



Sensitive monitoring of cell free DNA in liquid biopsies using Nanopore sequencing

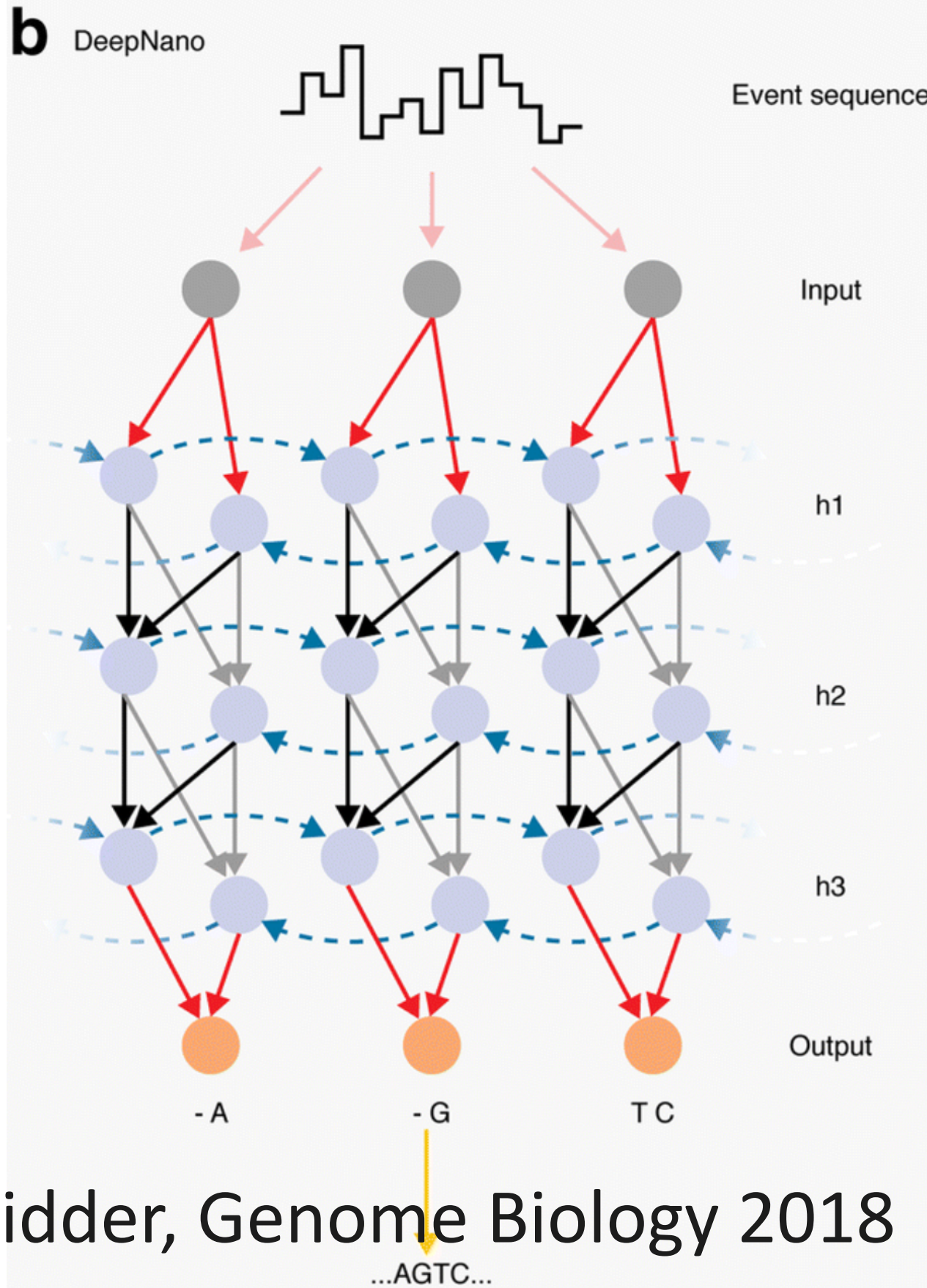
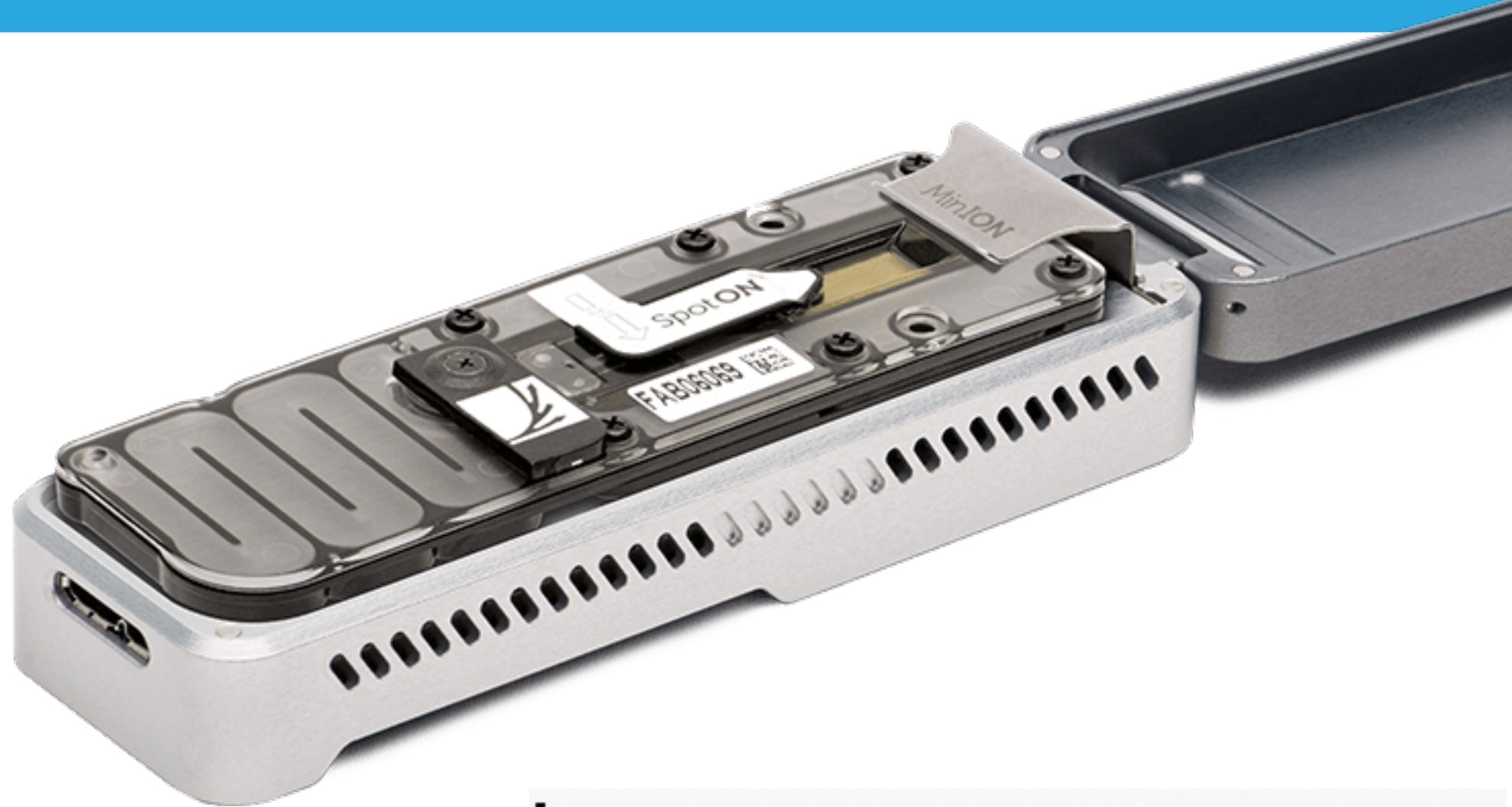
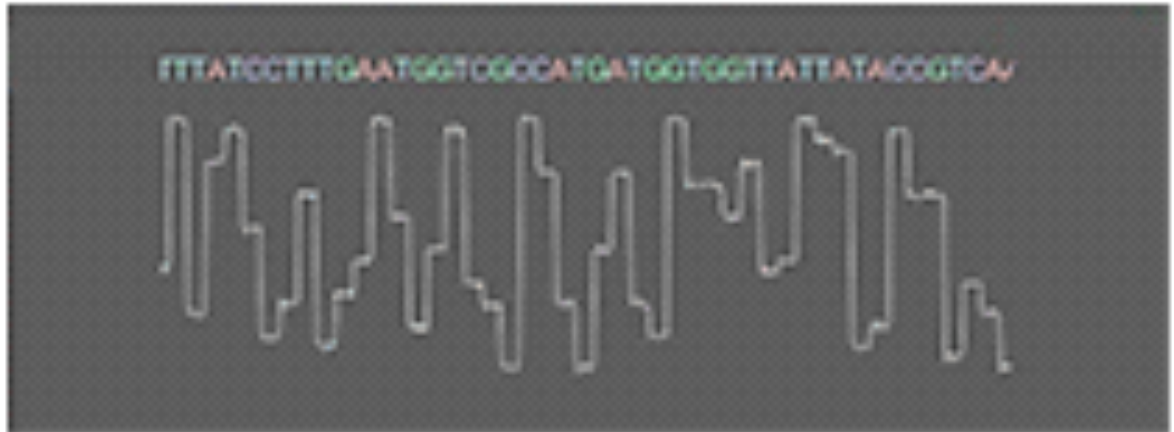
Jeroen de Ridder

Associate professor - Center for Molecular Medicine

Oncode Principal Investigator

Co-founder Cyclomics BV

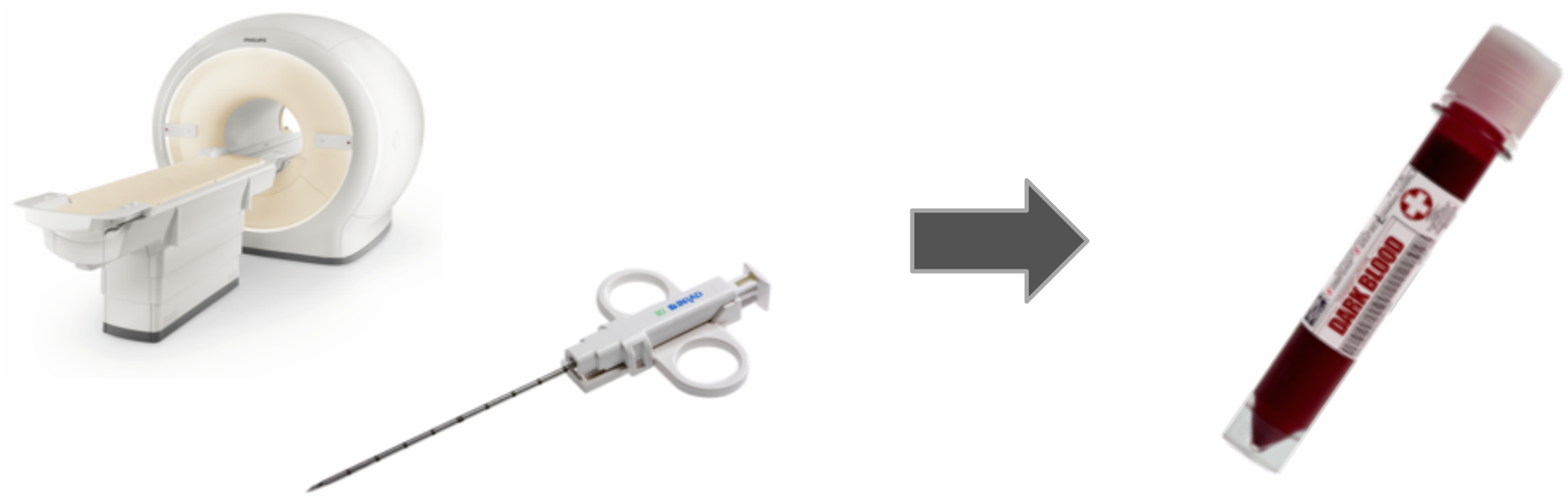
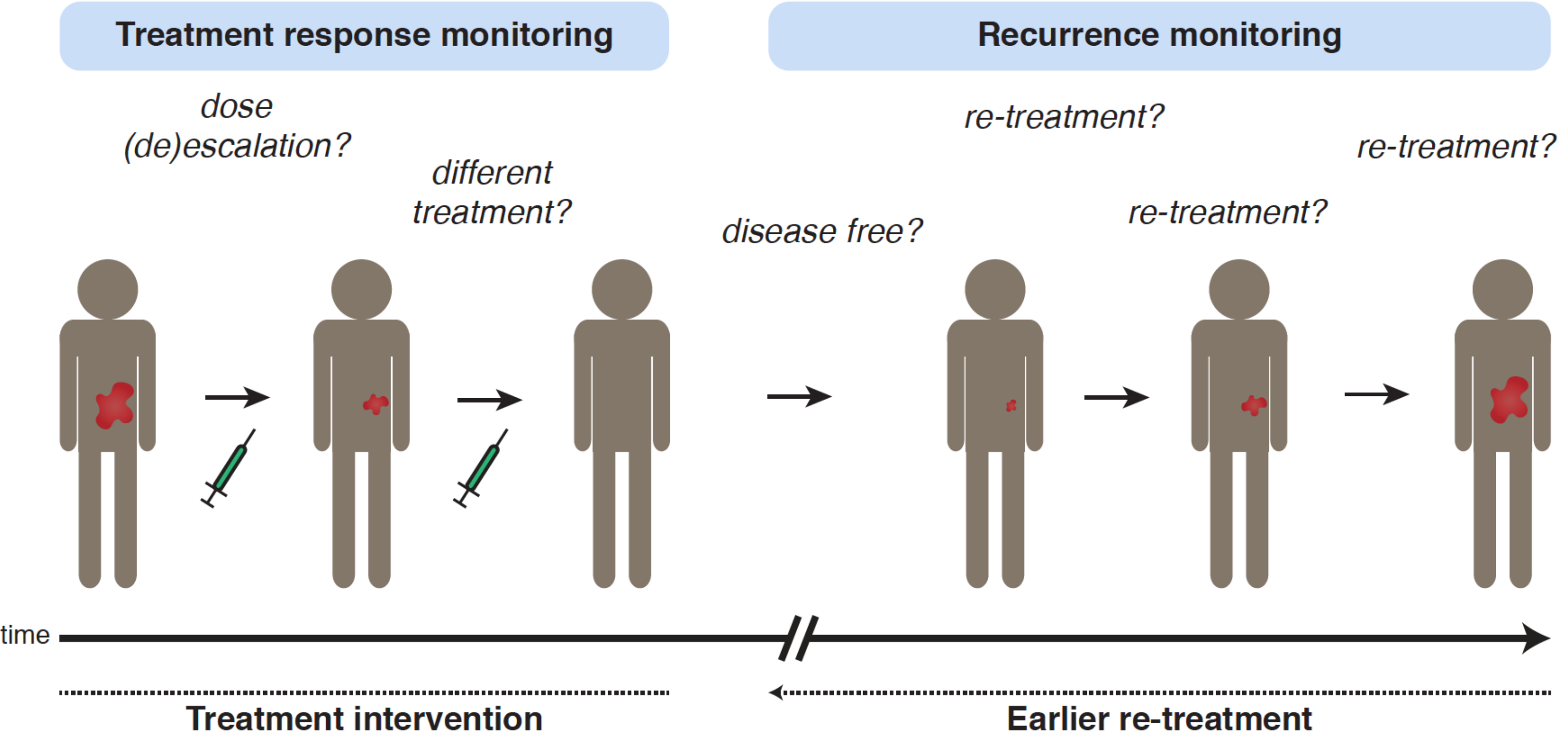
Nanopore sequencing









Recurrent Neural Network to perform basecalling

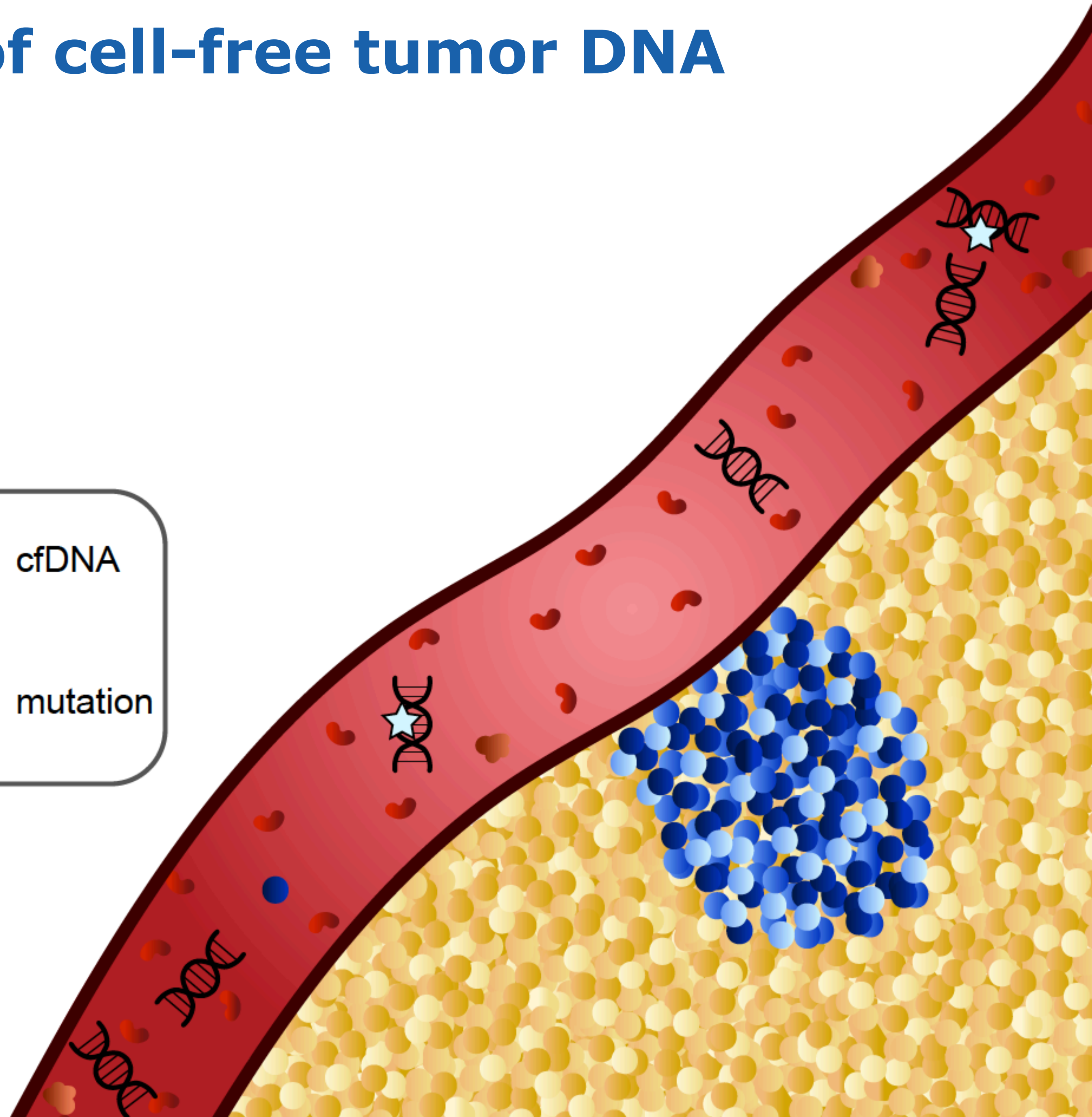
Reviewed in: Rang, Kloosterman & de Ridder, Genome Biology 2018

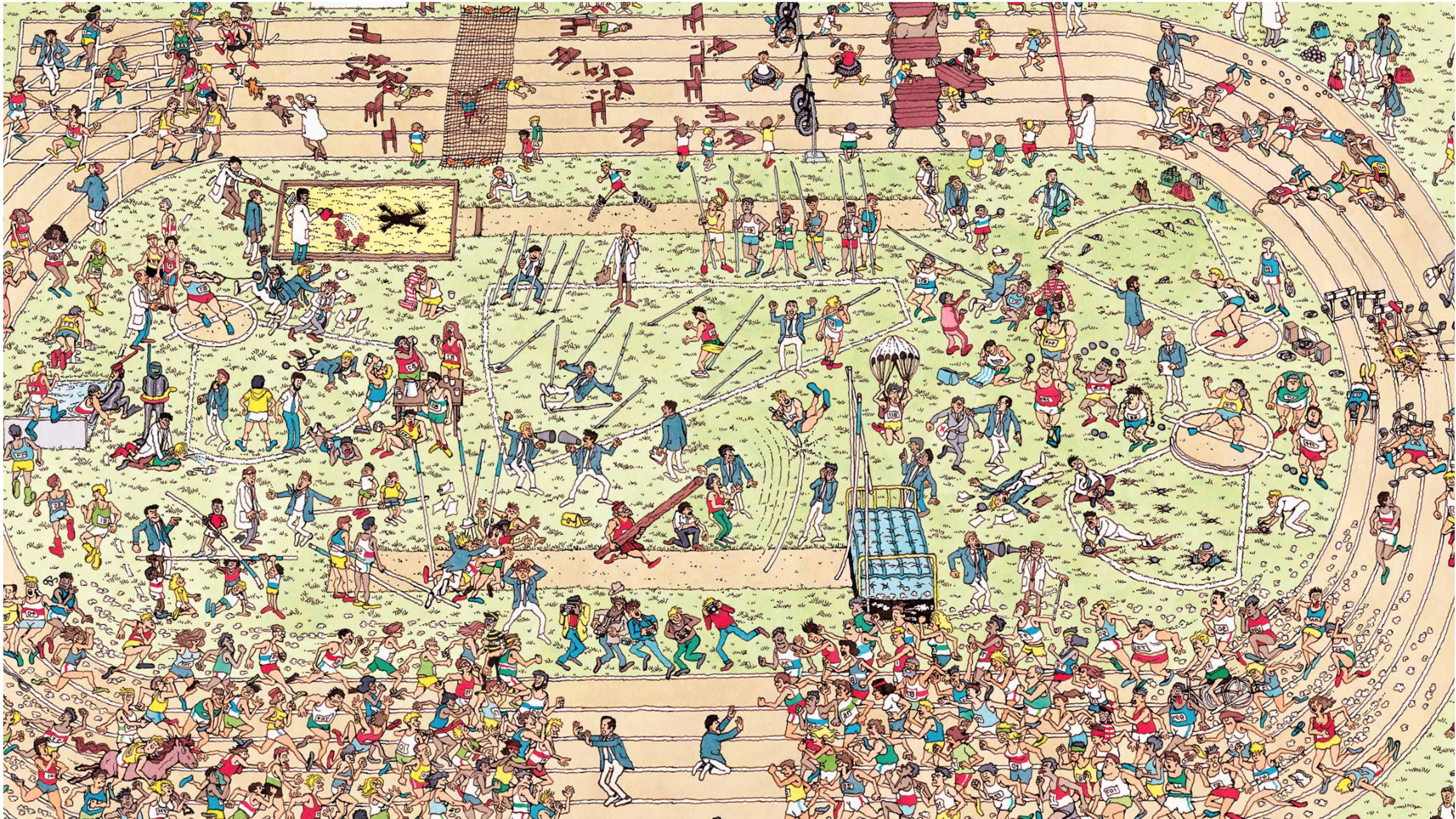
Challenges in cancer diagnostics



CyclomicsSeq - Detection of cell-free tumor DNA

	healthy cells		cfDNA
	tumor cells		mutation
	red blood cell		
	immune cell		





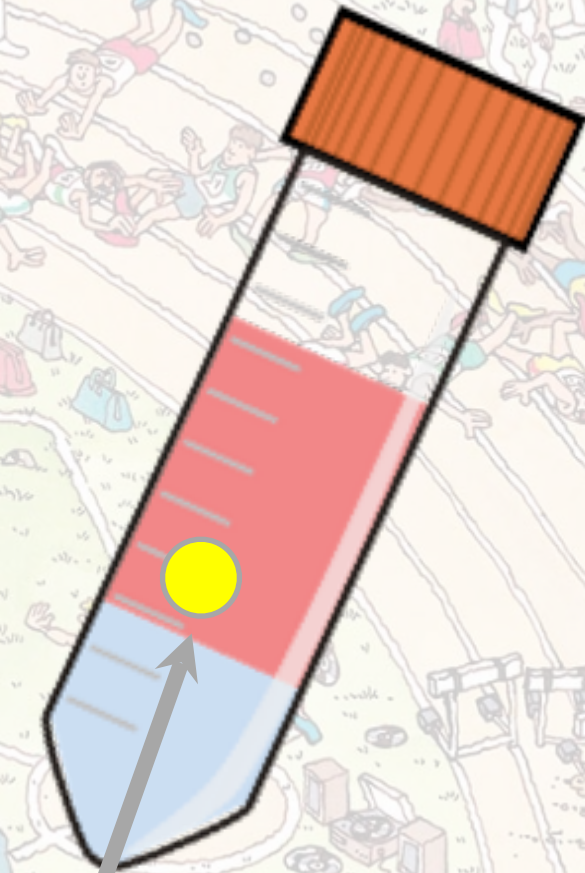
cfDNA molecules are small (~150bp)

Only a tiny fraction of cell free DNA molecules carry a specific mutation (~0.00000001%)

In one vial of blood there may be only 10-1000 molecules that carry a mutation!

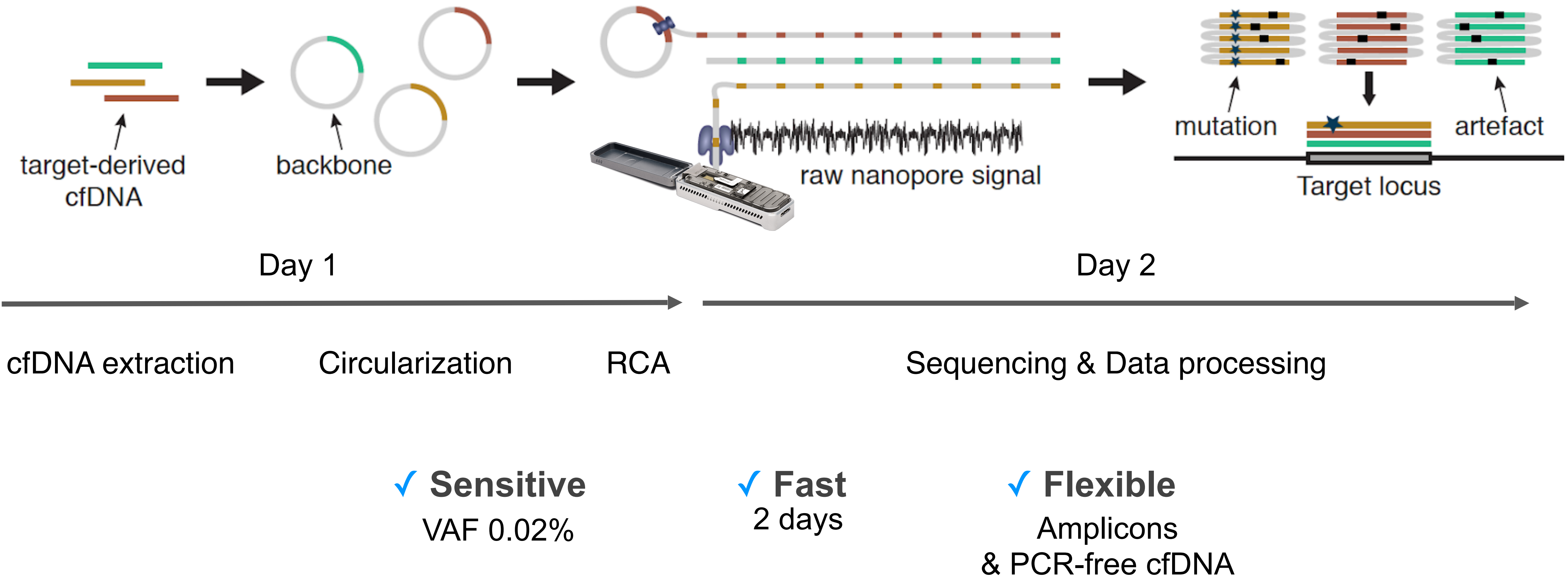
We need extremely sensitive detection methods

.. and cheap, and fast and point-of-care!



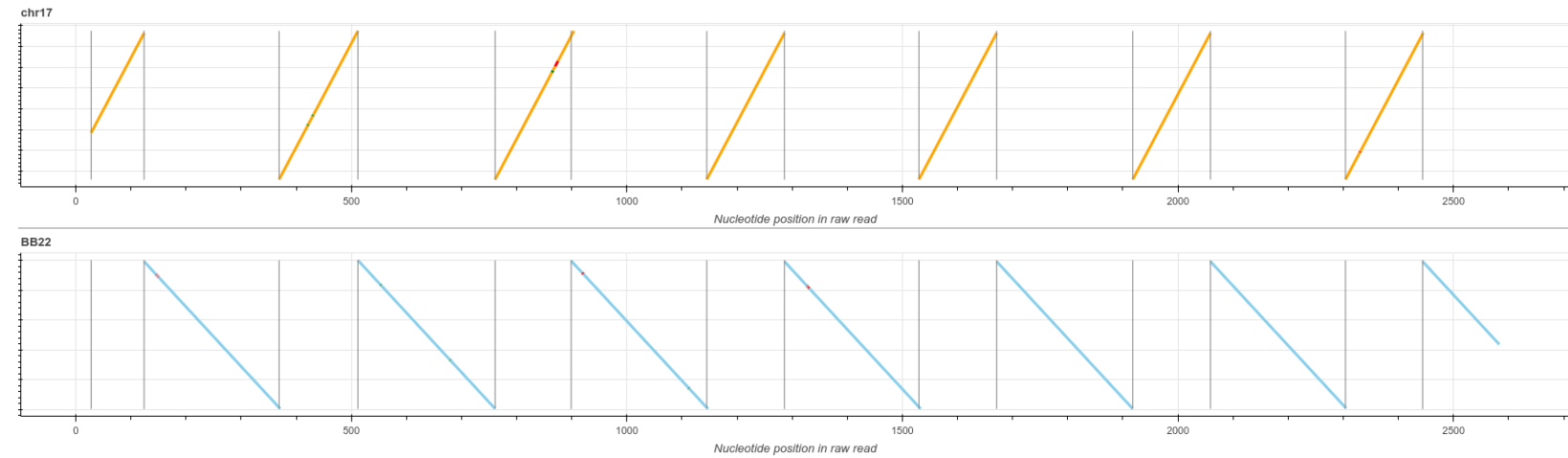
HERE is Waldo!

CyclomicsSeq - overview

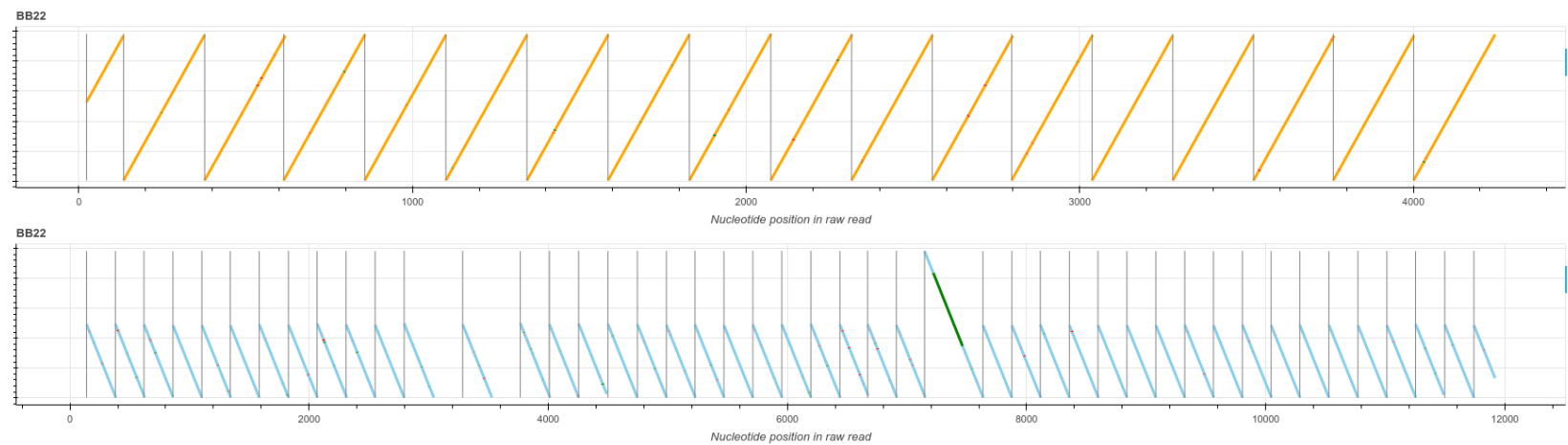


High-quality concatemers improves consensus accuracy

Alternating mapping to insert and backbone

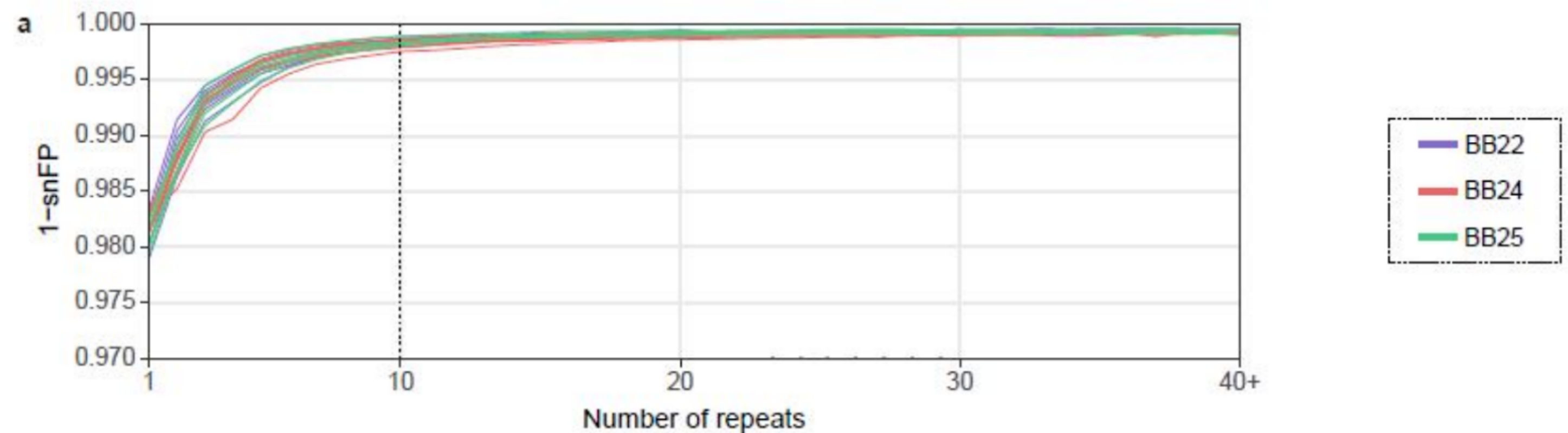
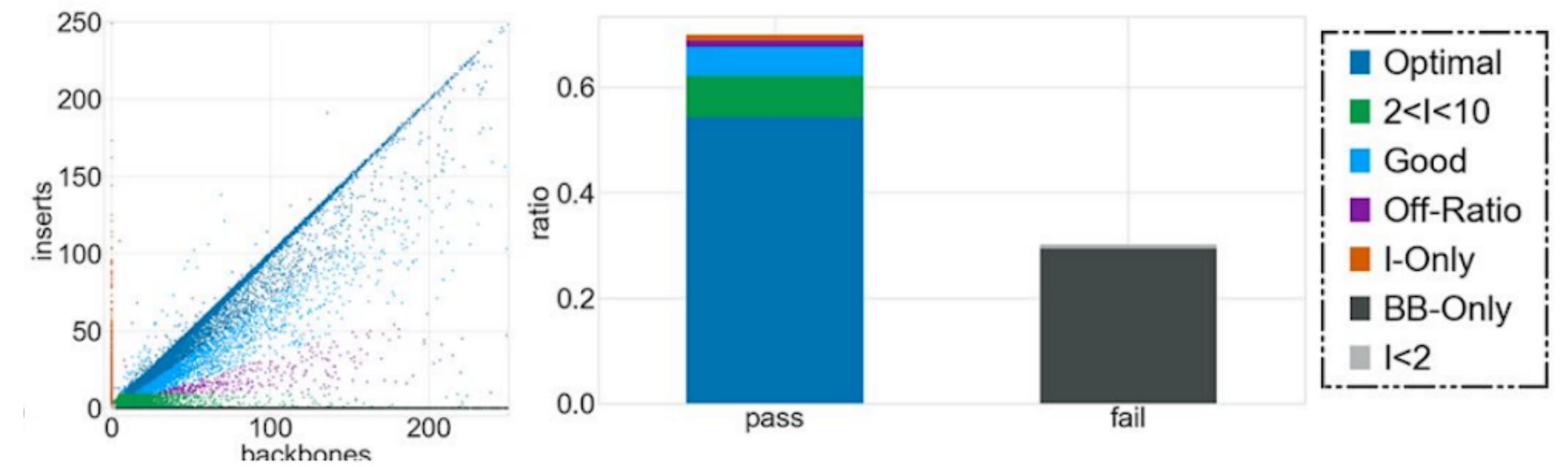


Some insert-only or backbone only

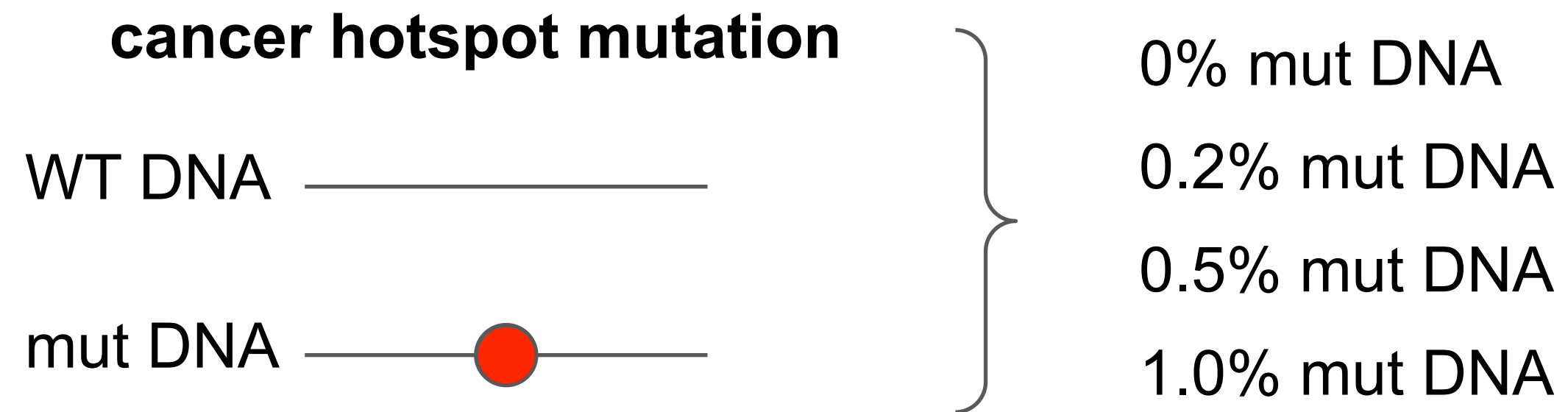


Highly accurate consensus calling with increasing number of repeat copies

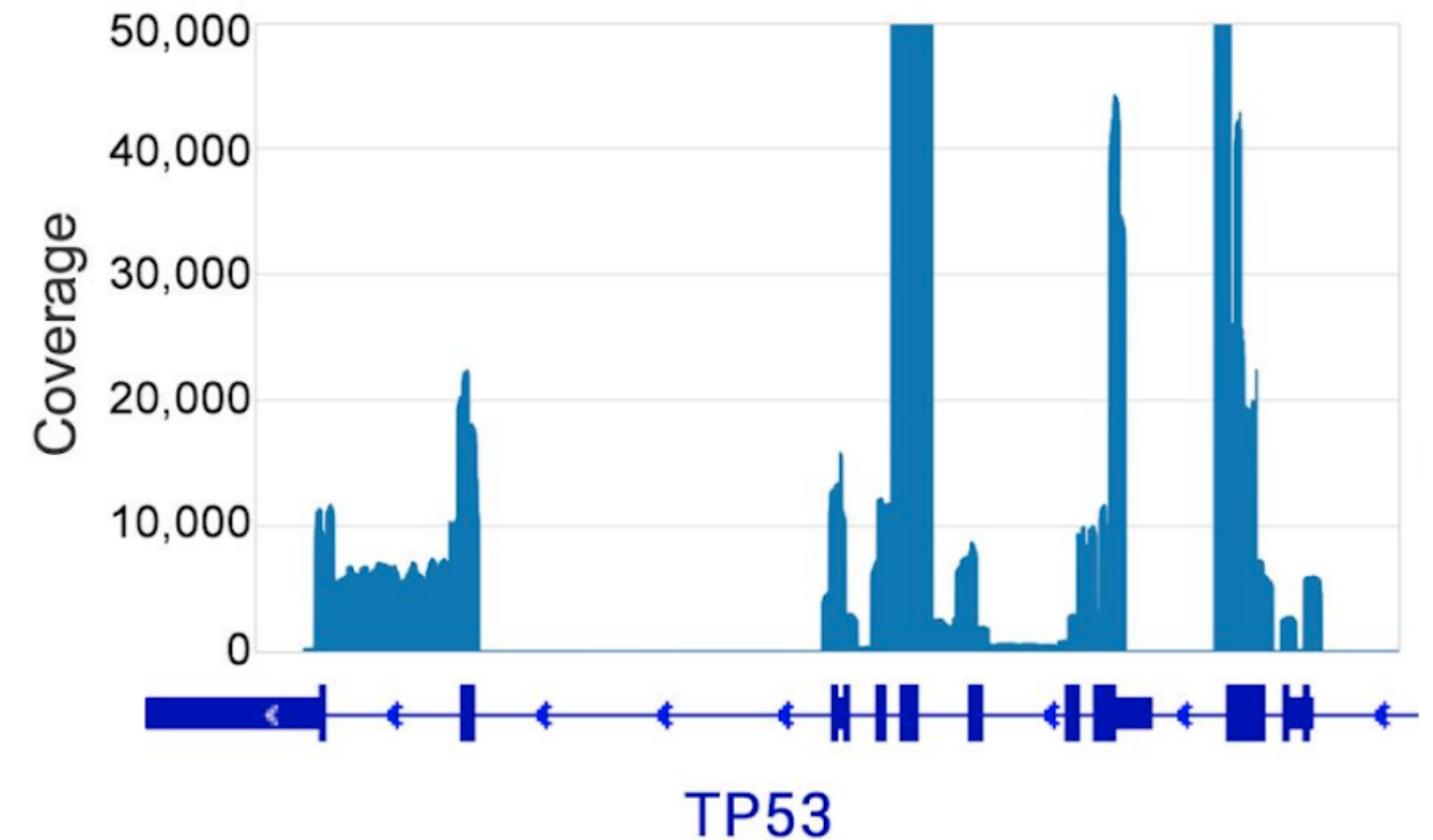
Single molecule accuracies!



CyclomicsSeq detects low VAF across whole gene



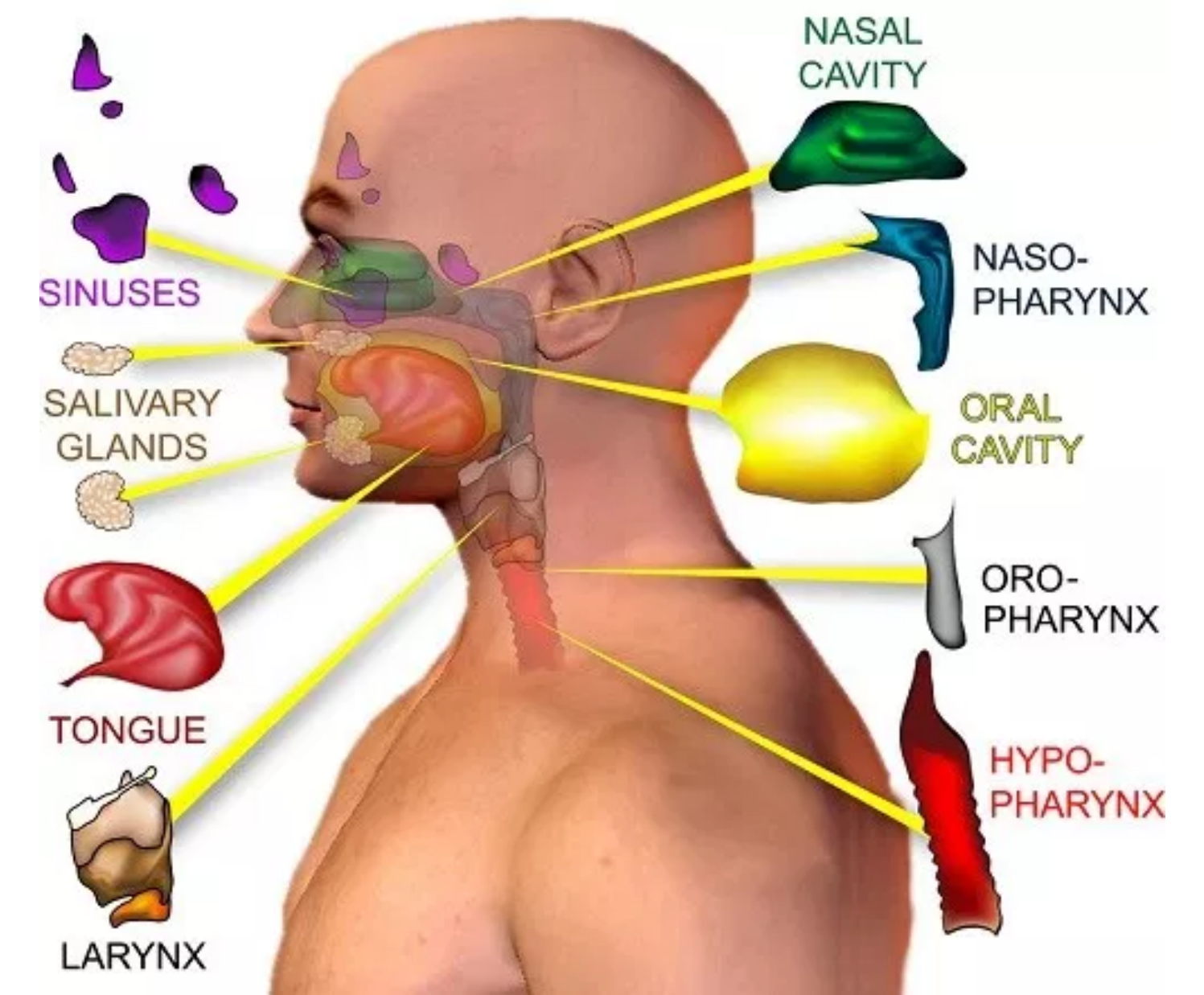
Dataset	Expected mut freq	Found mut freq
002	0.2 %	0.34%
005	0.5%	0.65%
010	1.0%	1.00%
WT	0%	Not called



- Multiplex PCR amplification of TP53 exons from ctDNA
- Coverage of all TP53 exons
- PCR-free enrichment technology under development

Use case: Head and neck cancer

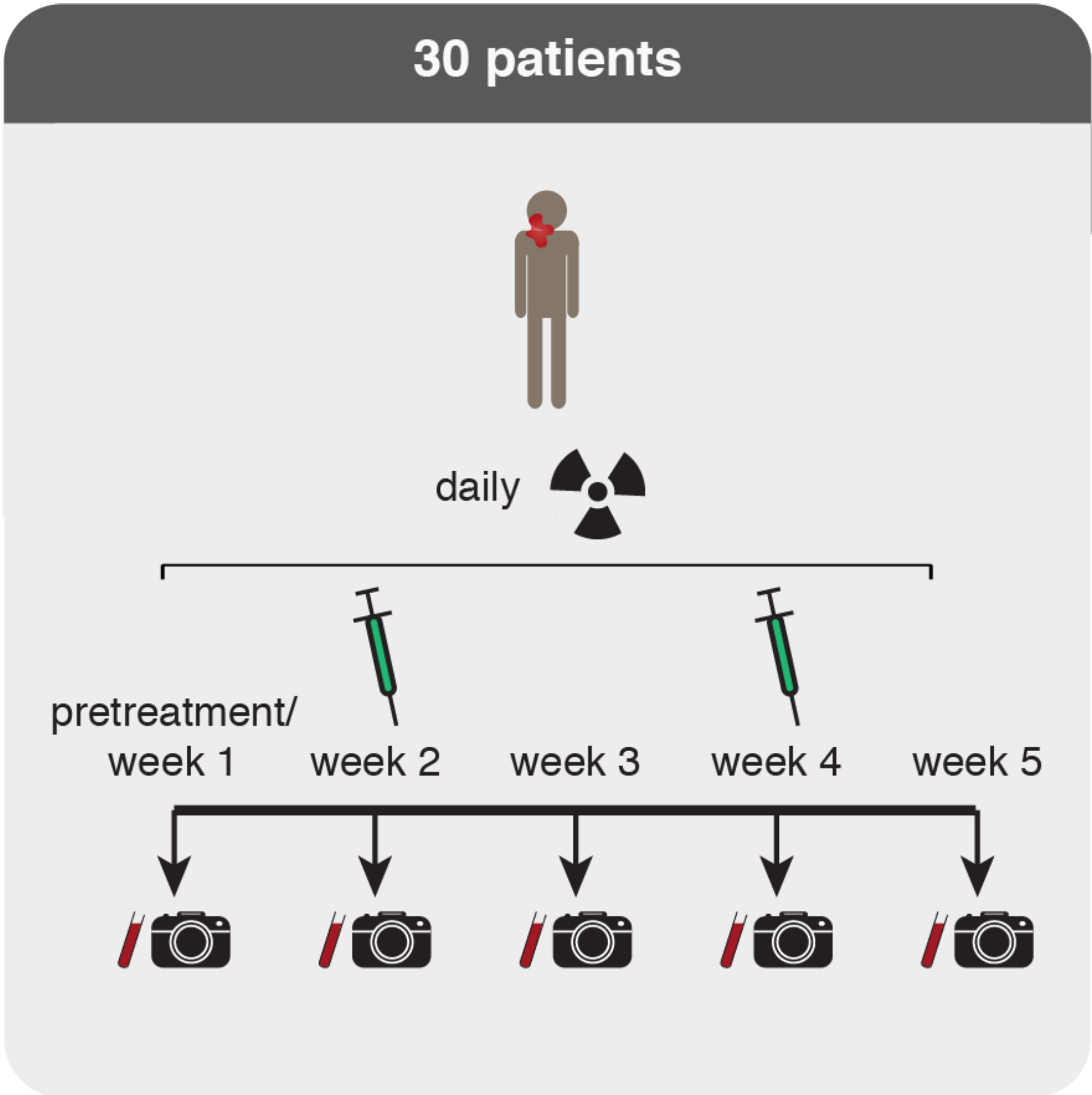
- Some numbers...
 - 600,000 cases per year (worldwide)
 - 380,000 deaths per year (worldwide)
- Treatment
 - 1st line – Radio(chemo)therapy -> 55% response
 - 2nd line – Salvage surgery -> possible in $\pm 50\%$
- Recurrence
 - $\sim 50\%$
- >90% of HPVneg tumors carry TP53 mutations




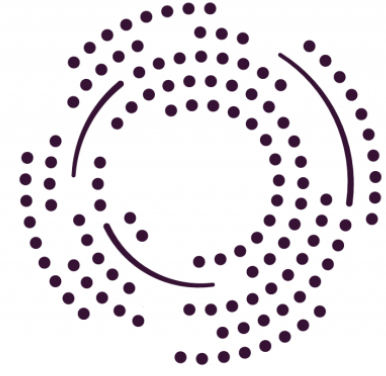
“Detection of head and neck tumors is often based on a ‘gut feeling’ interpretation”

Dr. Willems, UMCU, Pathologist

Use case: Head and neck cancer



→ 
CyclomicsSeq
+ ddPCR

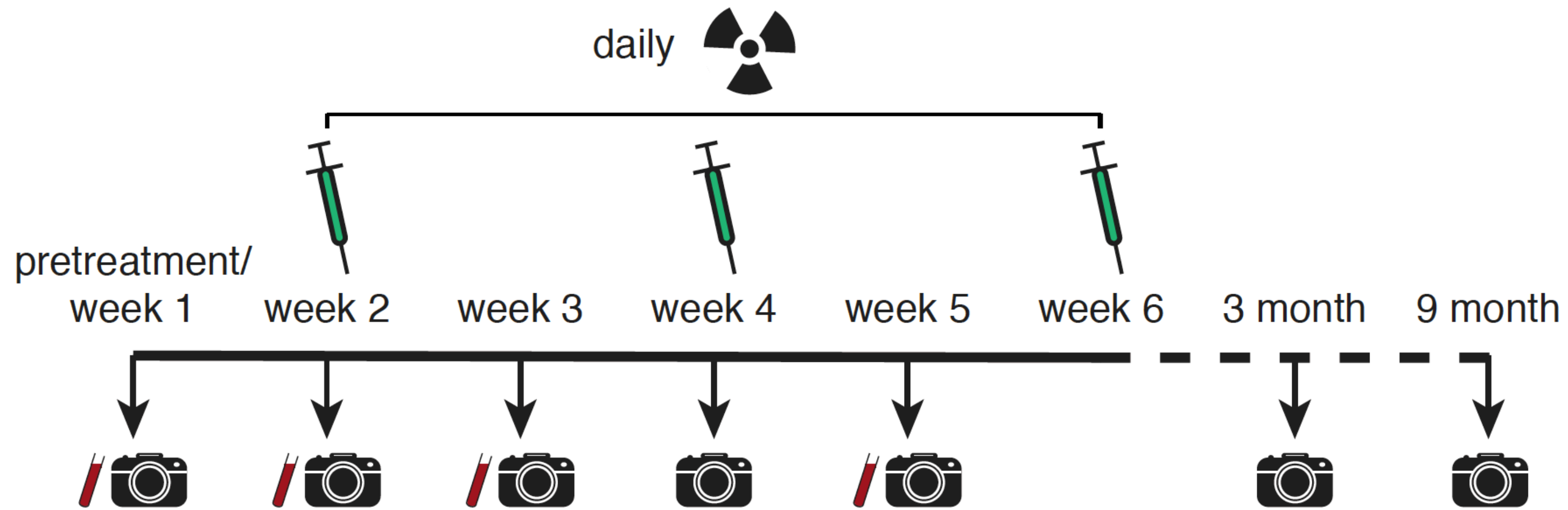


**Oncode
Institute**

Clinical Proof of Concept

Patient 1

- Point mutation TP53:
 - 17:7577121 G>A
 - VAF = 0.6 (in tumor)

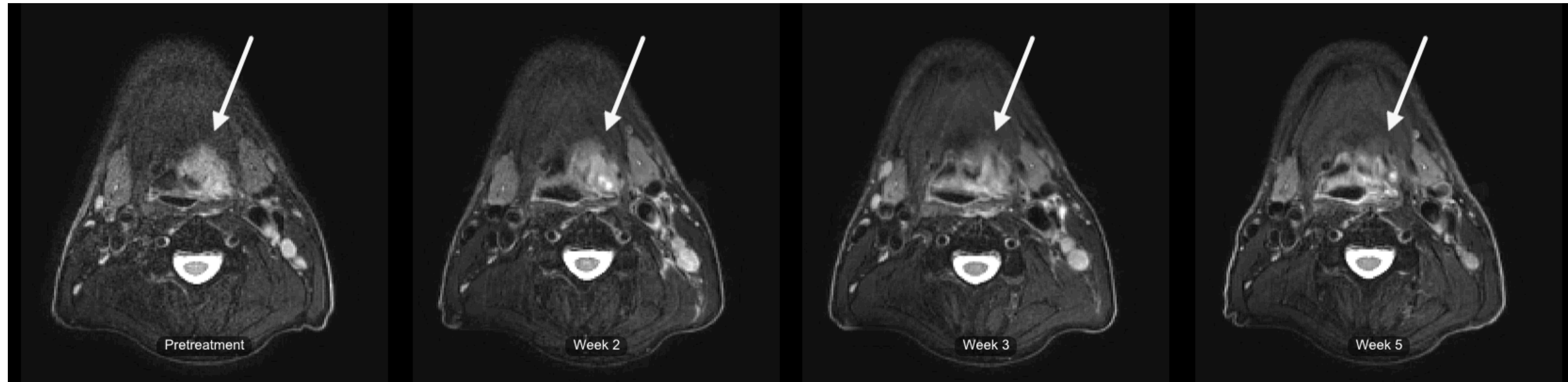
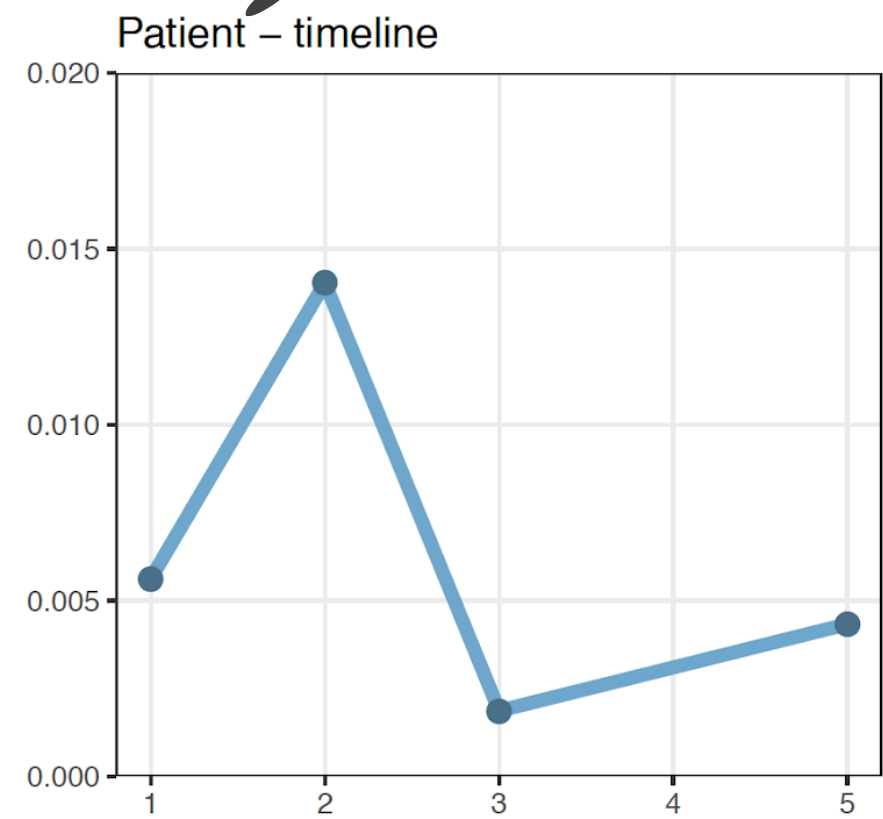


Stage II (oropharynx, left)

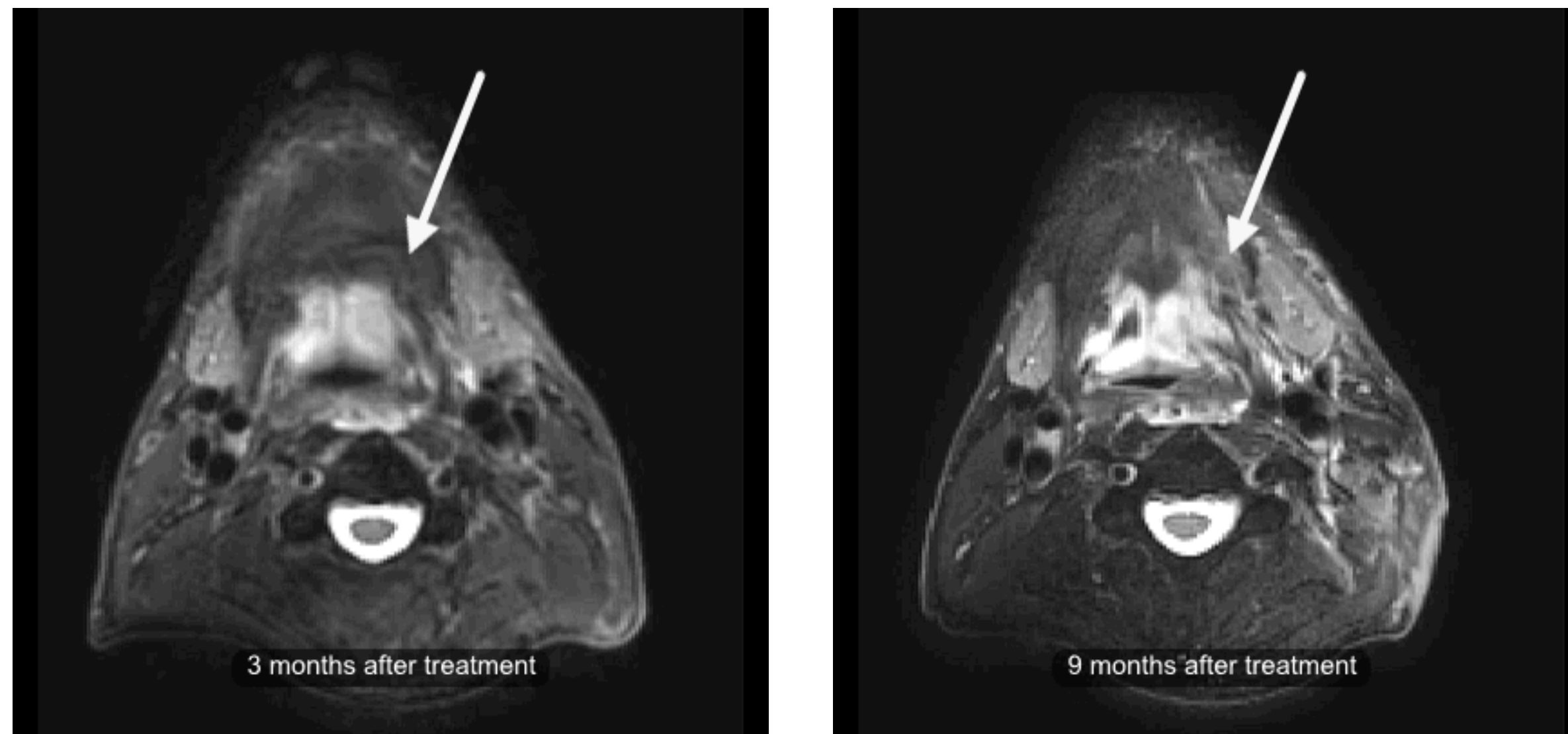
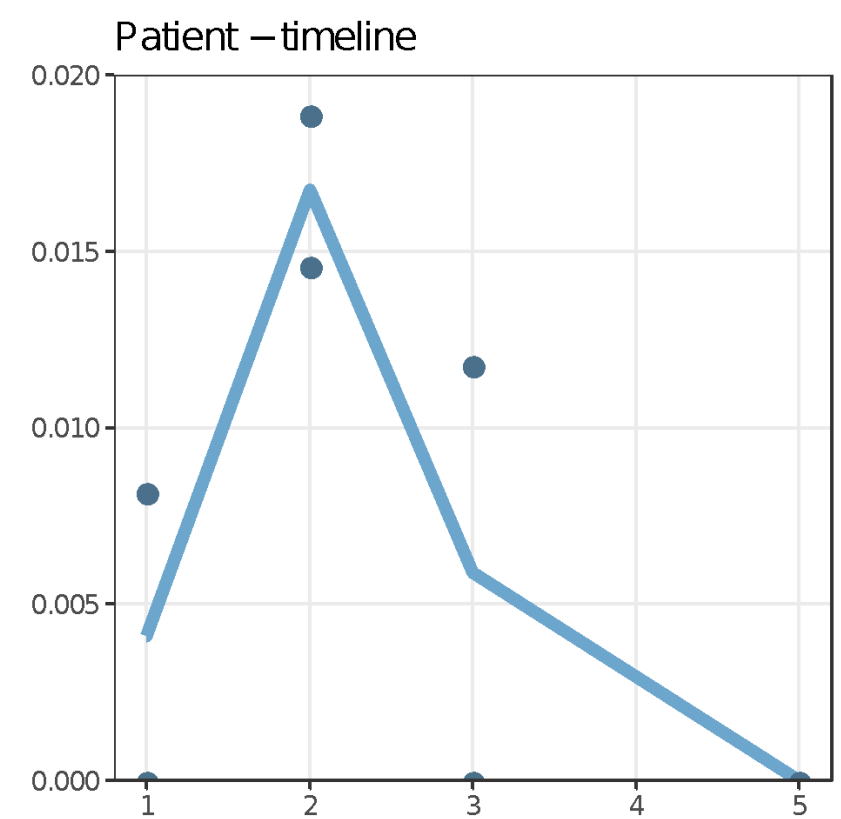


Residual disease (nodal)

MRI



ddPCR



CyclomicsSeq detected the residual disease **5 weeks** after treatment

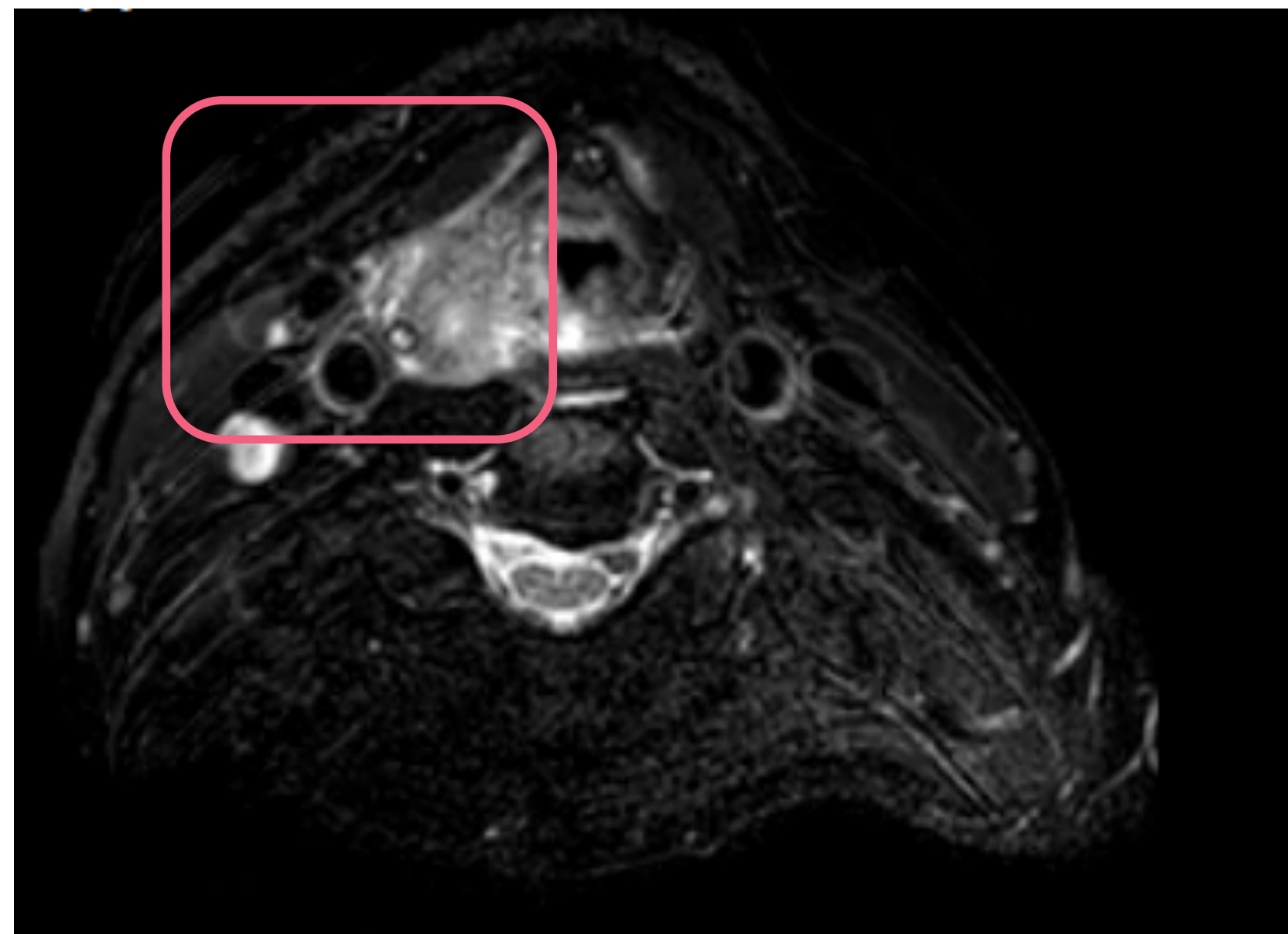
ddPCR **failed** in detecting the mutation

MRI showed clear signs of tumor growth only **after 9 months**

Patient 2

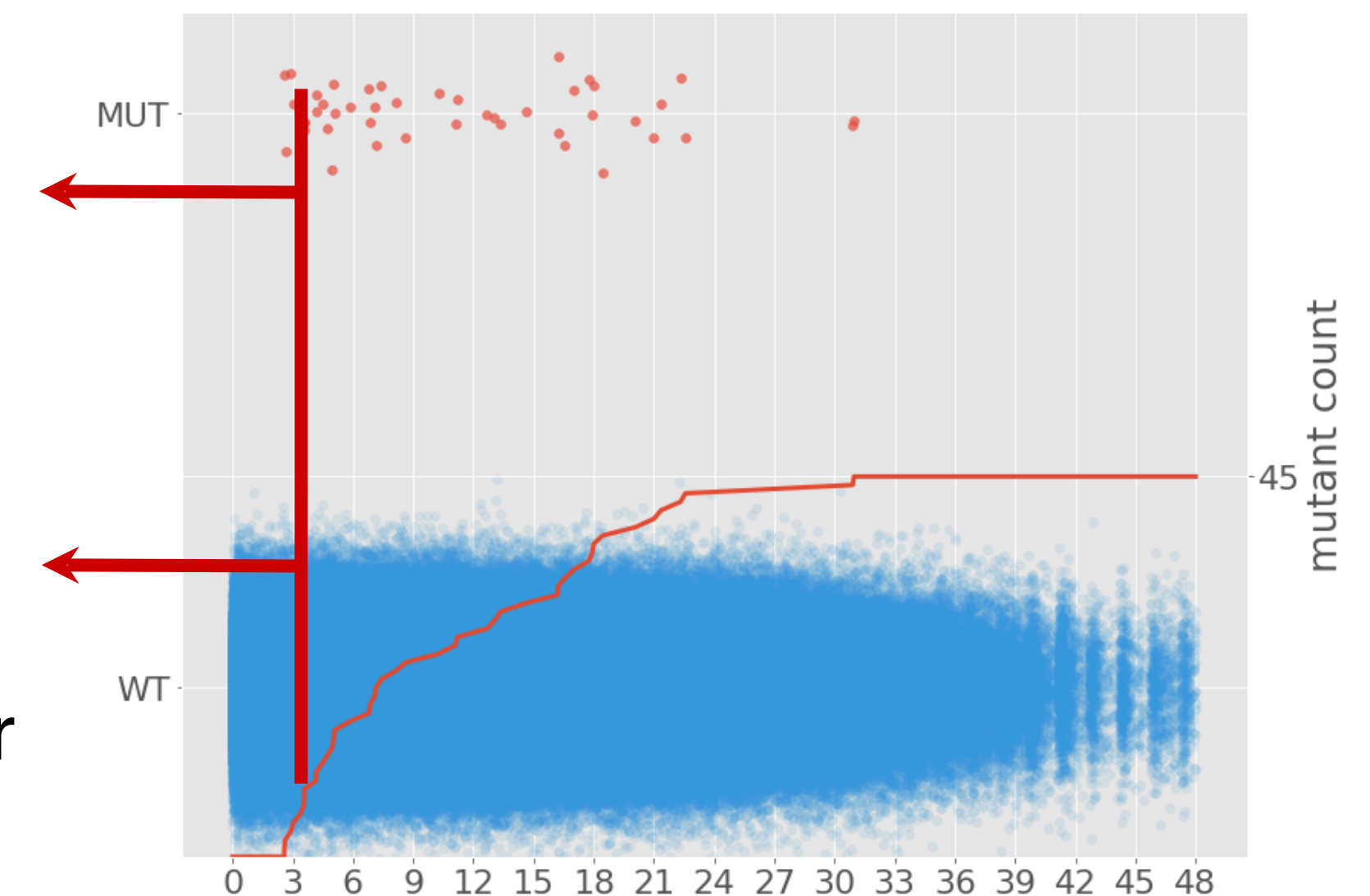
- Mutation TP53:
 - 17:7577095-7577123 Deletion
 - VAF = 0.34 (in tumor)

Stage IV (hypopharynx, right)

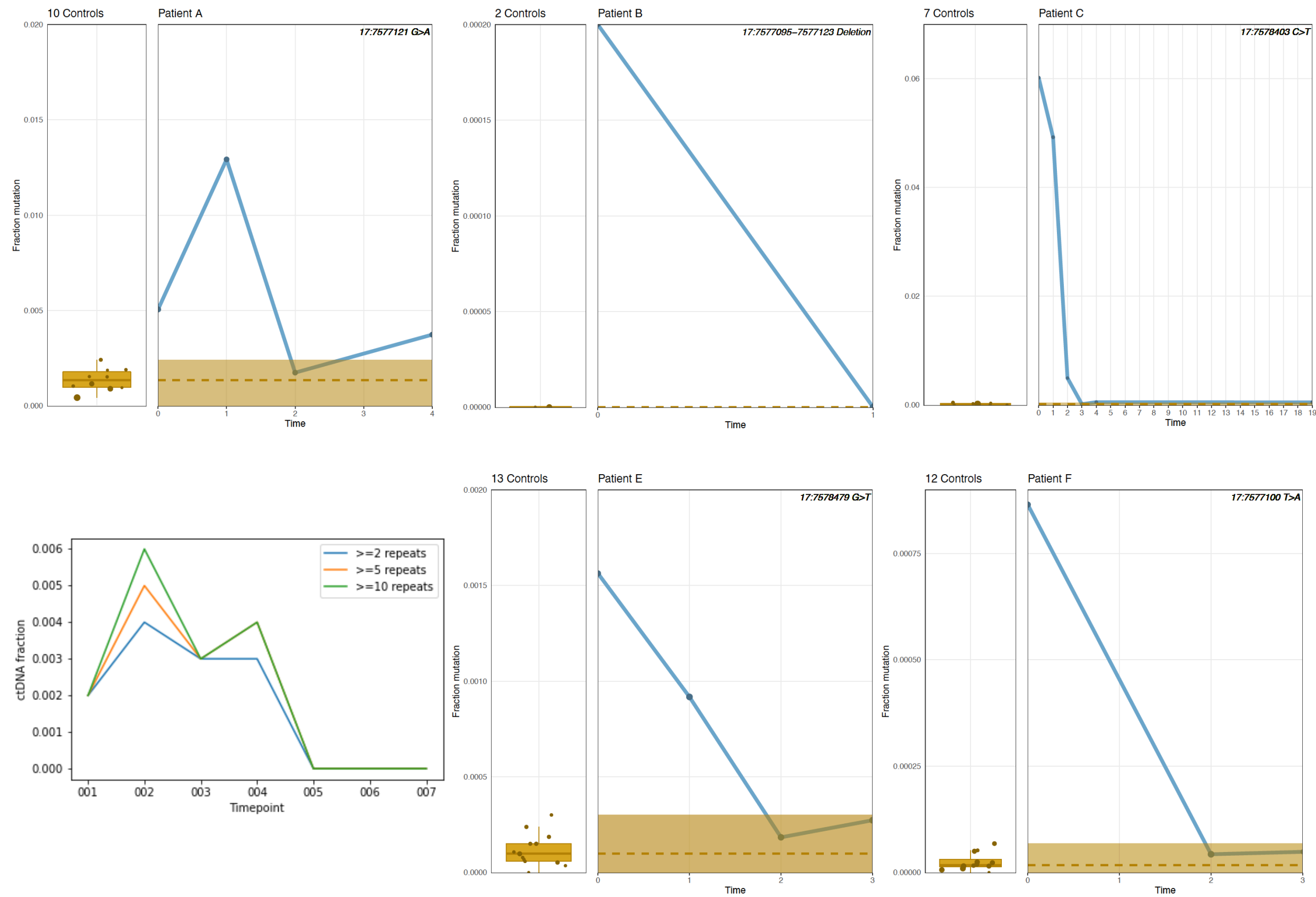


Real time
accumulation
of mutation
evidence

Detection of
the tumor after
only 3h of
sequencing



ctDNA levels correlate with patient outcome



Sample	Mutation	Timepoints	ddPCR?	MRI?	Outcome
Patient A	SNV	0,1,2,4	Y	Y	Nodal recurrence: 10 months
Patient B	DEL(&SNV)	0,1	Y	Y	Death (rupture carotic artery)
Patient C	SNV	0,1,2,3,4,19	Y	N	1 year disease free
Patient D	DEL	0,1,2,3,4,5,20	N	N	1 year disease free
Patient E	SNV	0,1,2,3	N	Y	1 year disease free
Patient F	SNV	0,2,3	N	Y	Death (ischemic intestine)

Reaching the patient: spinning out from the UMC Utrecht



Wigard Kloosterman



Dami Rebergen



Alessio Marcozzi



Wilbert van Workum



Jeroen de Ridder



Myrthe Jager



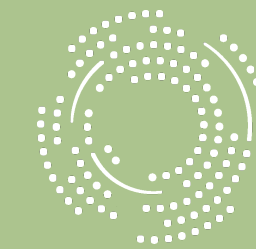
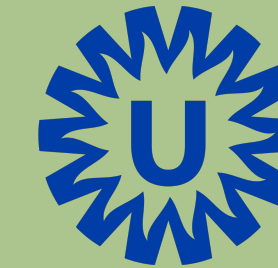
Martin Elferink



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Liting Chen



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Joris Vos
Lotje Zuur

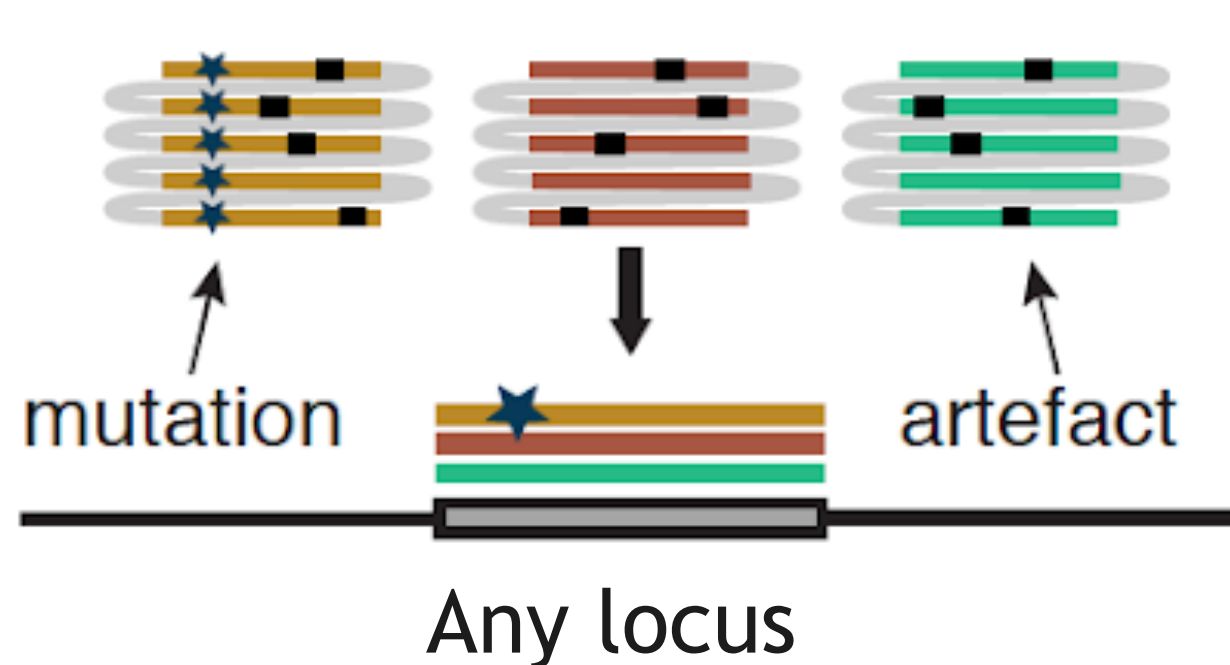
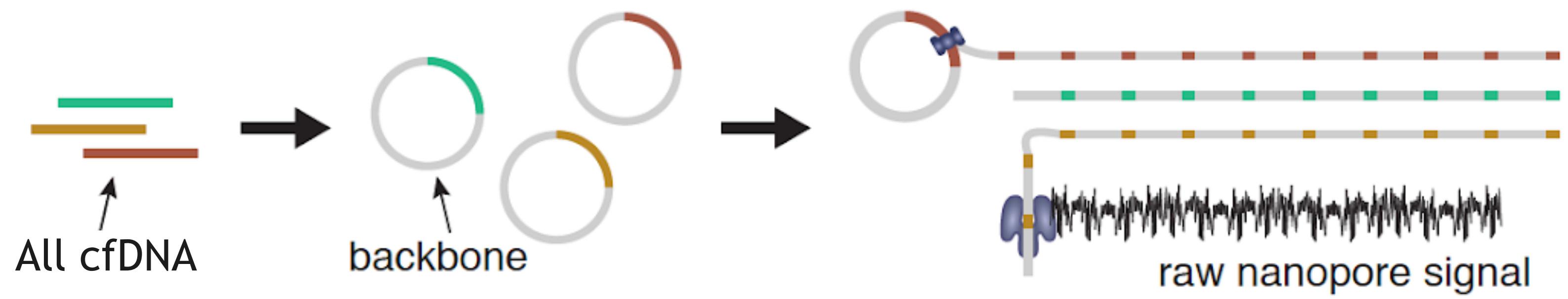


UMC Utrecht

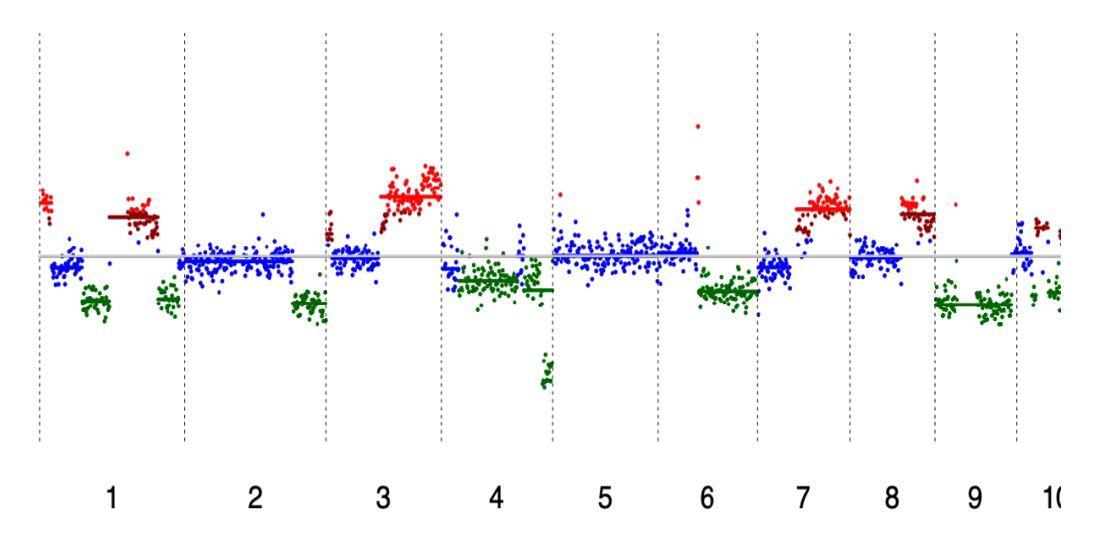
Lot Devriese
Stefan Willems

Boris Peltenburg
Joyce van Kuik
Joost van Ginkel

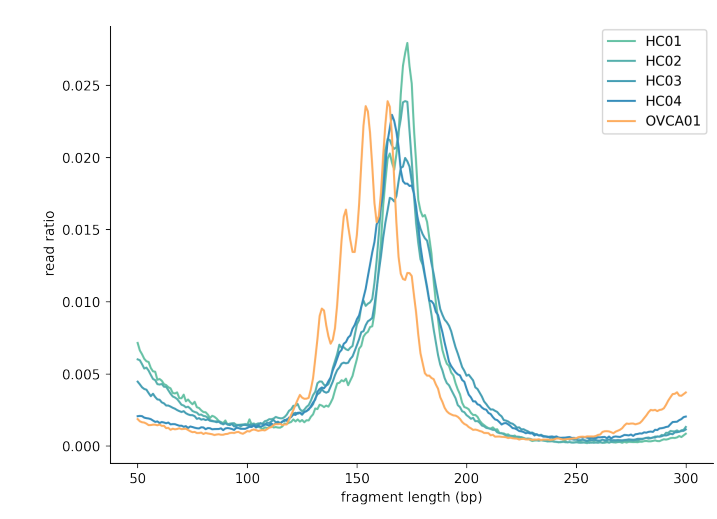
Leveraging all cancer cfDNA signals using genome-wide CyclomicsSeq



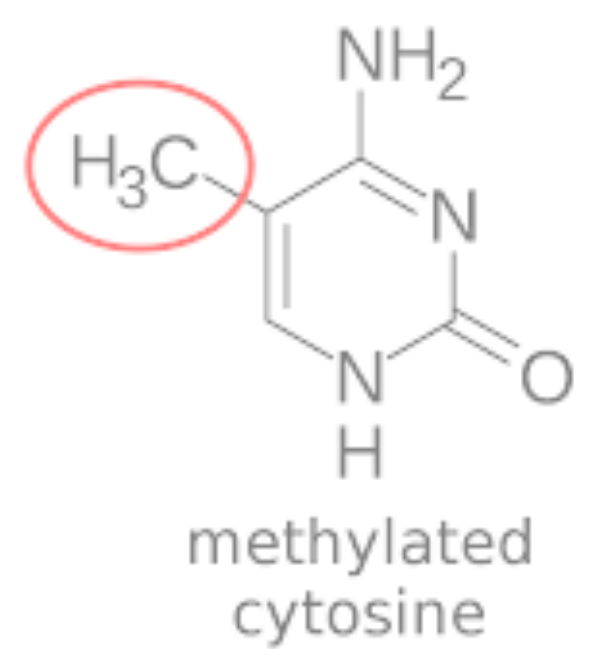
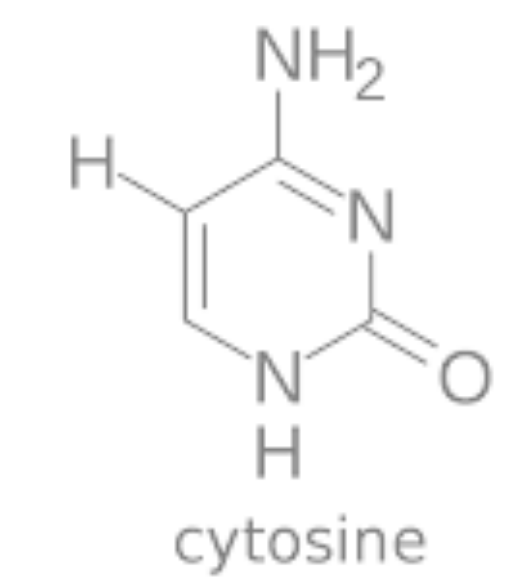
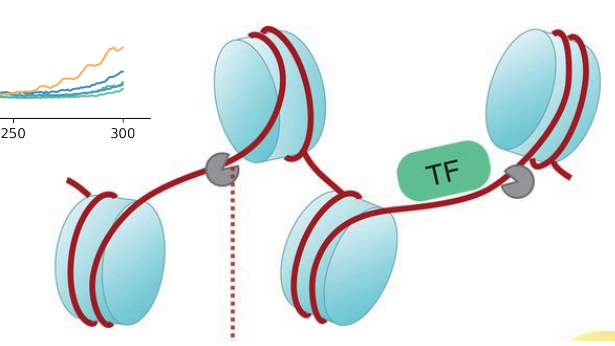
Somatic mutations



CNVs

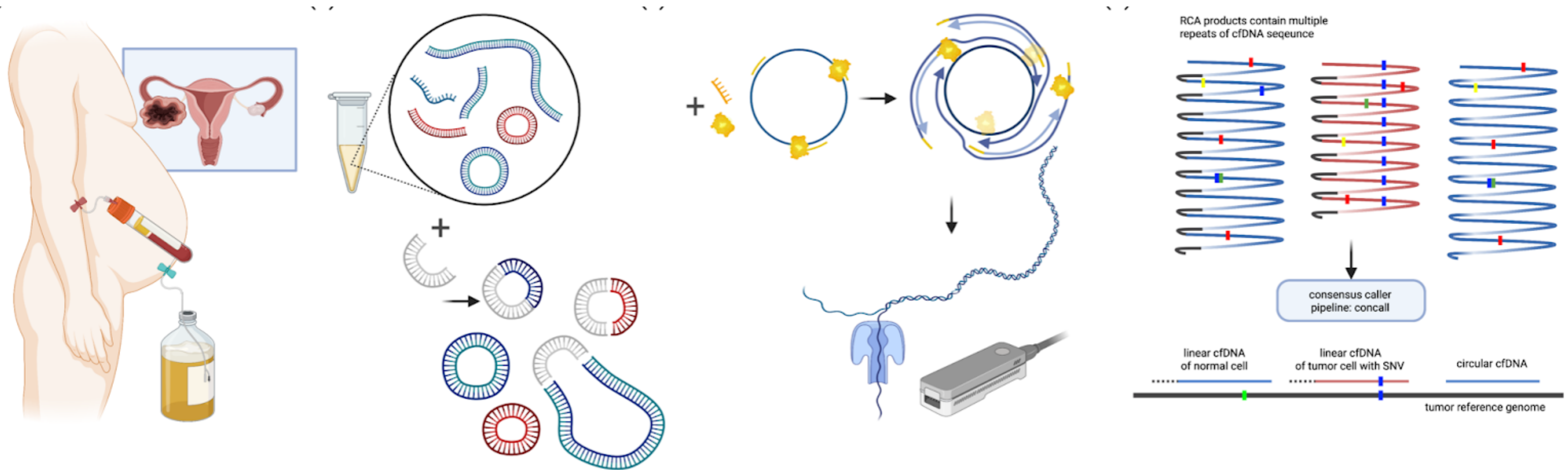


Fragment length



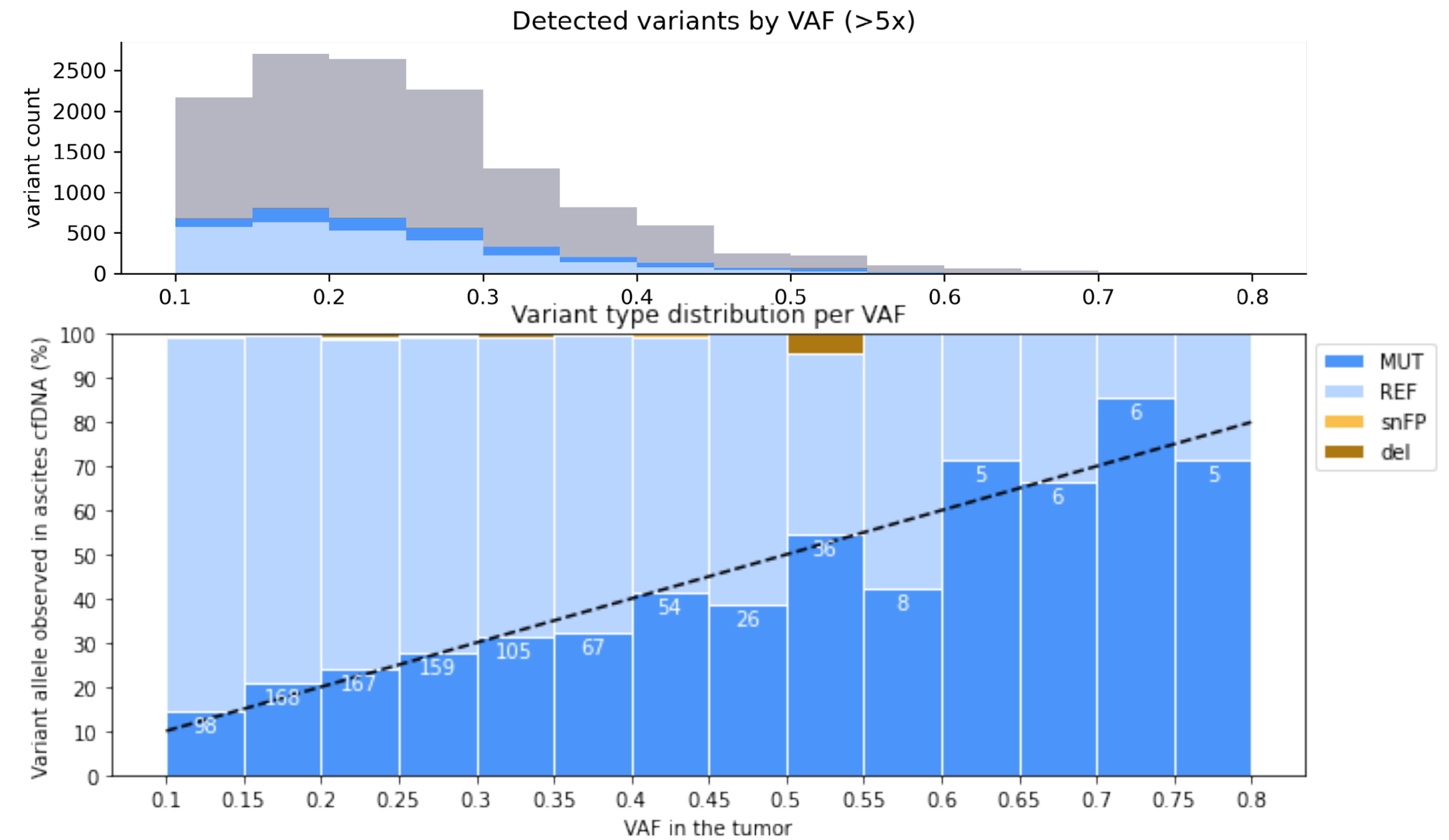
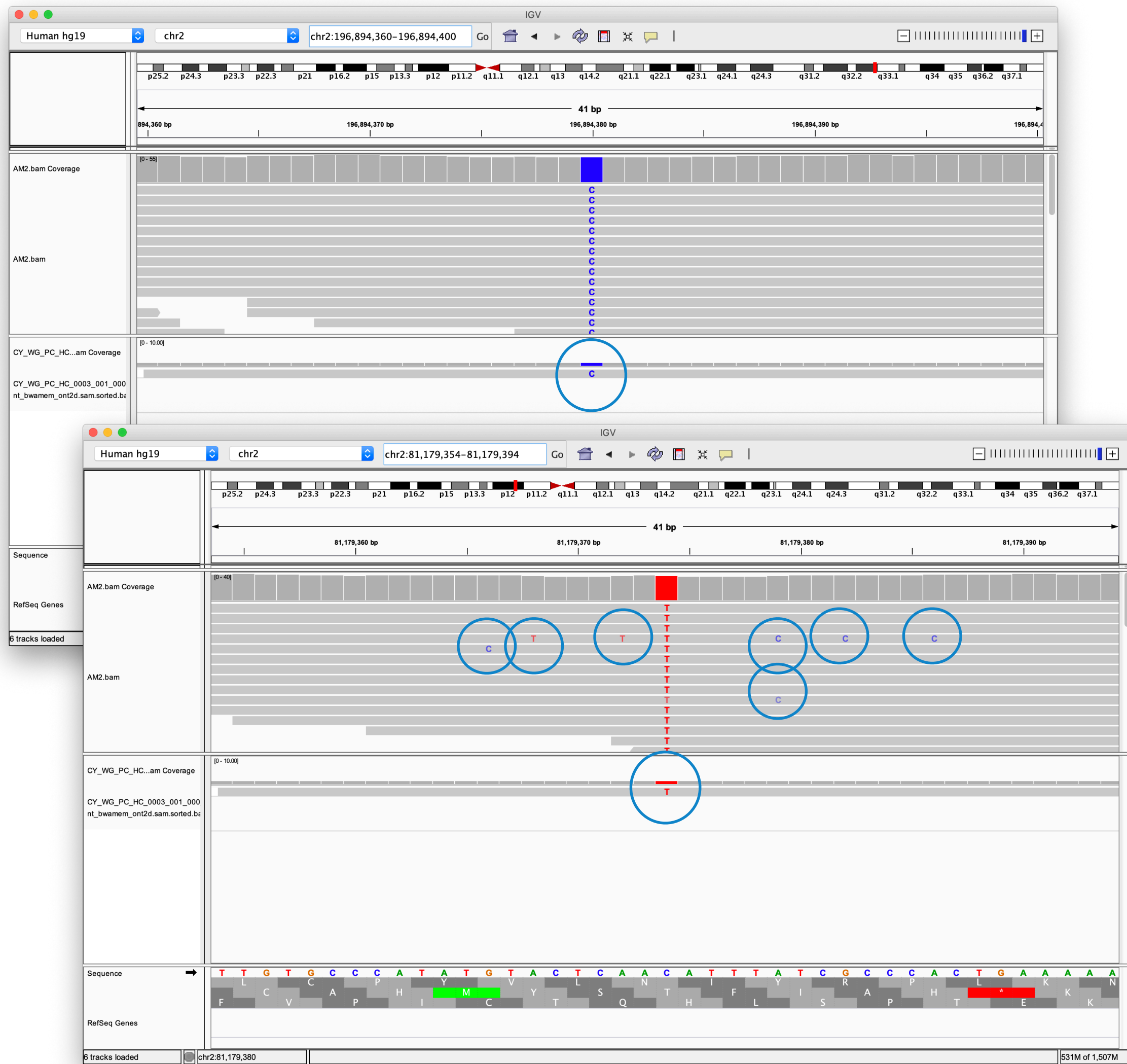
Methylation

Somatic mutation detection using genome-wide CyclomicsSeq



Evaluation of genome-wide CyclomicsSeq with ovarian cancer ascites samples

Genome-Wide CyclomicsSeq

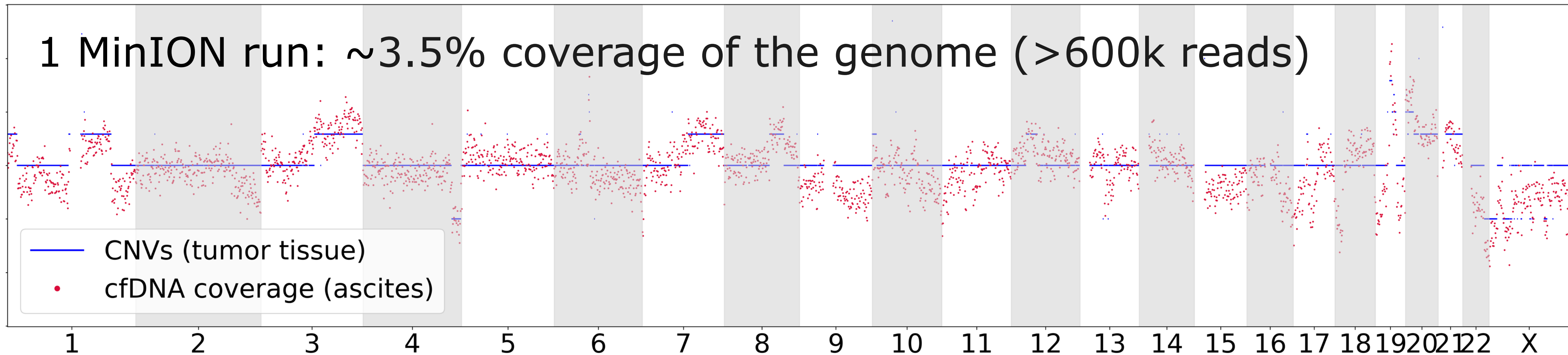


- 3917 sSNVs detected (out of 13231 sSNVs)
- VAF Detection follows allele frequency of the original tumor

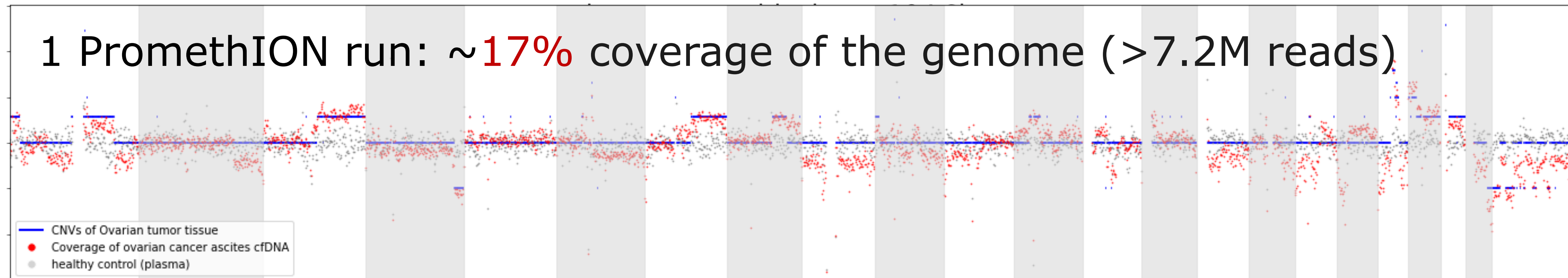
Coverage reveals tumor Copy Number Variations

cfDNA coverage in comparison with tumor CNAs

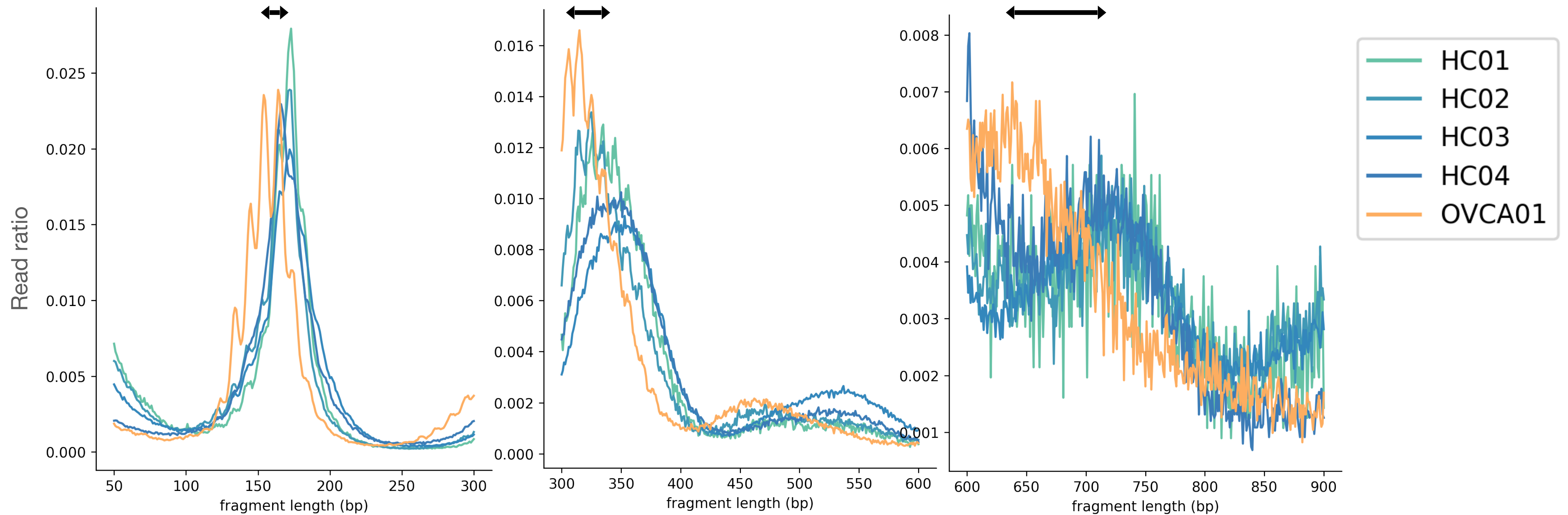
1 MinION run: $\sim 3.5\%$ coverage of the genome ($> 600k$ reads)



1 PromethION run: $\sim 17\%$ coverage of the genome ($> 7.2M$ reads)



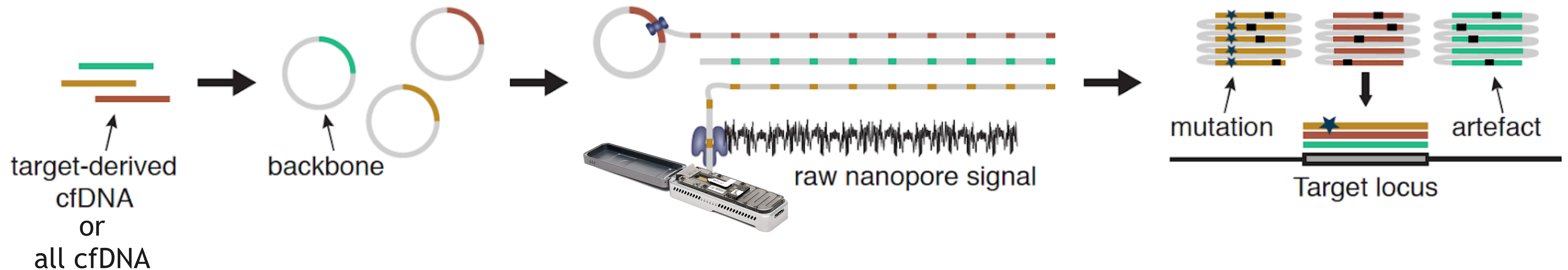
Analysis of cfDNA fragment length in ascites and healthy controls



Distance between peaks becomes larger for longer cfDNA fragments

Summary

- CyclomicsSeq enables accurate detection of cell free tumor DNA
- Genome-Wide CyclomicsSeq allows multi-modal interrogation of the cell free DNA



The lab

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Myrthe Jager
Adrien Melquiond
Luca Santuari
Emmy Wesdorp
Li-ting Chen

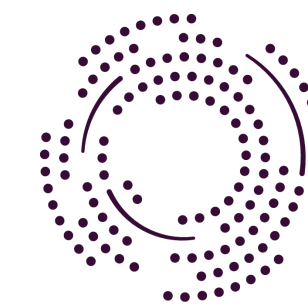
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Nicolle Besselink



Wigard Kloosterman
Dami Rebergen
Alessio Marcozzi



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Joyce van Kuik
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Lot Devriese
Stefan Willems
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David Stoddart
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