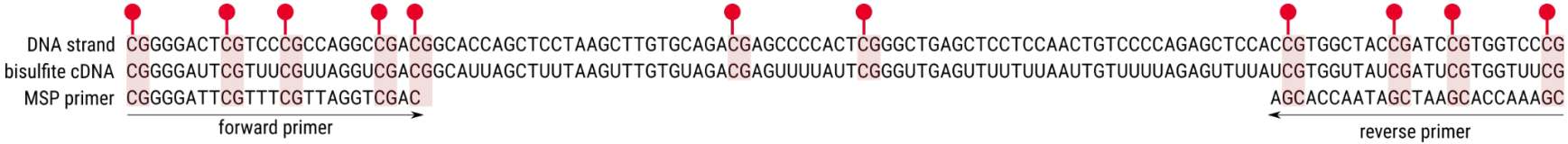
HNSCC = Nose, Mouth, Throat



Background: Methylation-Specific PCR (MSP)



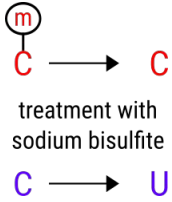




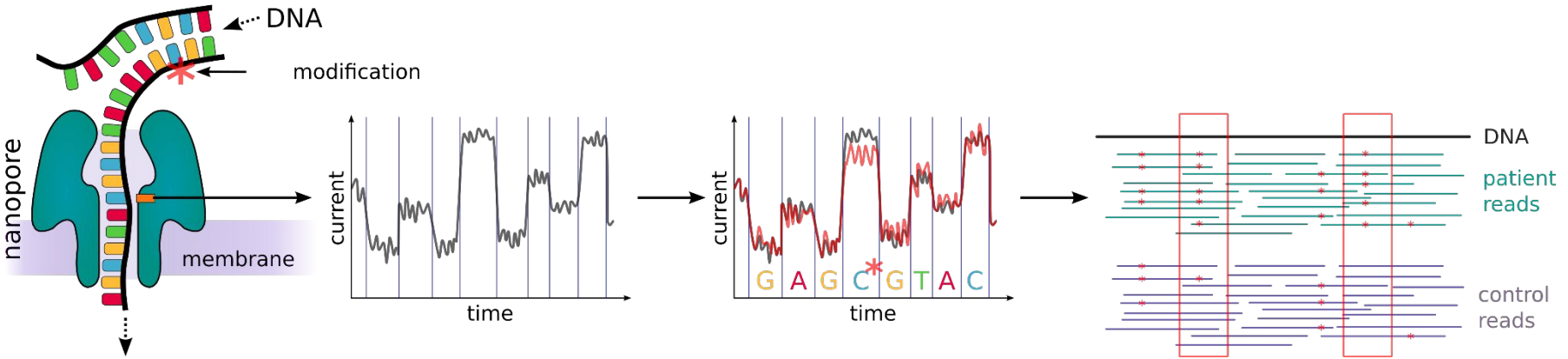
MSP needs regions which show methylations in tumor samples but not in control samples

➔ **completely** unmethylated in controls (ideally)

➔ further primer design constraints (primer length, distance, nucleotide composition,...)

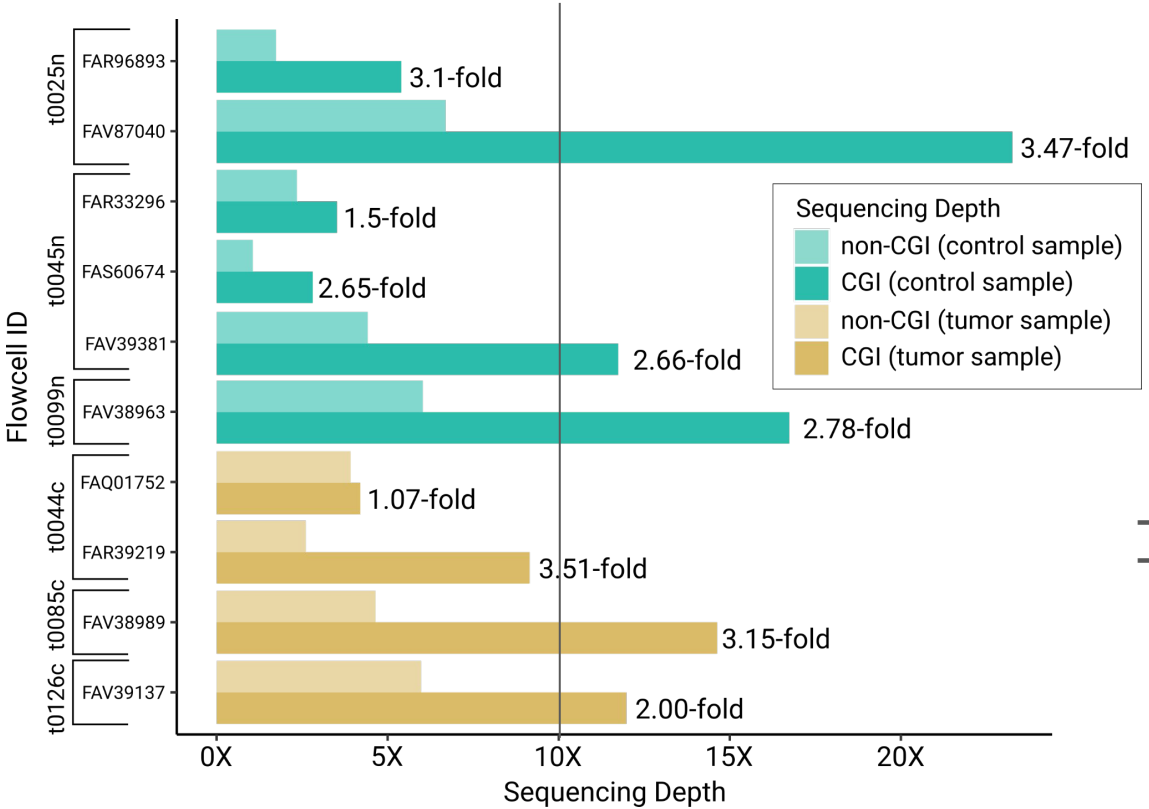


Background: Whole genome nanopore sequencing (enriched for CpG islands)



	Sample	Sex	Age	HPV	Tumor	Surgery
Control	T-0025-N	m	59 y	neg	0 %	UPPP
	T-0045-N	m	22 y	neg	0 %	UPPP
	T-0099-N	m	57 y	neg	0 %	UPPP
Tumor	T-0044-C	m	53 y	pos	80 %	HNSCC
	T-0085-C	m	74 y	neg	80 %	HNSCC
	T-0126-C	m	59 y	neg	70 %	HNSCC

Enrichment on CpG islands (CGIs) by using Adaptive Sampling

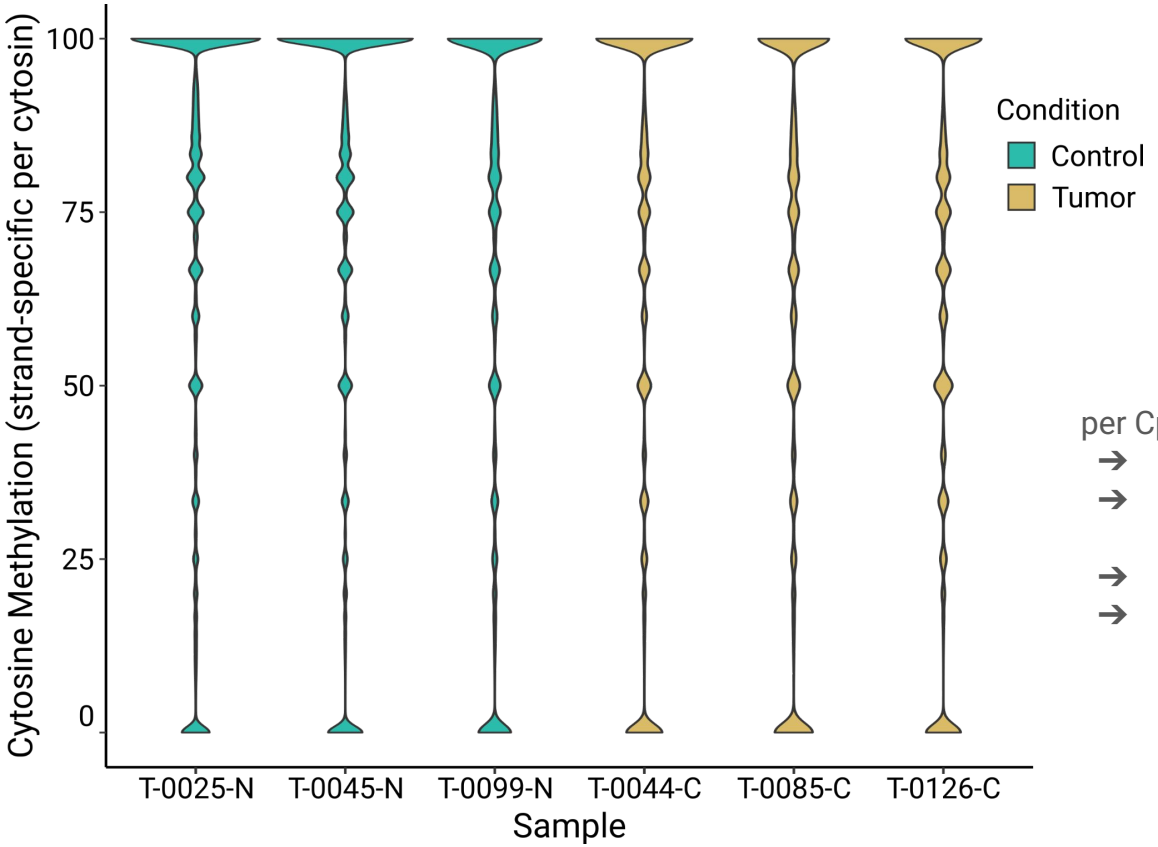


	Sample	HSA depth	CGI depth
Control	t0025n	8.44X	28.66X
	t0045n	7.83X	18.07X
	t0099n	6.03X	16.74X
Tumor	t0044c	6.53X	13.35X
	t0085c	4.65X	14.63X
	t0126c	5.98X	11.98X

➔ max. 3.5-fold increased sequencing depth

➔ adaptive sampling resulted in increased sequencing depth on the CpG islands (at least 10X for all samples)

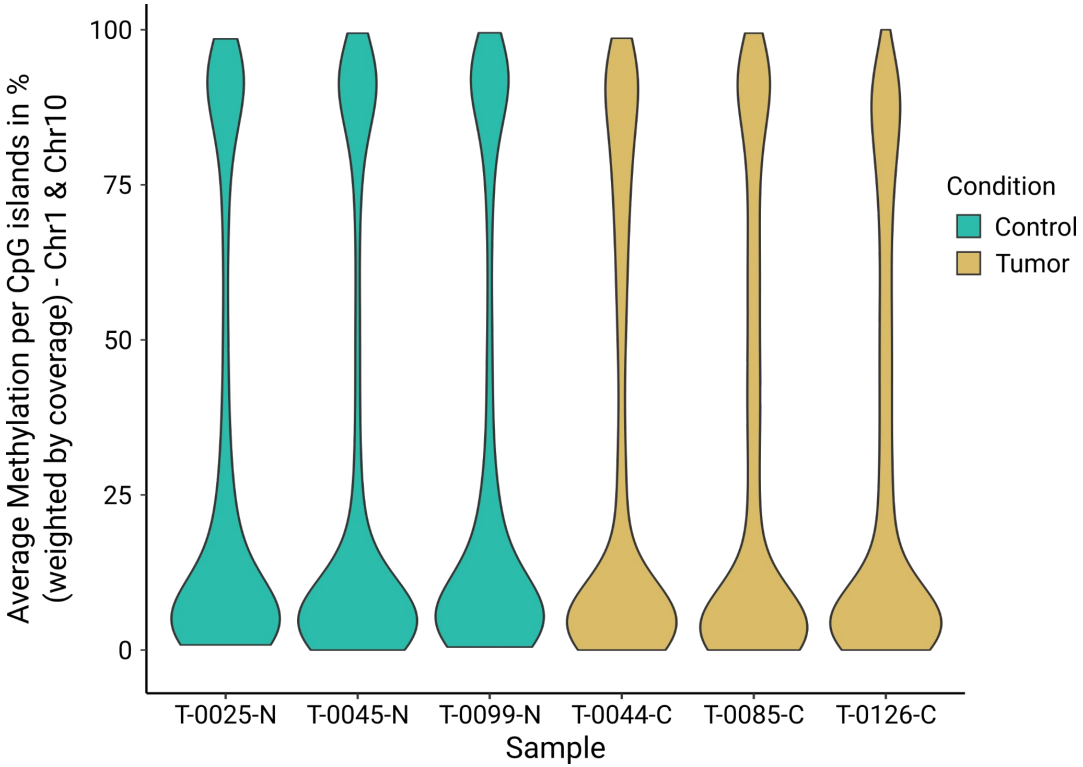
Nanopore sequencing - overall methylation



per CpG methylation information shows:

- ➔ most CpGs are highly methylated
- ➔ probably: artefacts (50%, 75%, 83%,..) based on low sequencing depth
- ➔ mean sequencing depth is only 5X
- ➔ tumor samples CpG methylated slightly shifted towards unmethylated CpGs

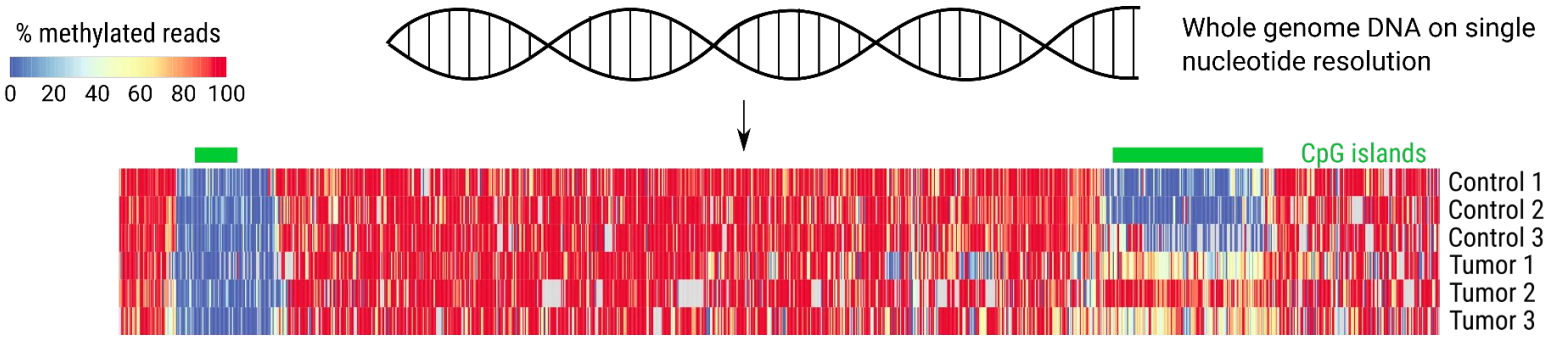
Nanopore sequencing - CpG island methylation



per CpG island methylation information shows:

- ➔ less artefacts and higher sequencing depth (mean sequencing depth = ??X)
- ➔ in contrast to genome-wide cytosine methylation, CGIs are rather unmethylated in both tumor and control samples

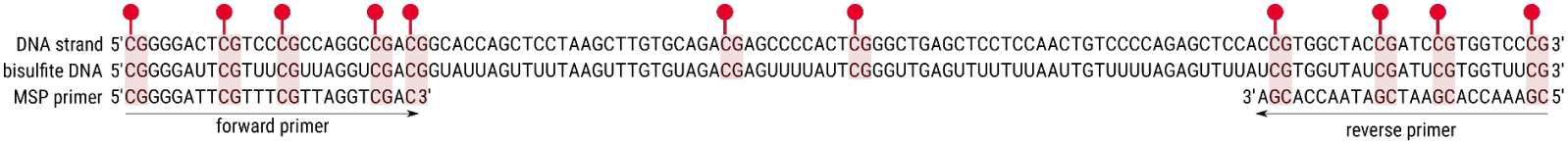
Prediction of MSP biomarker regions with diffONT



1. Filter CpGs with < 10% methylation in control samples
2. Filter CpGs with median(tumor methylation) > maximum(control methylation)
3. Filter Regions with min. 3 differential CpGs within max. 24 nt
4. Score Regions based on coverage and methylation in tumor samples
5. Select regions with two Primers within 400 nt

Scored MSP Primers

chro.	start	end	strand	#CpGs	length	score	GC	#nonCpGs
chr12	95548563	95548585	-	6	22	3995.61	0.91	1
chr12	95548565	95548585	-	5	20	3316.31	0.90	1
chr12	95548585	95548604	-	4	19	2826.14	0.68	2
chr12	95548739	95548760	-	8	21	5448.51	0.95	2
chr12	95548742	95548760	-	7	18	4763.69	0.94	2
				[...]				




chr12:95,546,311-95,551,005
p13.32 p13.2 p12.3 p12.1 p11.22 p11.1 q12 q13.11 q13.13 q13.3 q14.2 q15 q21.1 q21.2 q21.31 q21.32 q22 q23.1 q23.2 q23.3 q24.11 q24.21 q24.31 q24.32
4,695 bp
3x Control
3x Tumor
USP44
USP44
USP44
USP44

nature COMMUNICATIONS

ARTICLE

https://doi.org/10.1038/s41467-022-28158-2 OPEN

USP44 regulates irradiation-induced DNA double-strand break repair and suppresses tumorigenesis in nasopharyngeal carcinoma

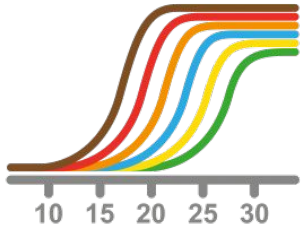
Yang Chen[1,3], Yin Zhao[1,3], Xiaojing Yang[1,3], Xianyue Ren[2,3], Shengyan Huang[1], Sha Gong[1], Xirong Tan[1], Junyan Li[1], Shiwei He[1], Yingqin Li[1], Xiaohong Hong[1], Qian Li[1], Cong Ding[1], Xueliang Fang[1], Jun Ma[1] & Na Liu[1]

"Here, we show that hypermethylation of *USP44* promotes radiotherapy resistance in NPC. *USP44* is hypermethylated in NPC, which is associated with its downregulation"

(Chen, Yang, et al. "USP44 regulates irradiation-induced DNA double-strand break repair and suppresses tumorigenesis in nasopharyngeal carcinoma." *Nature Communications* 13.1 (2022): 501.)

Validation using Methylation-Specific PCR (MSP)

	Sample	F1	L8	USP44	K1	Z5	U1
Control	T-0025-N	20.82	8.01	9.29	12.87	20.82	4.71
	T-0045-N	19.03	16.40	9.74	19.03	13.85	8.08
	T-0099-N	11.93	7.13	8.76	9.34	9.92	9.45
Tumor	T-0044-C	0.73	0.46	1.24	0.18	3.41	1.97
	T-0085-C	2.91	1.56	2.42	2.58	4.98	1.75
	T-0126-C	7.16	1.83	7.14	19.80	4.02	2.09

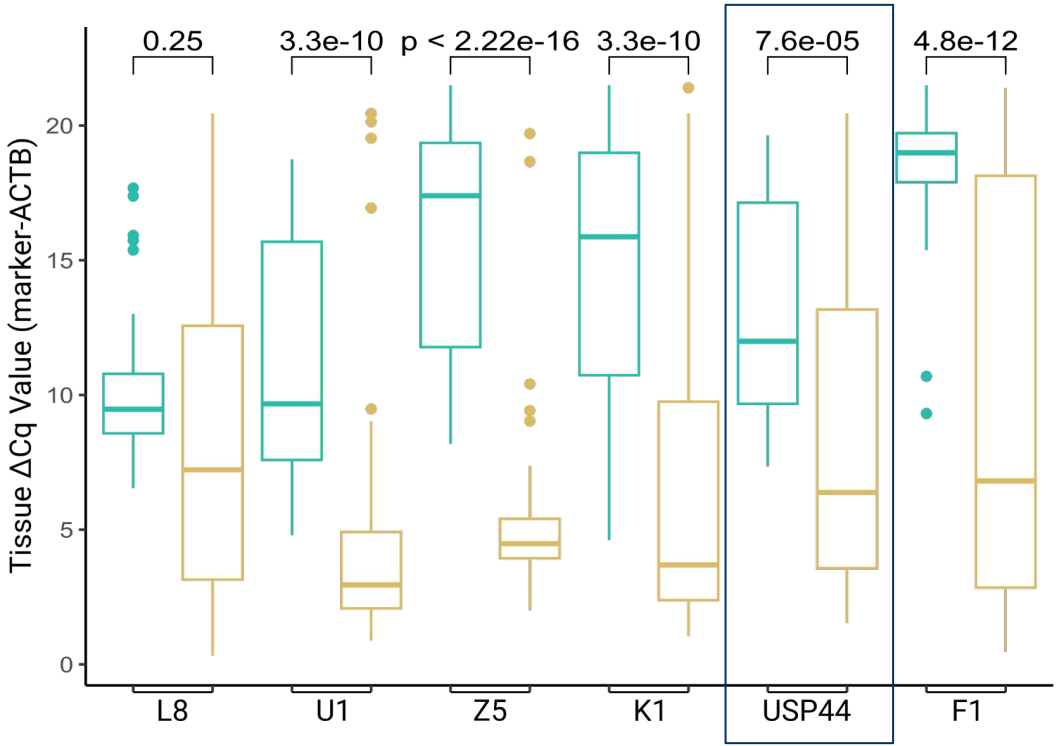


Cq value = # cycles until amplification above threshold

ΔCq value = Cq marker – Cq reference gene (ACTB)

- ➔ ΔCq values in tumor samples lower than in control
- ➔ more amplification in the tumor samples
- ➔ MSP confirms selected regions
- ➔ MSP discriminates between tumor and control

Validation using Methylation-Specific PCR (MSP)

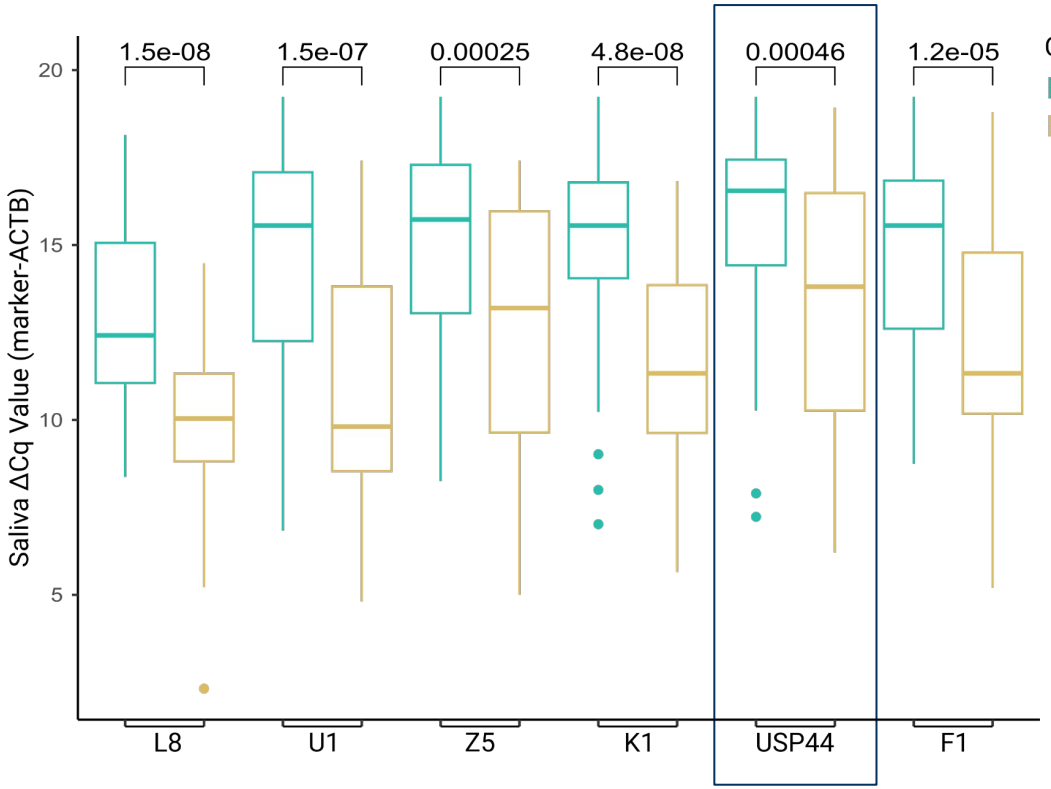


→ Results can be validated in additional tissue samples using methylation-specific PCR

Marker	Cutpoint	Youden	Sensitivity	Specificity	AUC
F1	13.97	0.6624	0.7059	0.9565	0.8252
L8	5.87	0.4510	0.4510	1.0000	0.5838
USP44	7.04	0.5490	0.5490	1.0000	0.7217
K1	7.81	0.6841	0.7059	0.9783	0.8235
Z5	7.37	0.9020	0.9020	1.0000	0.9646
U1	4.63	0.7451	0.7451	1.0000	0.8806

Youden's J statistic = sensitivity + specificity -1

Methylation-Specific PCR (MSP) in saliva – same same, but different



in saliva:
→ significantly different ΔCq values between tumor and control samples as well
→ BUT: worse sensitivity & specificity
→ not usable as diagnostic test (for screening)

Marker	Cutpoint	Youden	Sensitivity	Specificity	AUC
F1	11.67	0.4929	0.60	0.89	0.7450
L8	10.46	0.4929	0.60	0.89	0.8149
USP44	15.42	0.3409	0.64	0.70	0.7069
K1	13.20	0.5504	0.71	0.84	0.8083
Z5	11.33	0.3460	0.49	0.86	0.7157
U1	11.57	0.5016	0.64	0.86	0.7974

Summary

- Low Coverage Nanopore Sequencing data can usefully predict differentially methylated region between tumor and control samples
- diffONT can predict regions, which are usable in methylation-specific PCR
- methylation-specific PCR support nanopore sequencing results
- transferring results from tissue into saliva is difficult

Outlook

- Compare against publicly available methylation data (TCGA)
- Check publicly available gene expression data for analyzed regions (TCGA)

Thank you for your attention.

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Comments and Questions?

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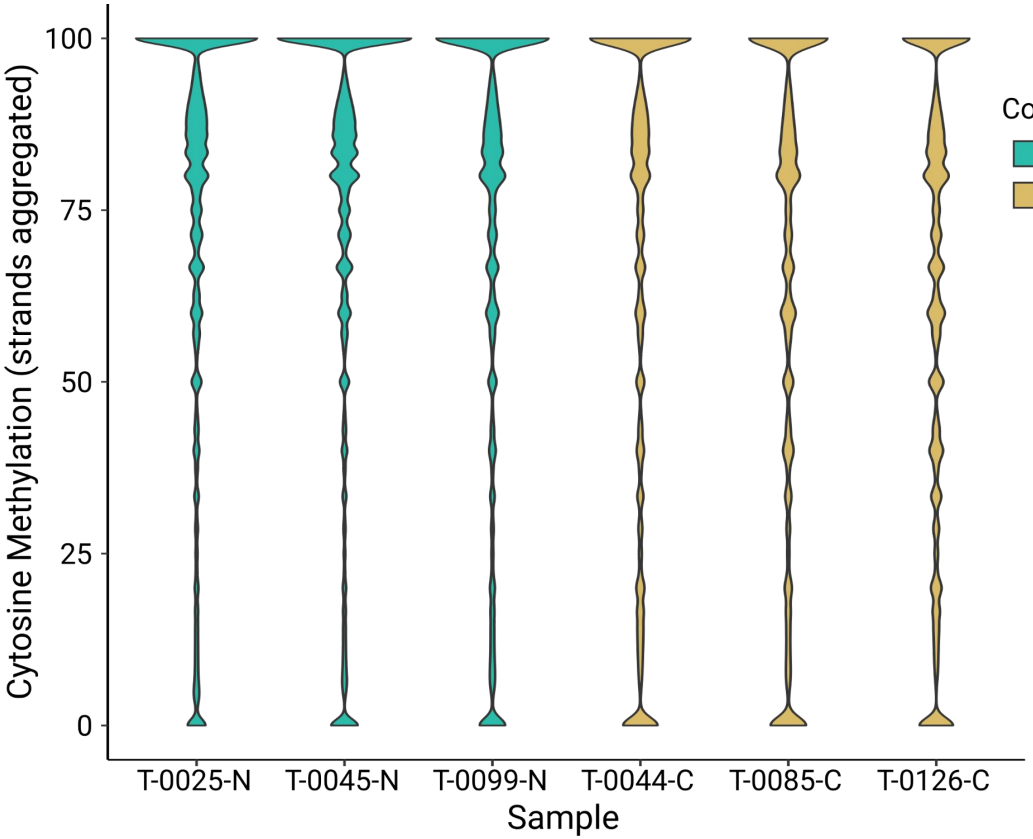
Backup-Slides

Adaptive Sampling → increased sequencing depth on CGIs

	FC ID	Sample	Wash	Adaptive	Yield (Gb)	HSA	CGI	Enrichment
Control	FAR96893	T0025N	1x	Yes +2000nt	6.46	1.74	5.40	3.10
	FAV87040	T0025N	1x	Yes +2000nt	24.82	6.70	23.26	3.47
	FAR33296	T0045N	1x	after wash	5.45	2.35	3.52	1.50
	FAS60674	T0045N	0x	Yes +2000nt	4.11	1.06	2.81	2.65
	FAV39381	T0045N	1x	Yes +2000nt	16.11	4.42	11.74	2.66
	FAV38963	T0099N	1x	Yes +2000nt	24.40	6.03	16.74	2.78
Tumor	FAQ01752	T0044C	2x	No	13.49	3.92	4.20	1.07
	FAR39219	T0044C	1x	Yes +2000nt	9.68	2.61	9.15	3.51
	FAV38989	T0085C	1x	Yes +2000nt	17.13	4.65	14.63	3.15
	FAV39137	T0126C	1x	Yes +2000nt	23.50	5.98	11.98	2.00

Nanopore sequencing - overall methylation



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