Sequencing cell-free DNA

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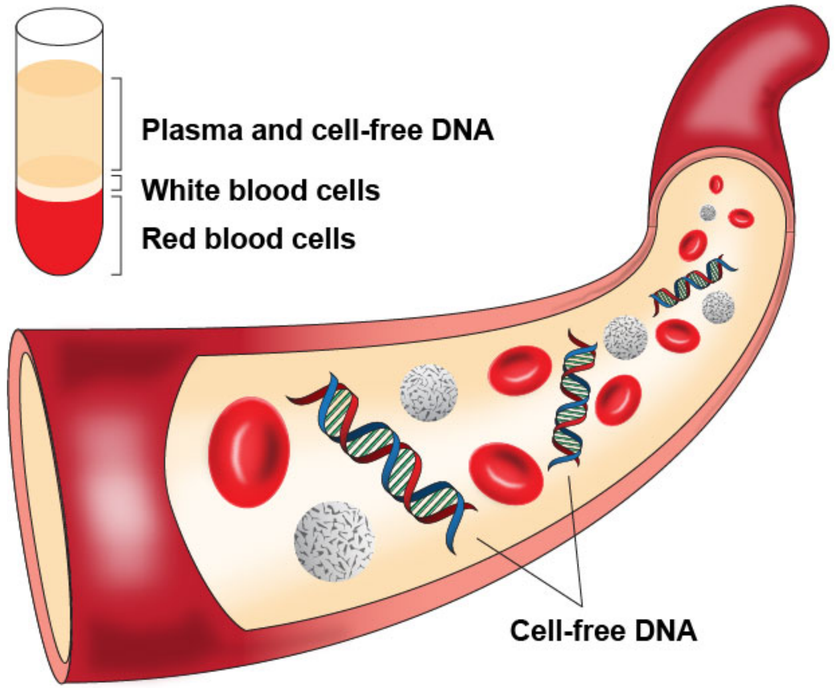
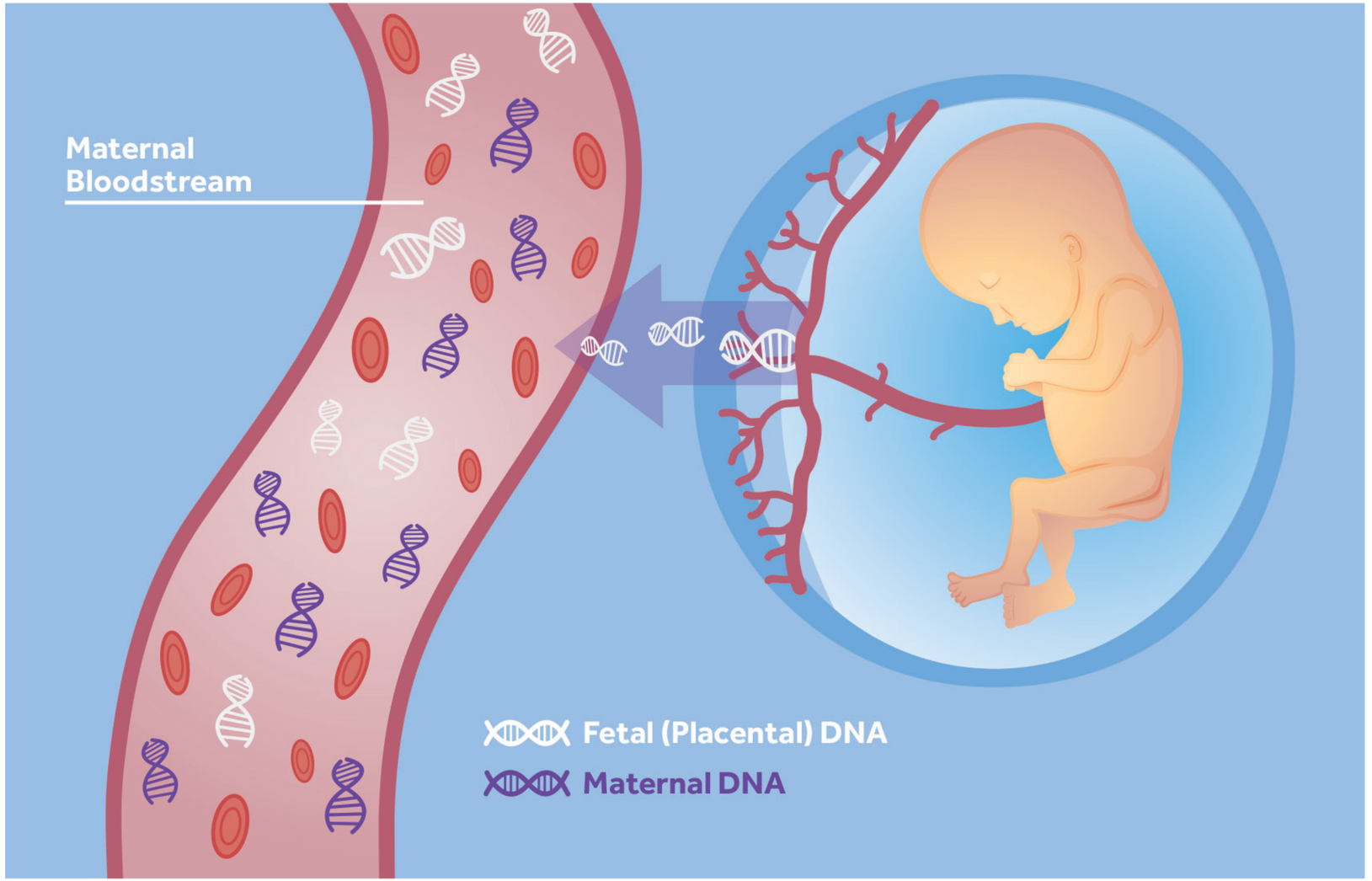
Cell-free DNA (cfDNA)



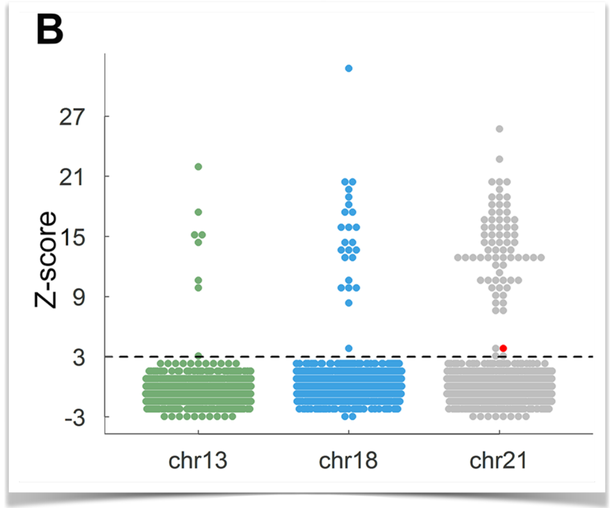
cfDNA half life: <2 hours

Cell-free fetal DNA (cffDNA)

Can be used to perform Non-invasive Prenatal Testing



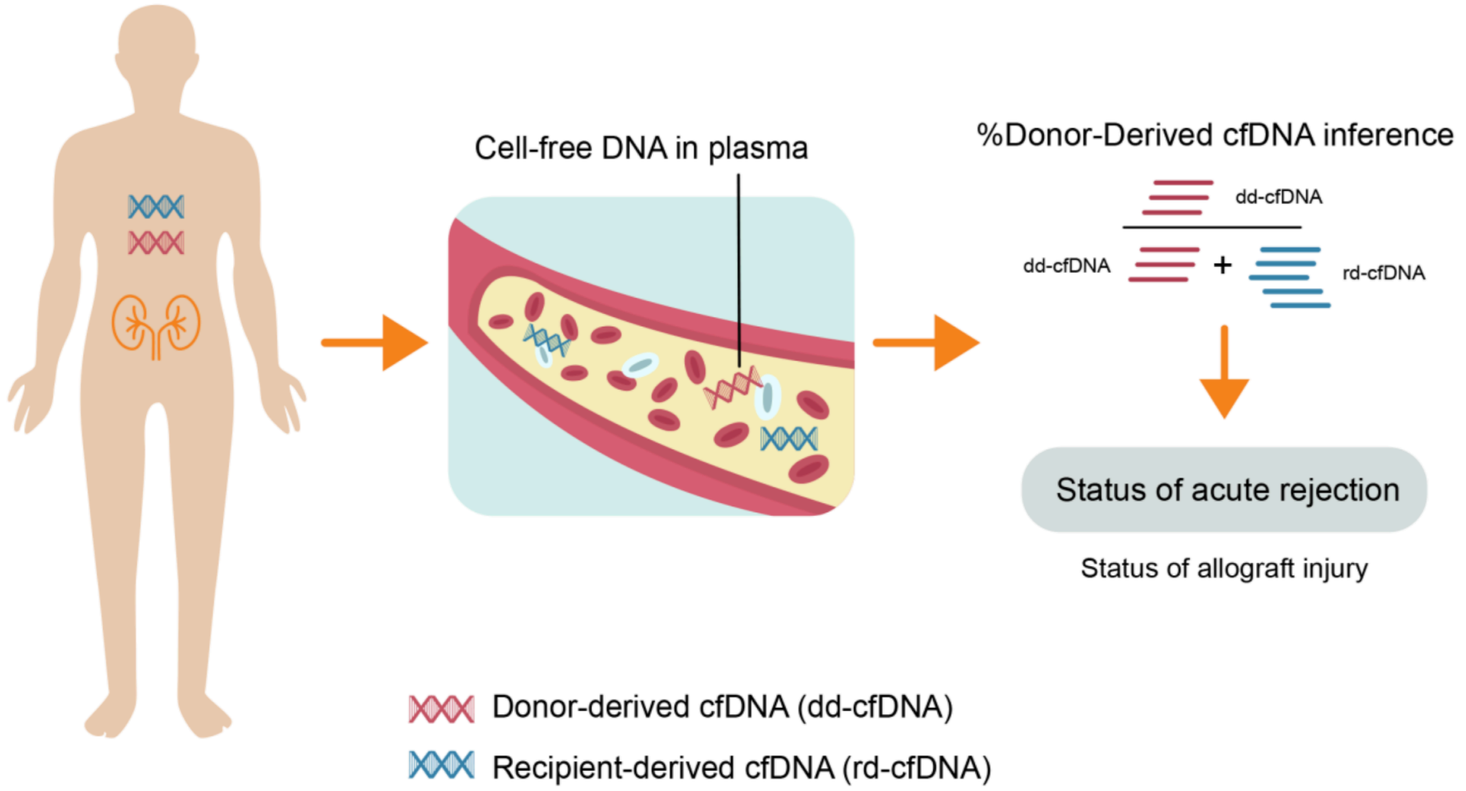
Currently used to detect aneuploidy in fetuses



Can also be used to detect monogenic disorders in fetuses

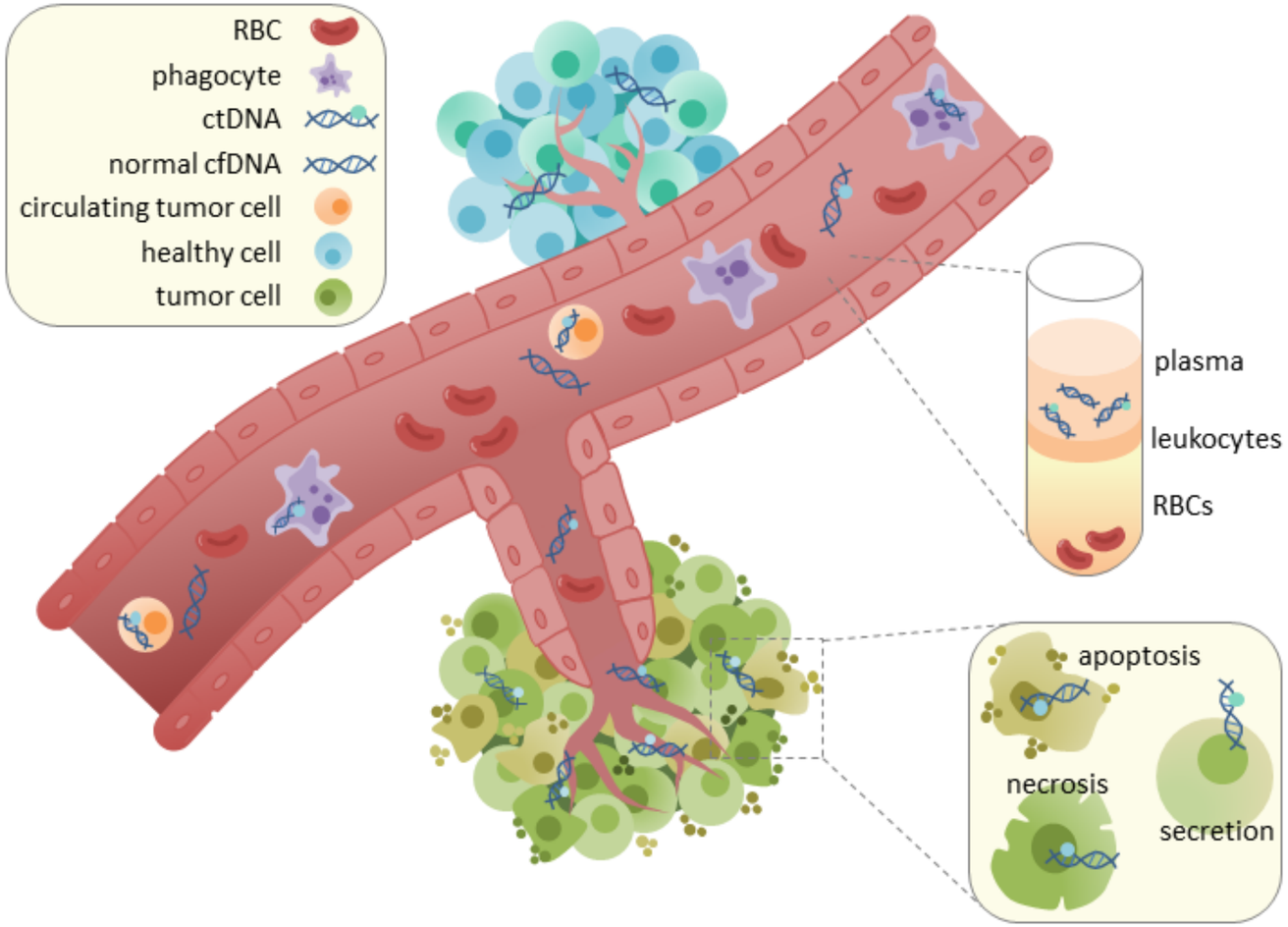
donor-derived cfDNA

Can be used to detect allograft rejection



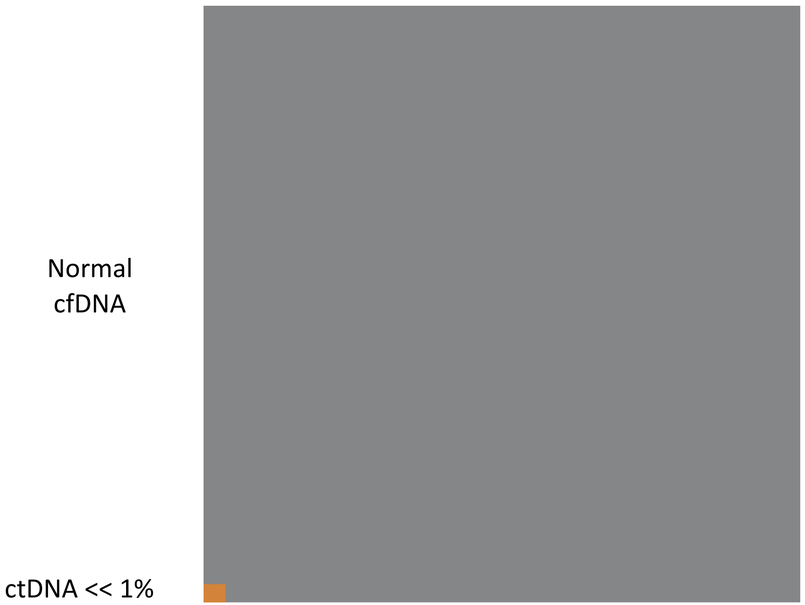
Circulating Tumor DNA (ctDNA)

Can be used for detecting and monitoring Cancer

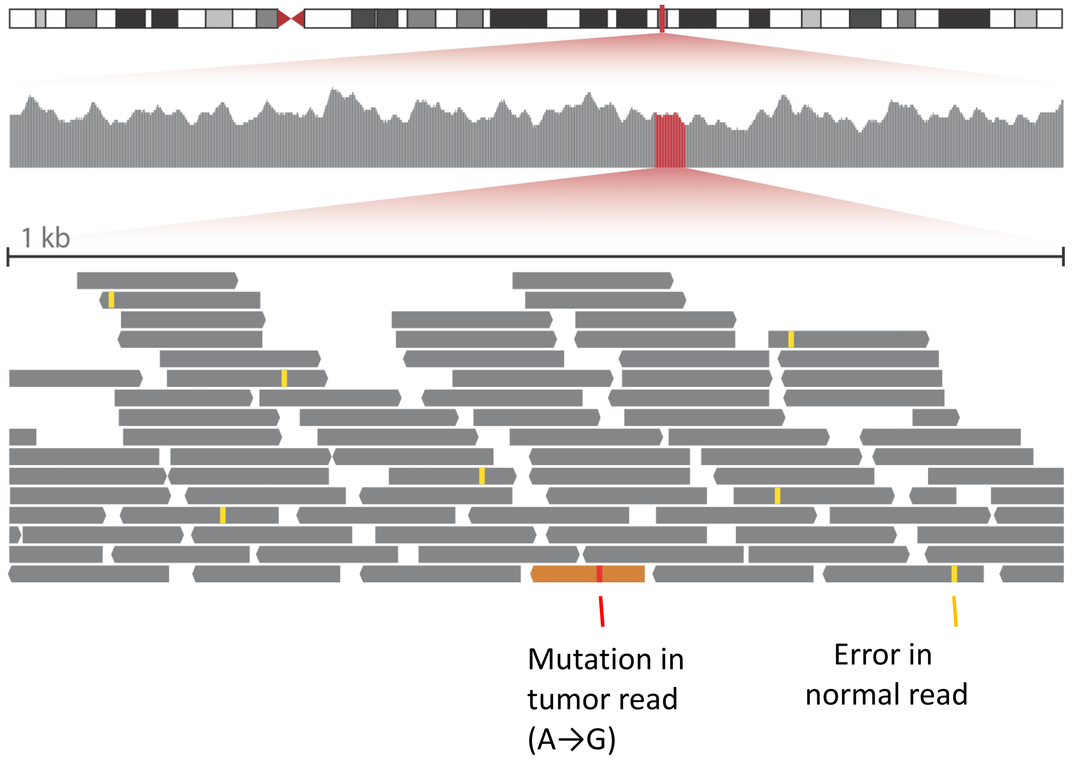


Key challenge in the analysis of ctDNA

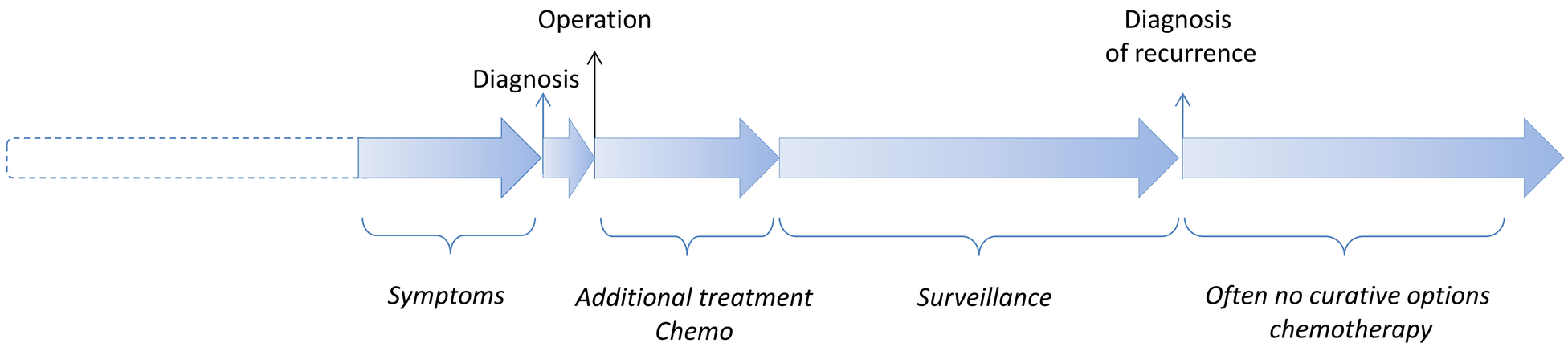
Minute amounts of circulating tumor DNA



Circulating free DNA data



Clinical opportunities of ctDNA

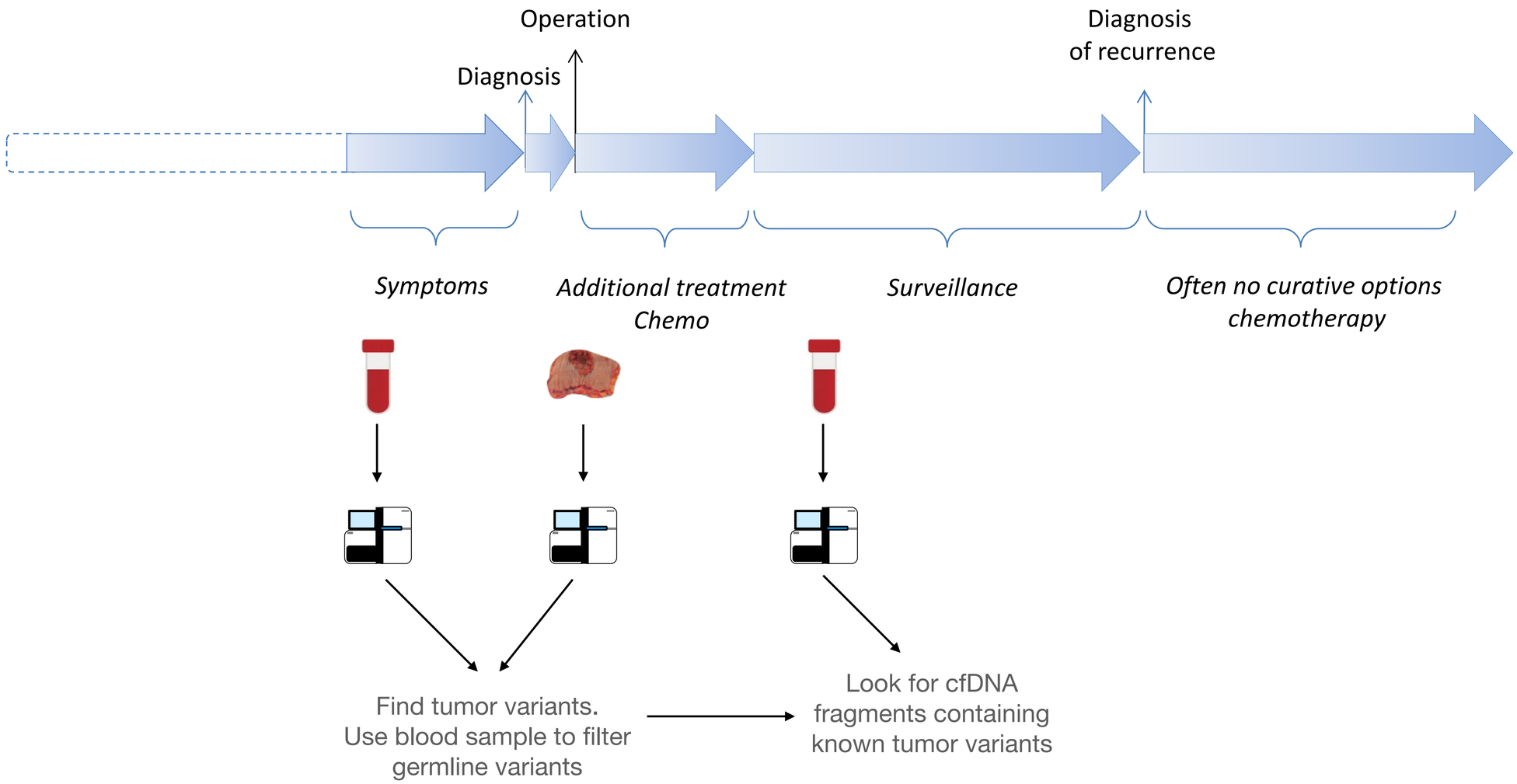


Early detection of cancer

Determine if there is tumor left after surgery and decide if additional treatment is needed

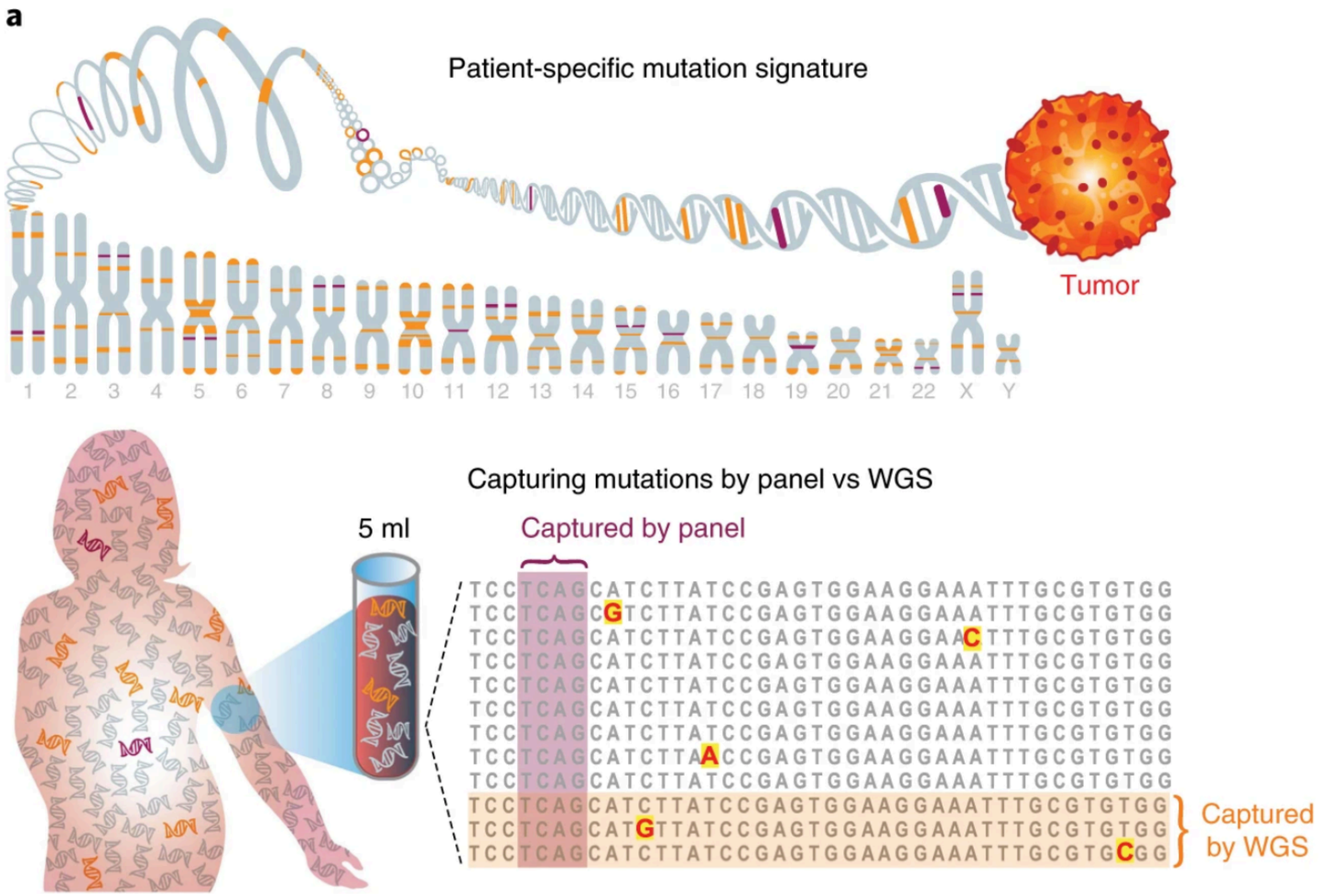
Monitor patients with regular tests to make sure we detect recurrence early.

Tumor informed analysis



Tumor informed analysis

Two strategies: Deep or Wide?

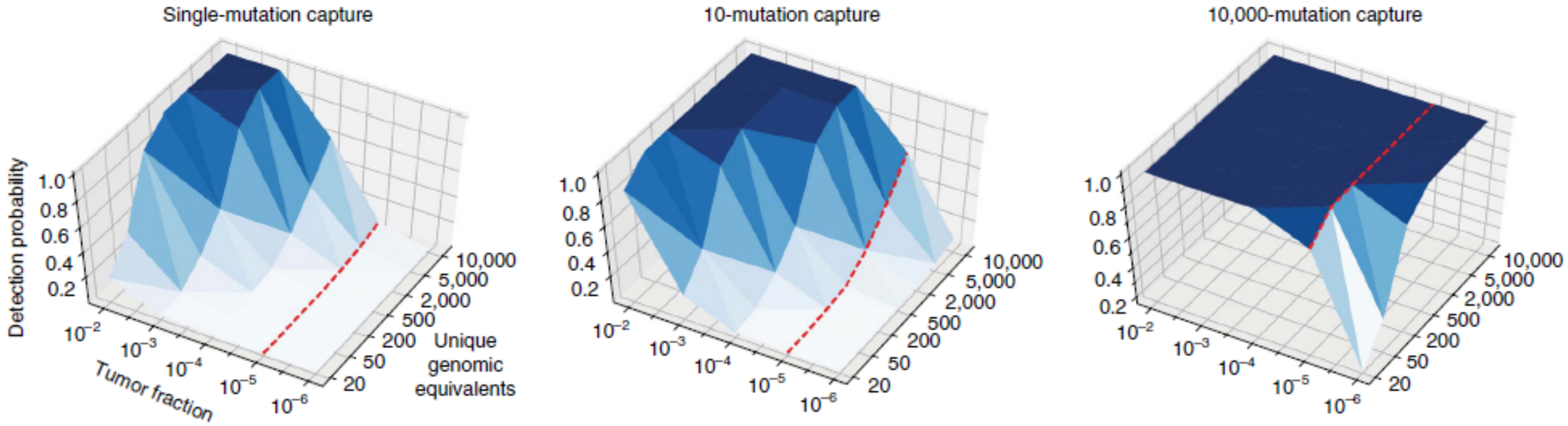


Zviran et al Nat. Medicine, 2020

Tumor informed analysis

Factors affecting sensitivity of ctDNA detection

- Tumor fraction of the total cfDNA amount
- Number of genome equivalents examined (plasma volume)
- Number of markers

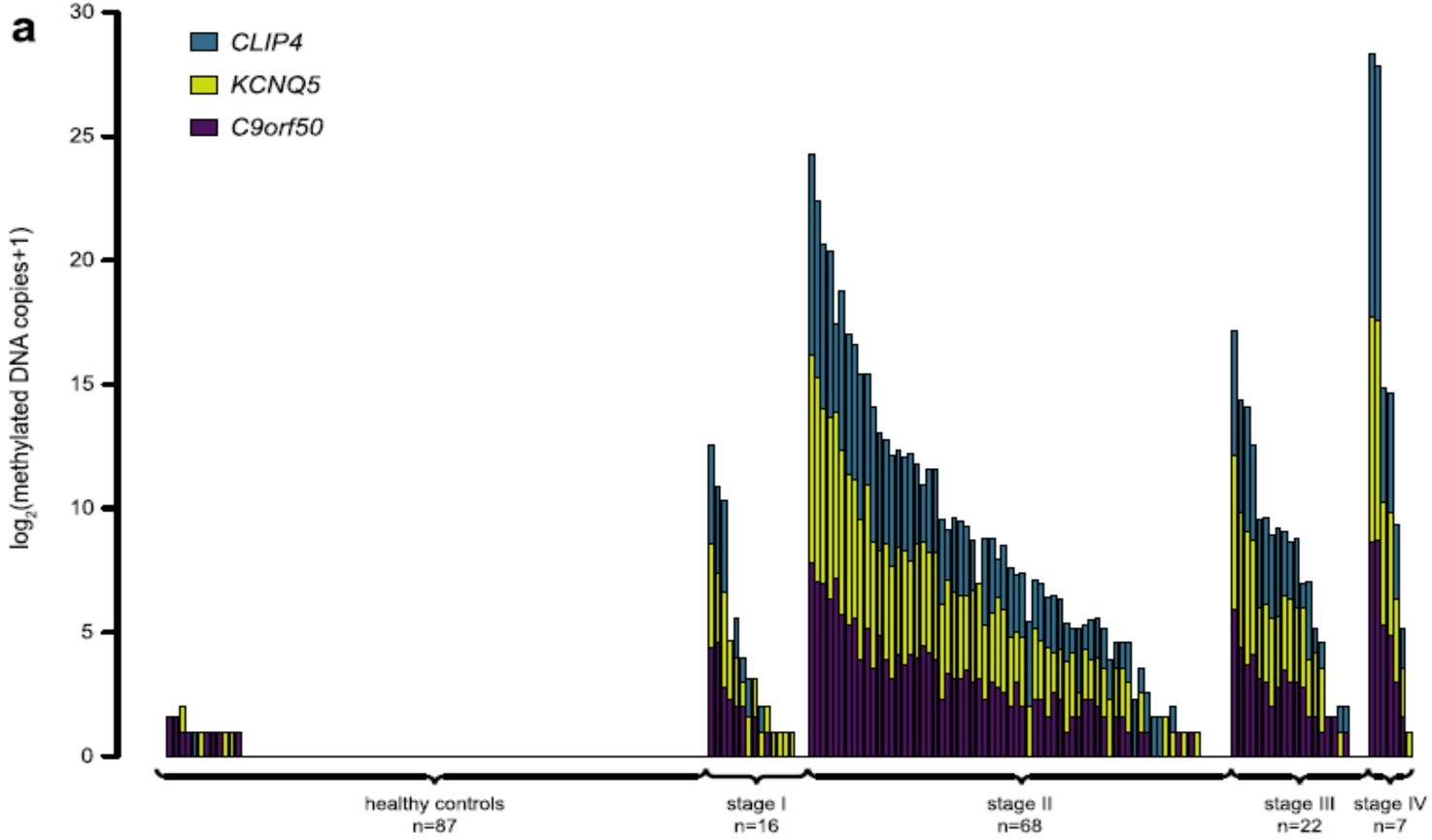


Zviran et al Nat. Medicine, 2020

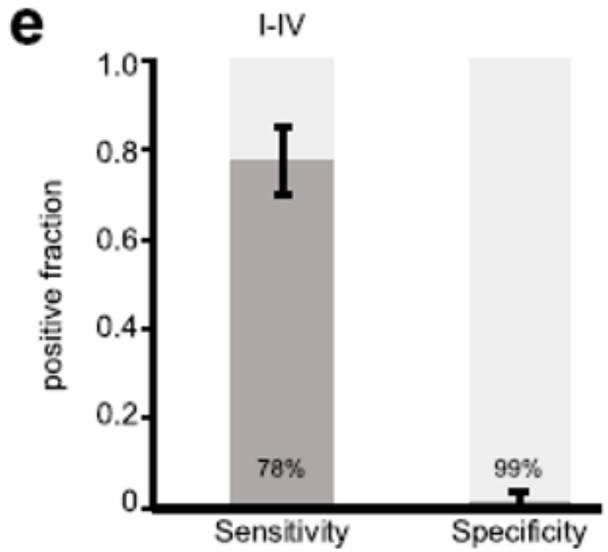
Tumor agnostic approches

Panel of known driver mutations

Example: Test methylation of three genes known to be methylated in cancers:



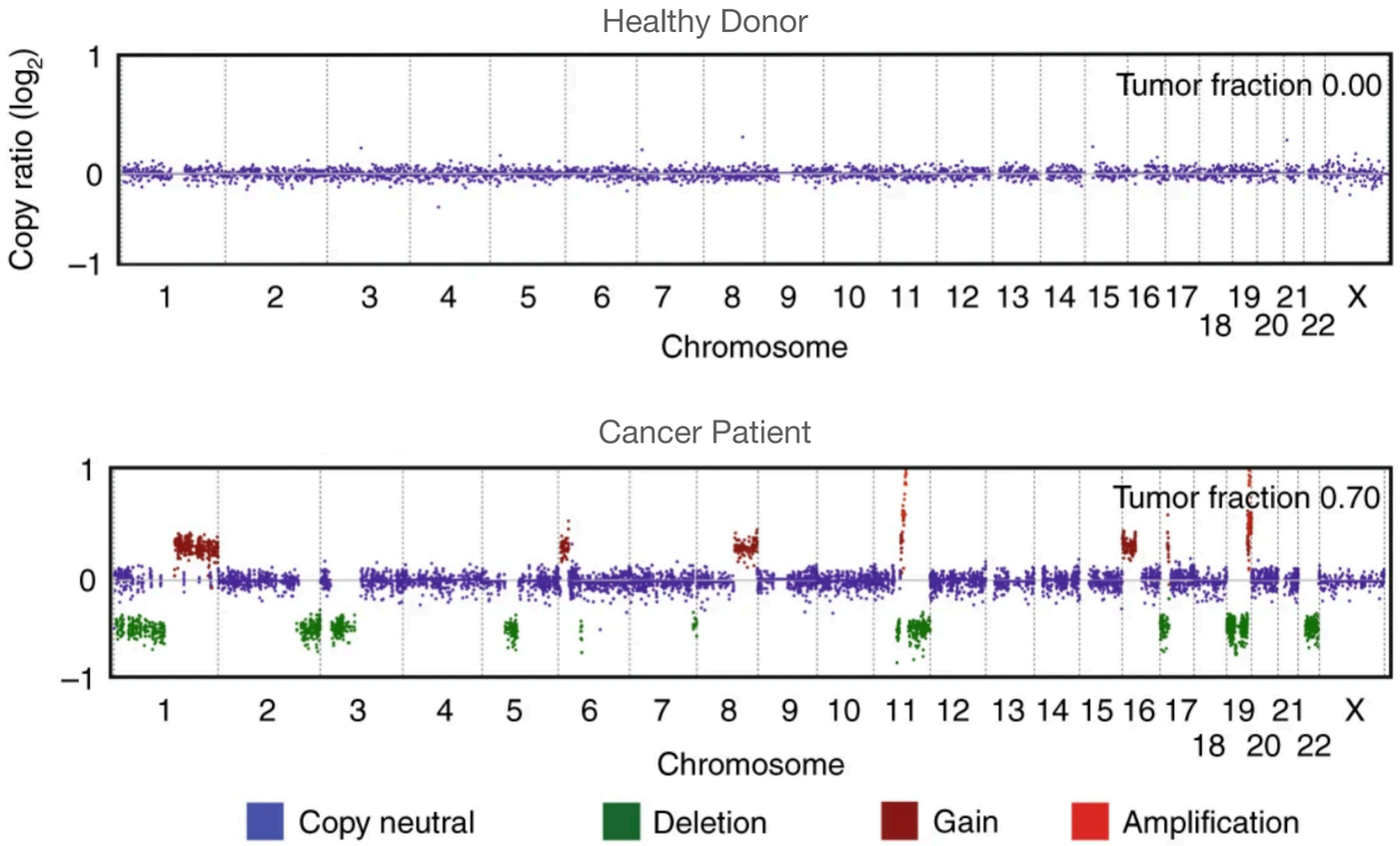
TriMeth test 2/3:



Jensen et al, Clinical Epigenetics, 2019

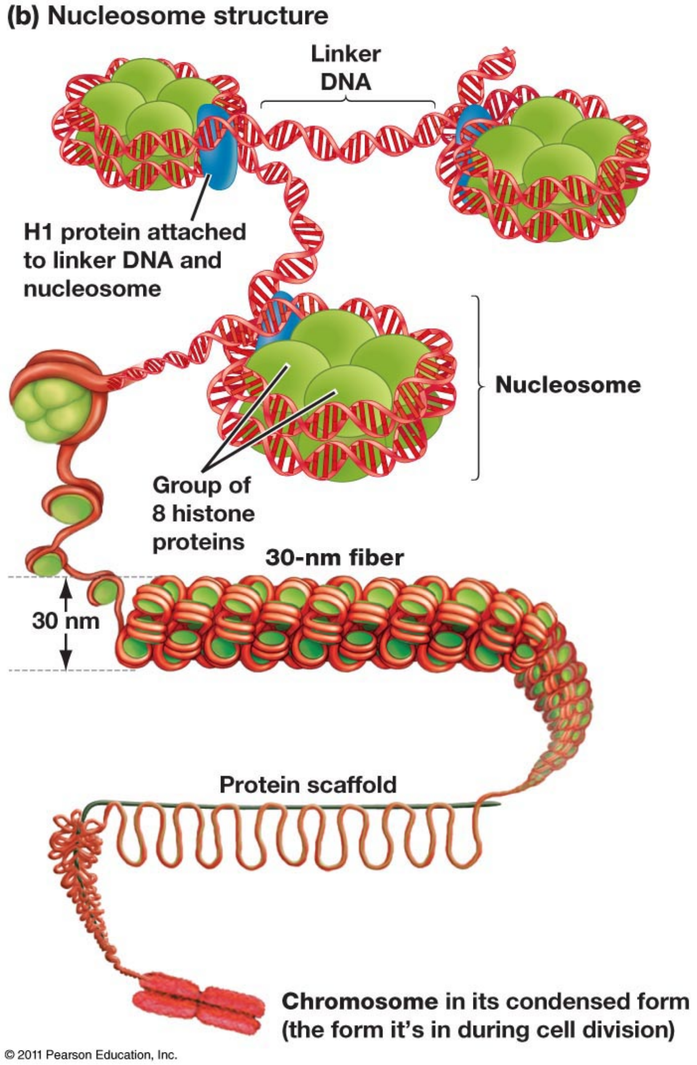
Tumor agnostic approches

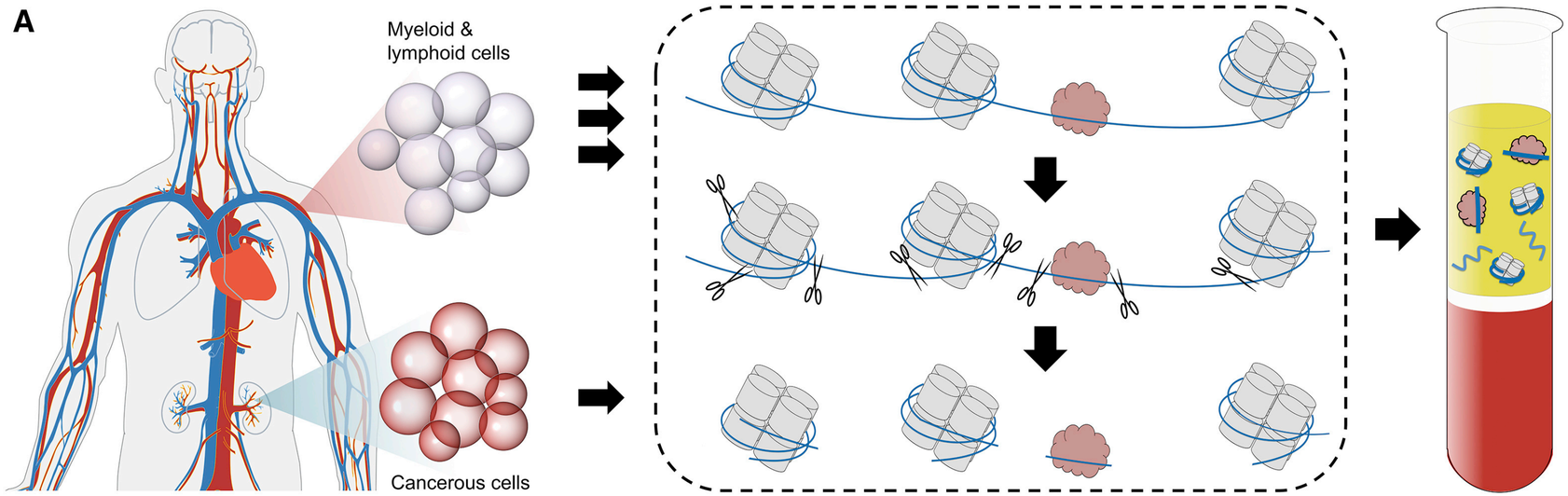
Finding Copy Number Variants (CNVs)



Adalsteinsson et al Nat. Communications, 2017

Extra info besides genetic variants

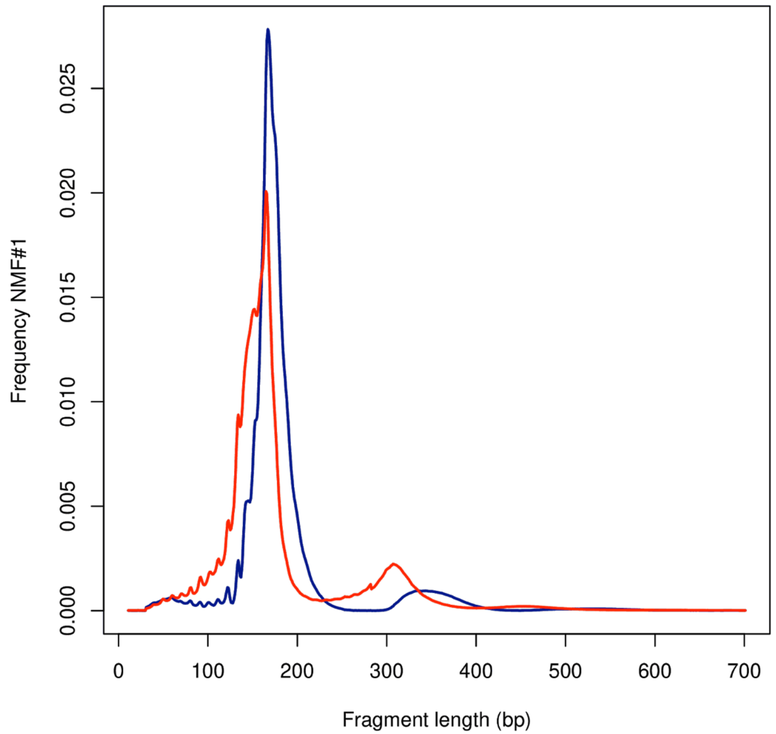




Snyder et al, Cell, 2015

Unlike normal sequencing the fragmentation is not random. I contains information about the epigenetic state of the cell the fragment comes from.

Differences in fragment length

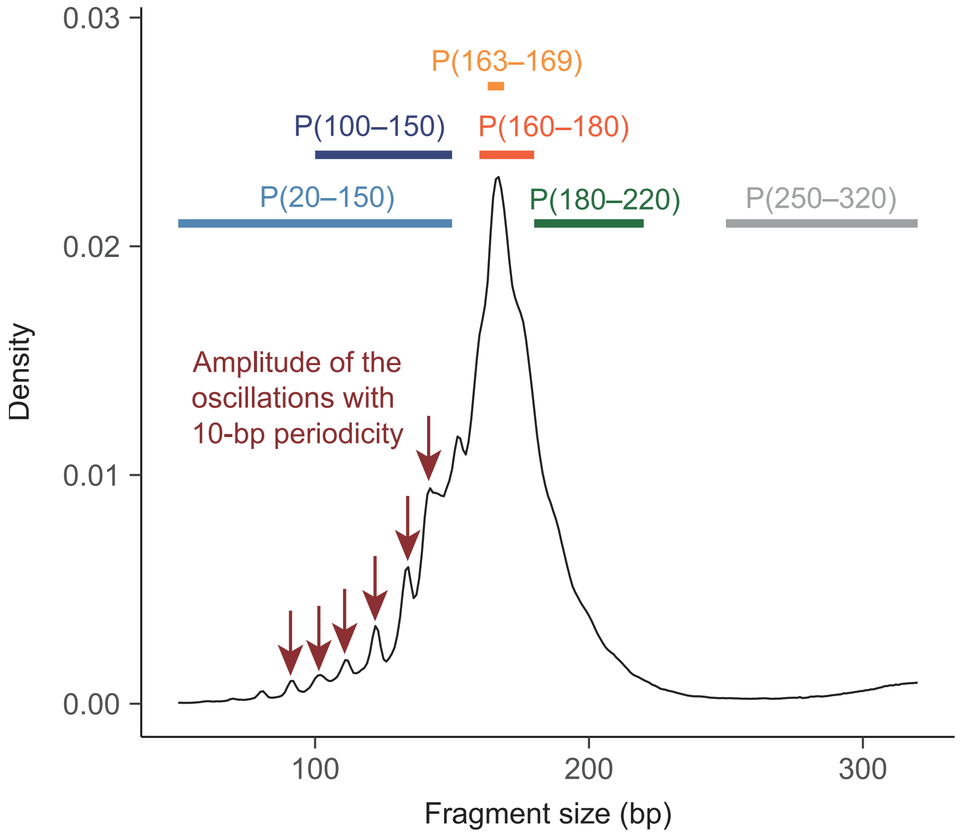


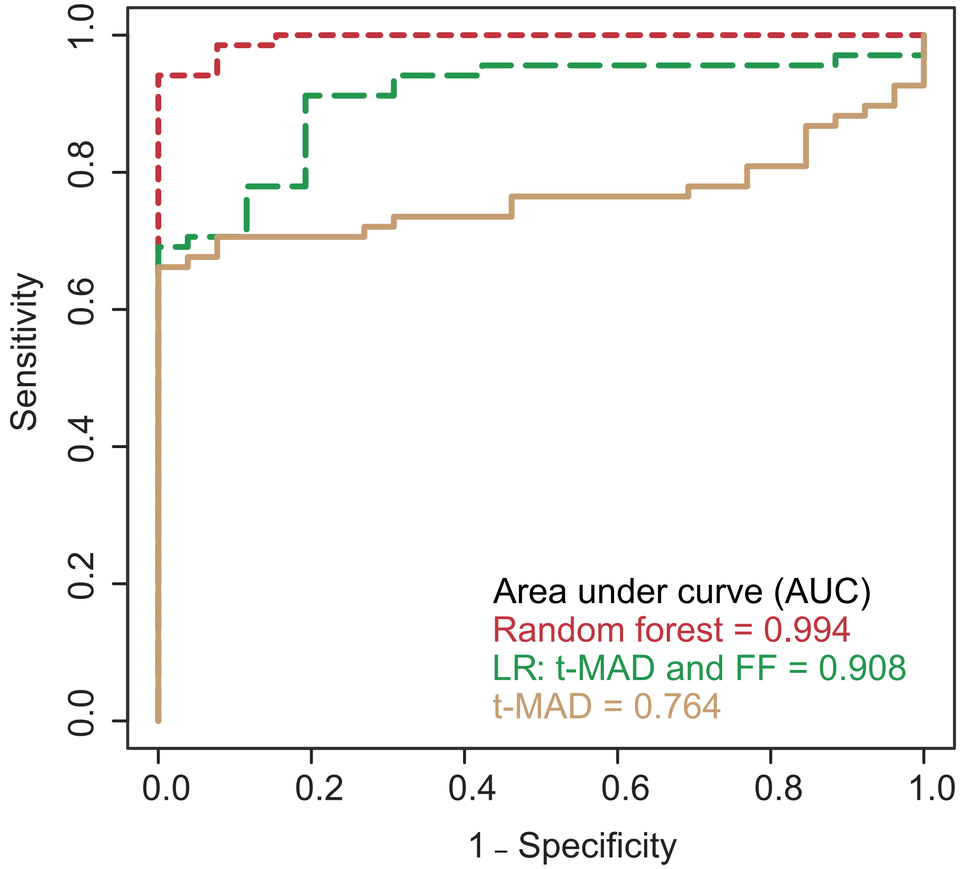
Circulating tumor DNA

Normal circulating free DNA

Differences in fragment length

Using Machine learning to classify samples





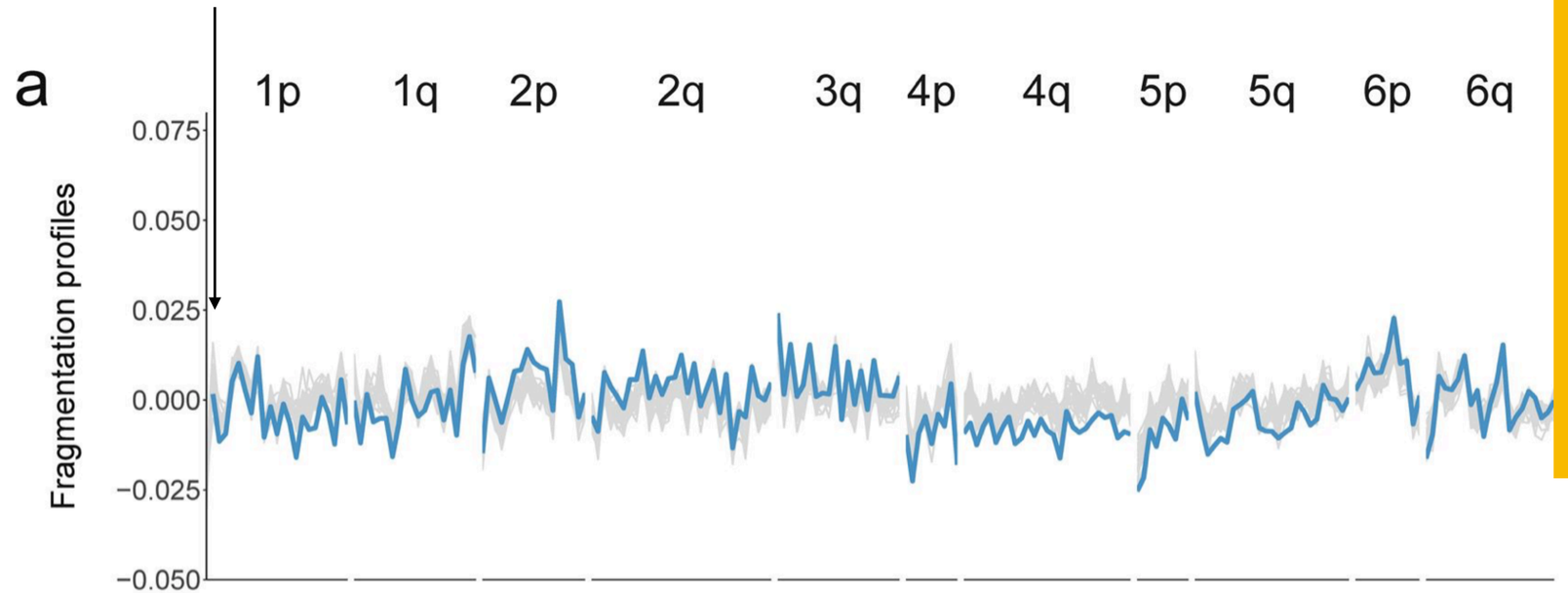
Mouliere et al., Sci Transl Med 2018

Differences in fragment length

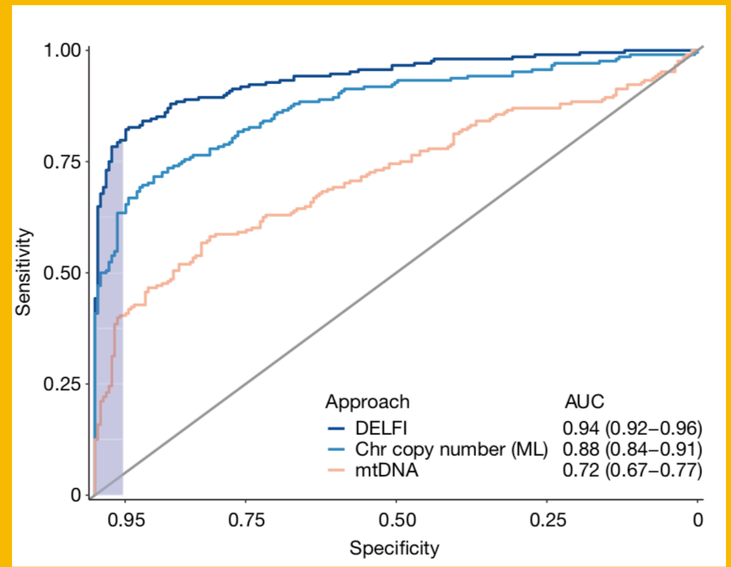
DELFI: DNA Evaluation of Fragments For early Interception

5 MB bins

ratio: small(100–150 bp) / large(151-220 bp)



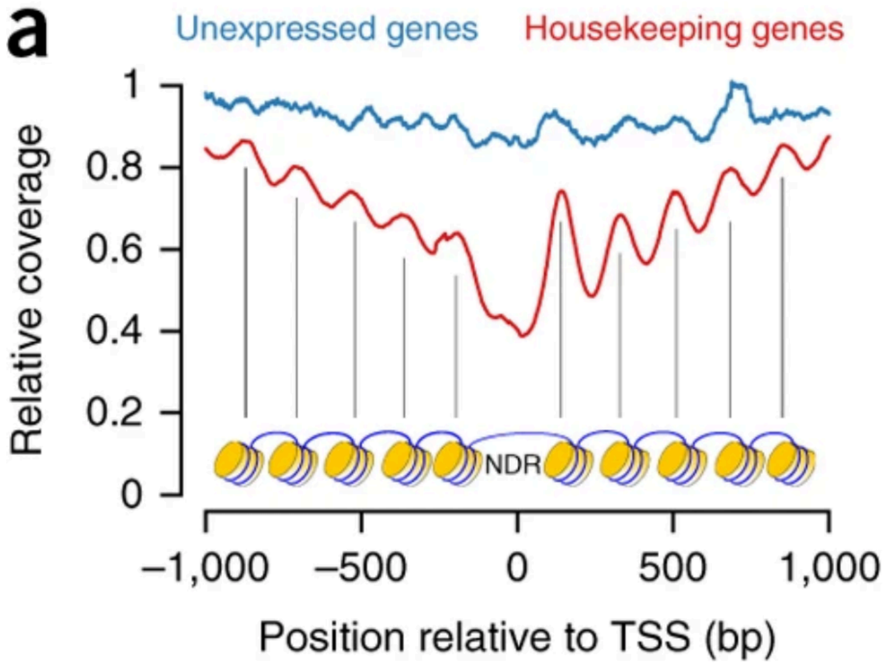
gradient tree boosting



Cristiano et al., Nature 2019

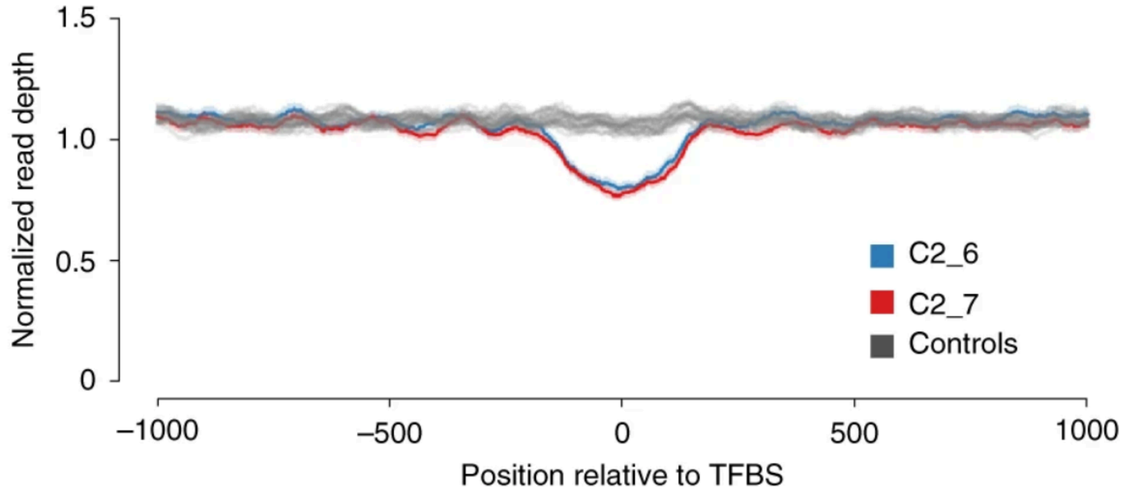
Other epigenetic information we can get from cfDNA

Lower coverage around the Transcription Start Site (TSS) of expressed genes



Ulz et al Nat. Genetics, 2016

Lower coverage around active Transcription Factor Biniding Sites (TFBS)

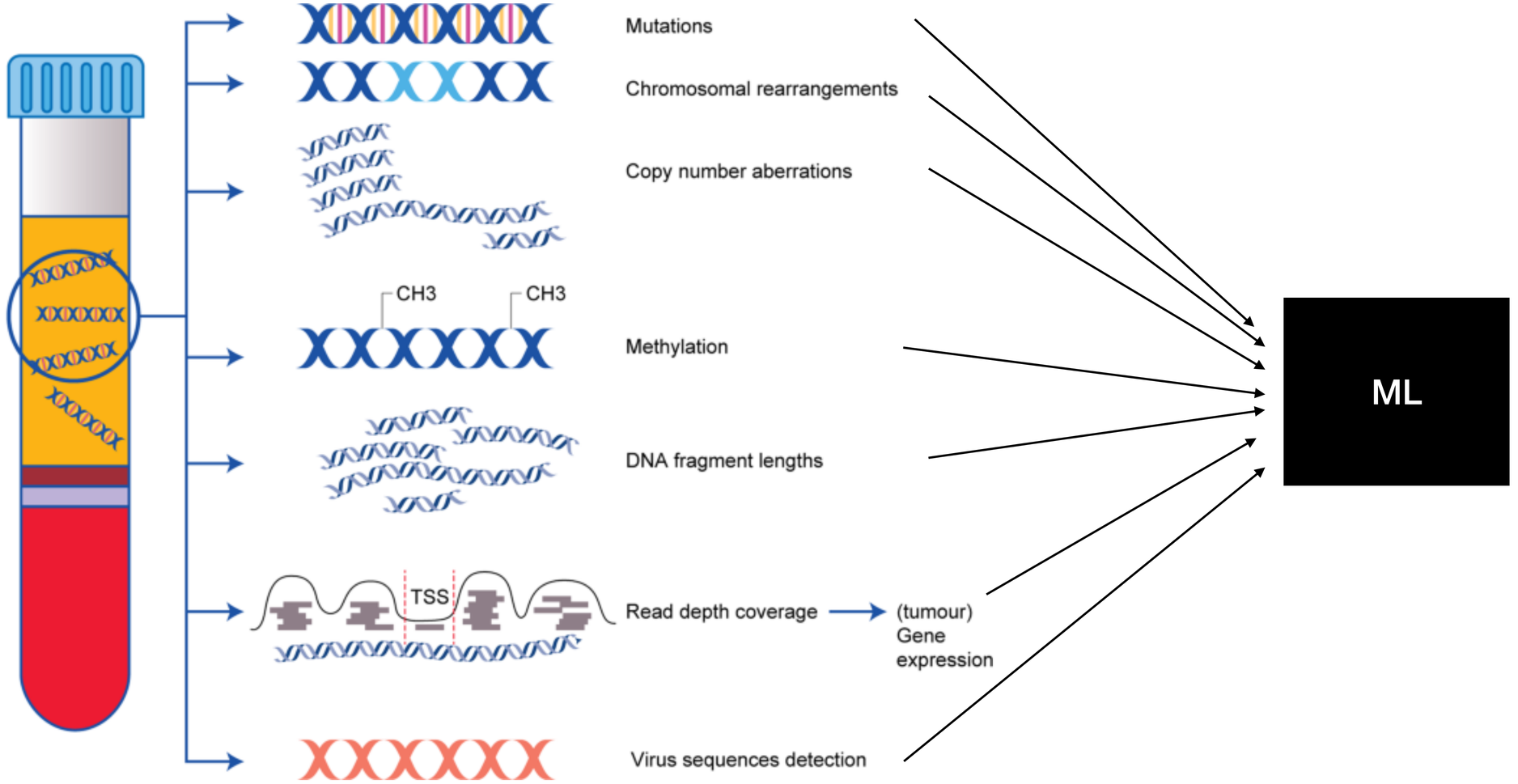


Ulz et al Nat. Communications, 2019

Overview of strategies

	Tumor Informed	Tumor Agnostic
Targeted	**Advantages:** Specificity **Challenges:** Few markers, Only known mutations Biopsy sampling risk Time and cost	**Advantages:** No tumor needed Fast and cheep **Challenges:** Few markers, Specificity / FDR control
Whole Genome Sequencing (WGS)	**Advantages:** Specificity Many Markers **Challenges:** Only known mutations Biopsy sampling risk Time and cost	**Advantages:** No tumor needed Fast Many possible features **Challenges:** New methods needed Specificity / FDR control

The future?



Tumor agnostic WGS strategy combining many different features

Keller et al BJC, 2020