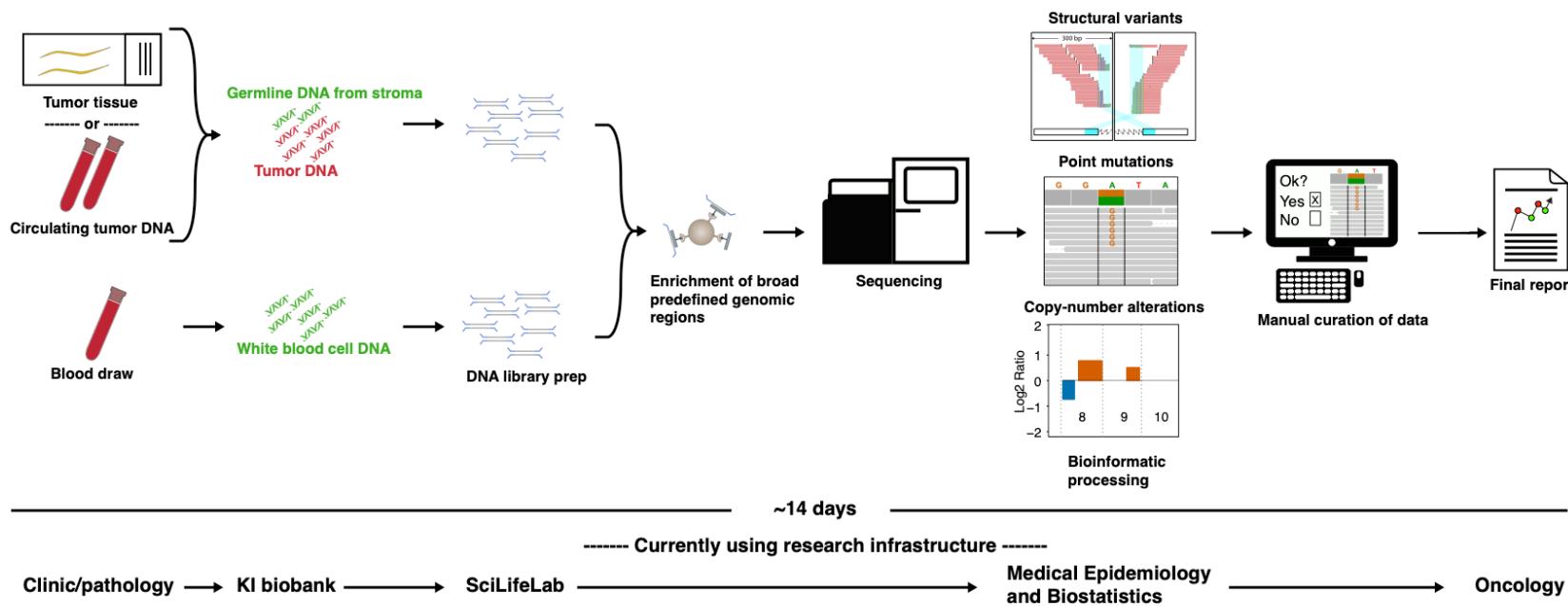


Practical considerations for performing cancer genomics



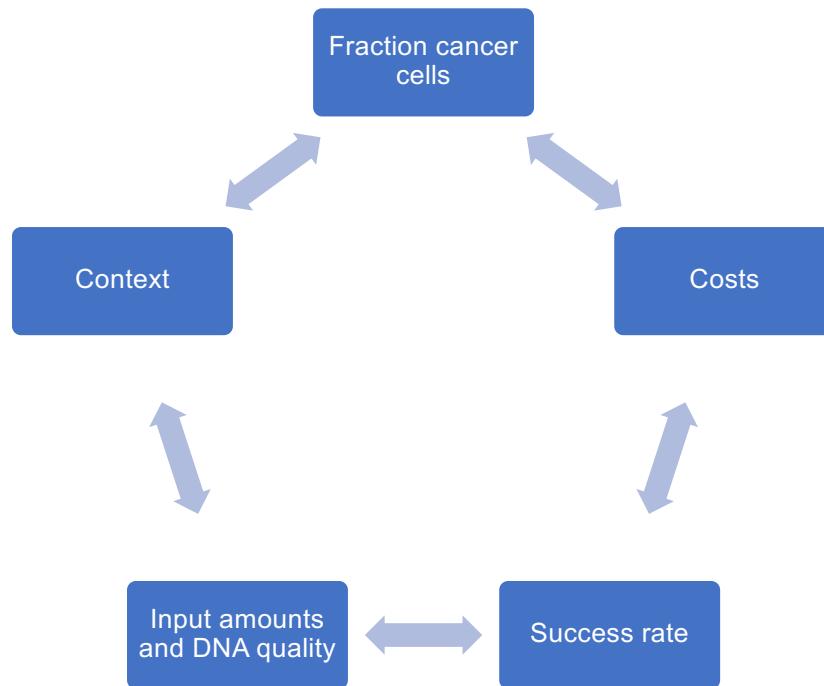
Karolinska
Institutet

Practical considerations for performing cancer genomics



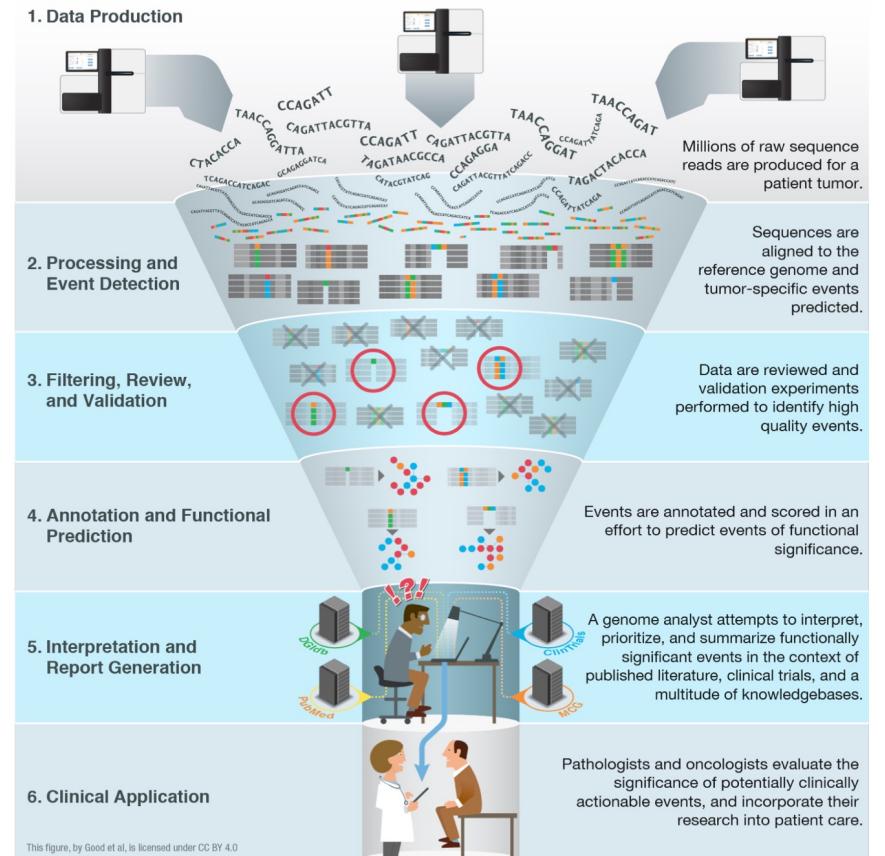
Examples given for DNA-seq but the same applies for RNA-seq

How to sequence depends on many things ..



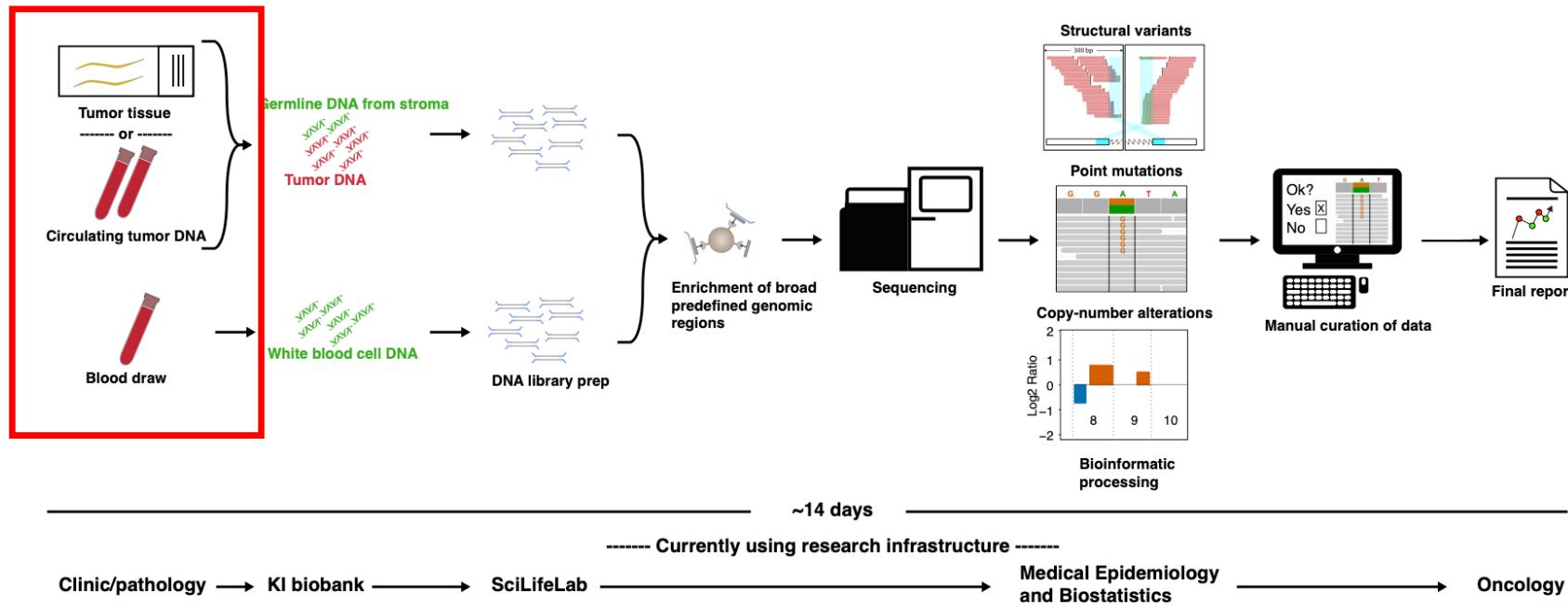
Context

- **Research**
 - Find new genomic features/associations in “large” cohorts
 - Accept a relatively high false positive rate
 - Turnover is flexible
- **Clinical**
 - Deliver on time – as high success rate as possible.
 - Predefined variants/events of interest
 - Not acceptable with false positives
 - Need to be honest about a potentially false negative situation
 - If cost-efficient, “research” data can be generated simultaneously



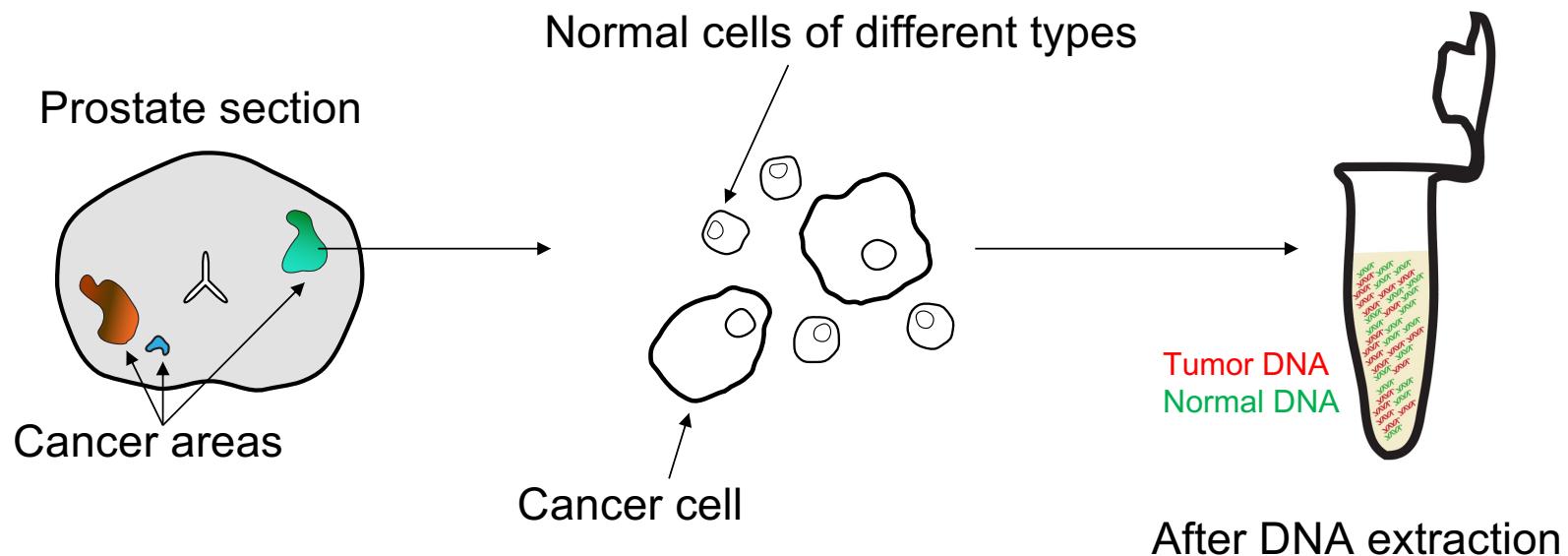
Good BM et al. Genome Biology 2014.

Practical considerations for performing cancer genomics

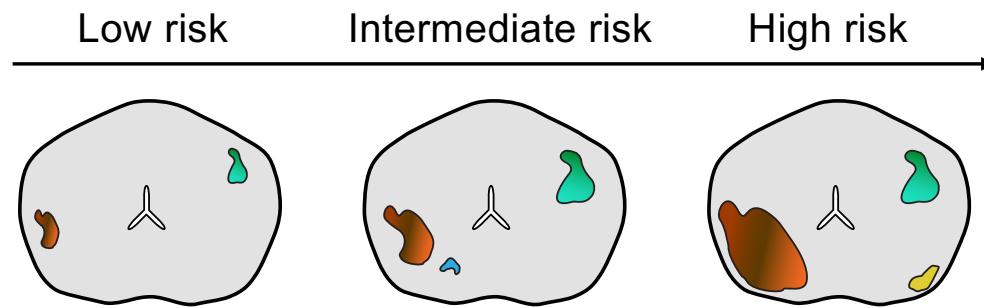


Starting material – heterogeneity and purity

- Never 100% pure cancer cells
- Vary from 0.0001 – 0.7

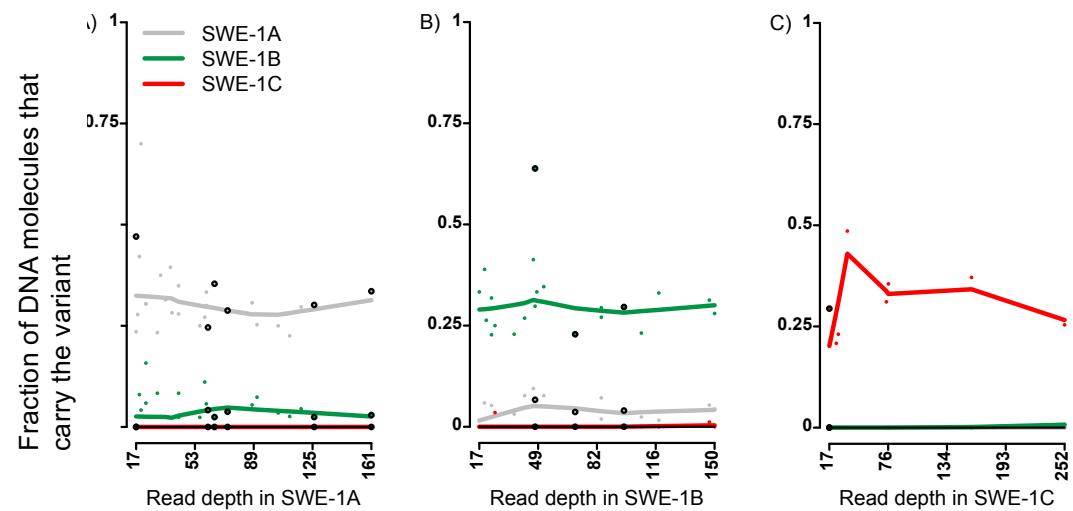
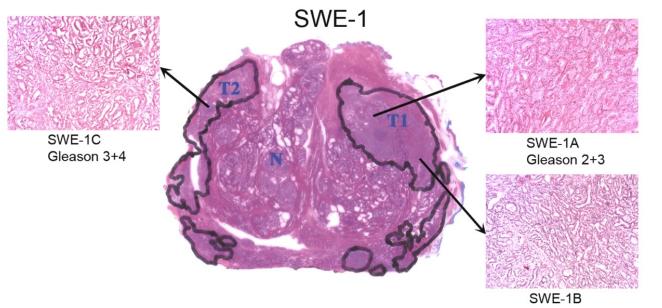


Tumor heterogeneity – primary cancer



Tumor heterogeneity – primary cancer

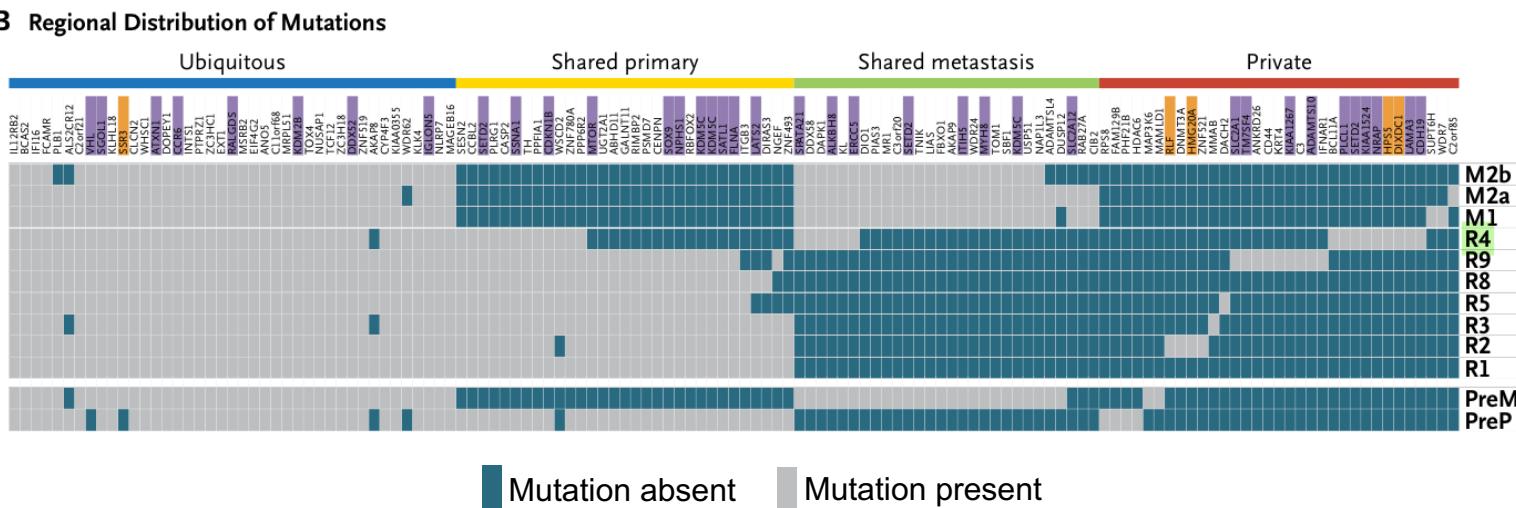
Non-aggressive localised prostate cancer may harbor somatically independent tumors.



Exome Sequencing of Prostate Cancer Supports the Hypothesis of Independent Tumour Origins, Euro Uro 2012

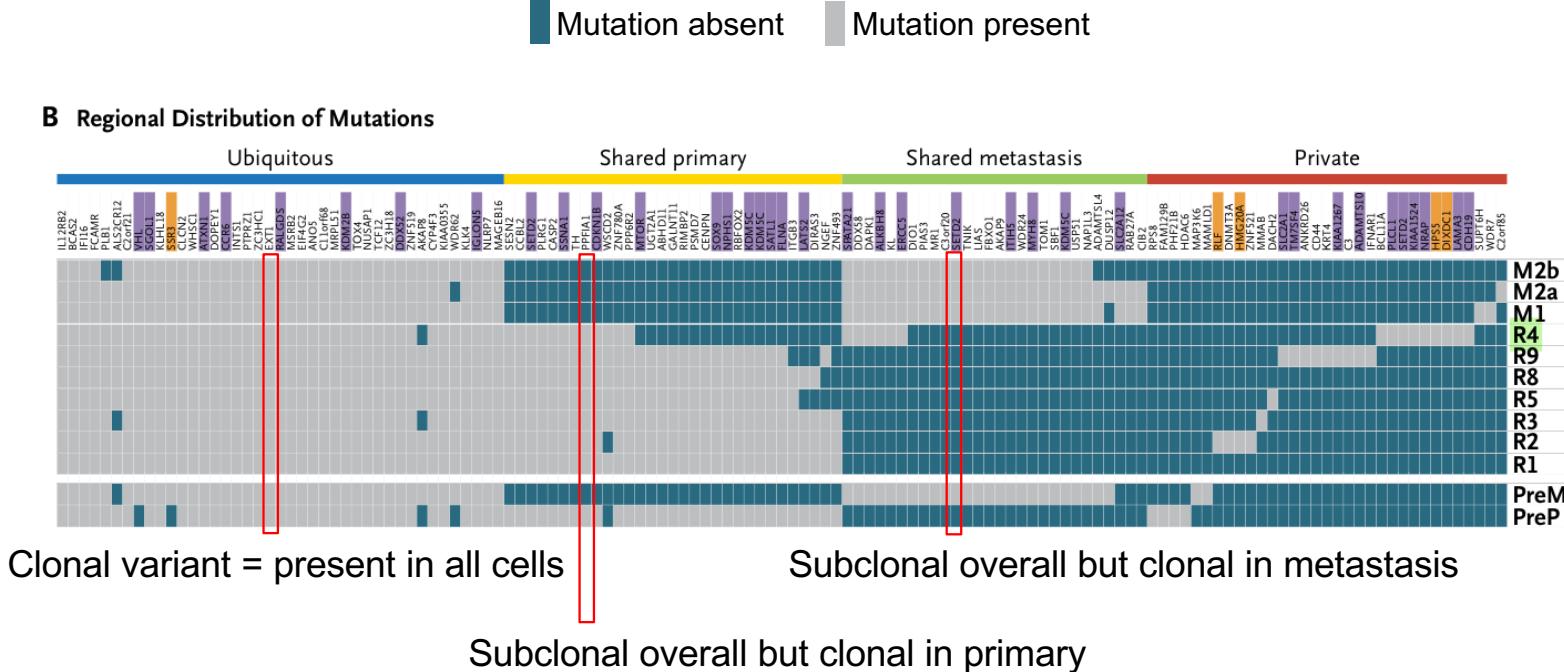
Tumor heterogeneity – advanced cancer

- Heterogeneity decreases with stage and grade



Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing, NEJM 2012

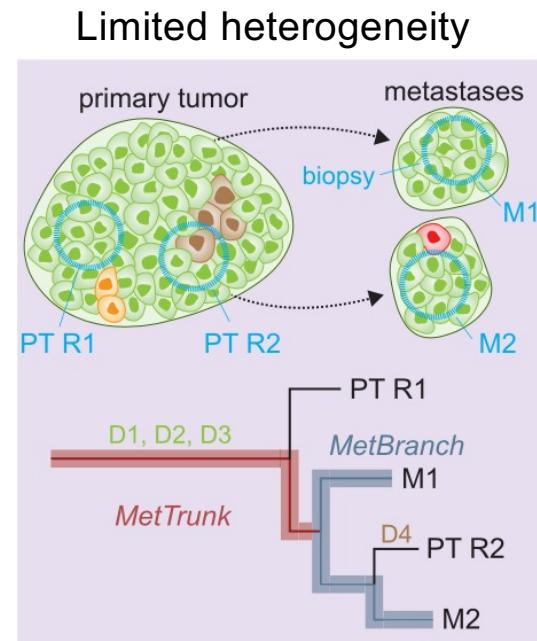
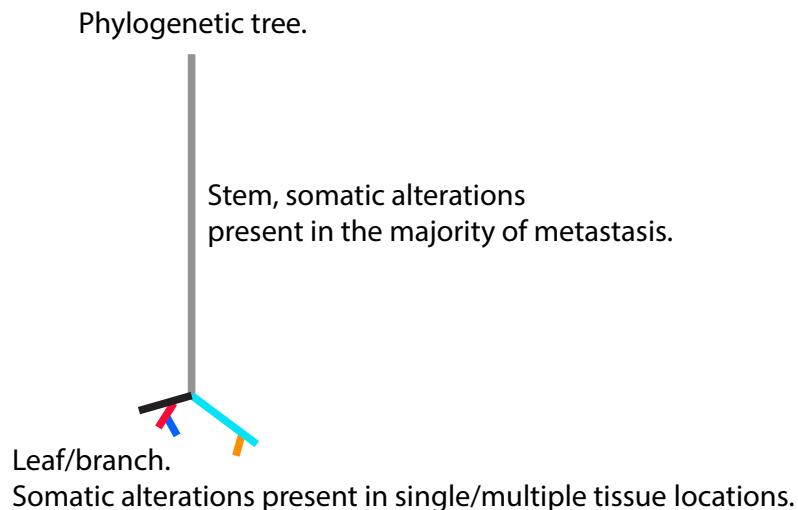
Tumor heterogeneity – advanced cancer



Intratumor Heterogeneity and Branched Evolution Revealed by Multiregion Sequencing, NEJM 2012

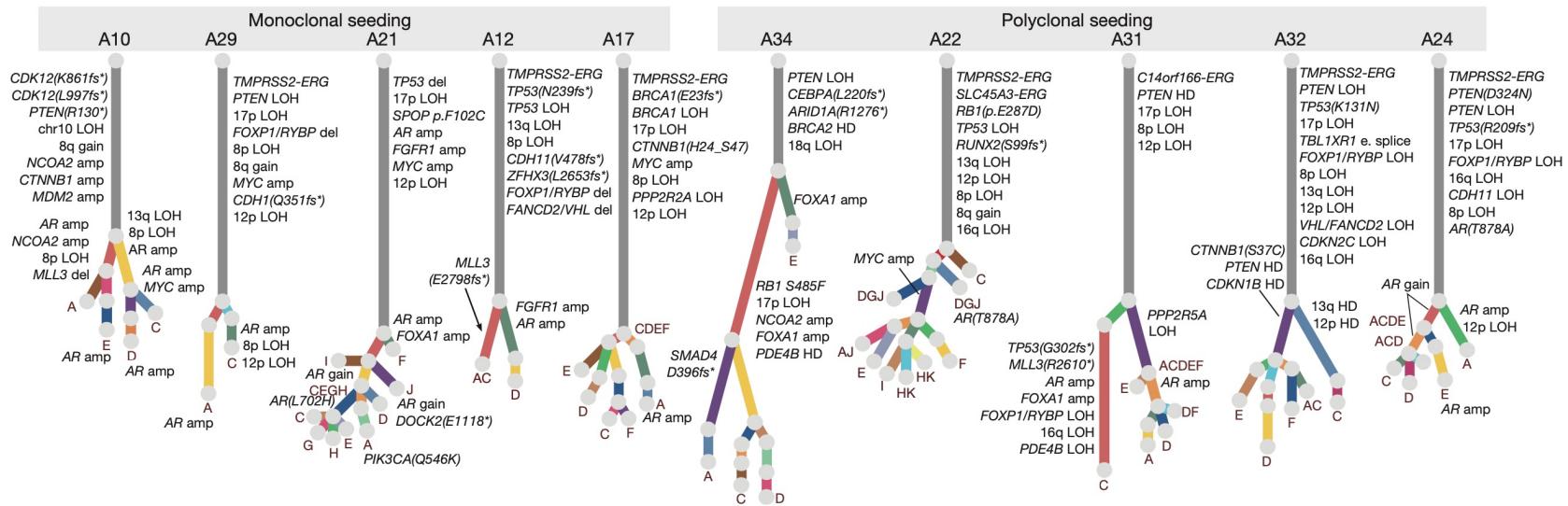
Tumor heterogeneity – de novo metastatic cancer

- De novo metastatic disease



Minimal functional driver gene heterogeneity
among untreated metastases, Science 2020

Tumor heterogeneity – late stage metastatic cancer



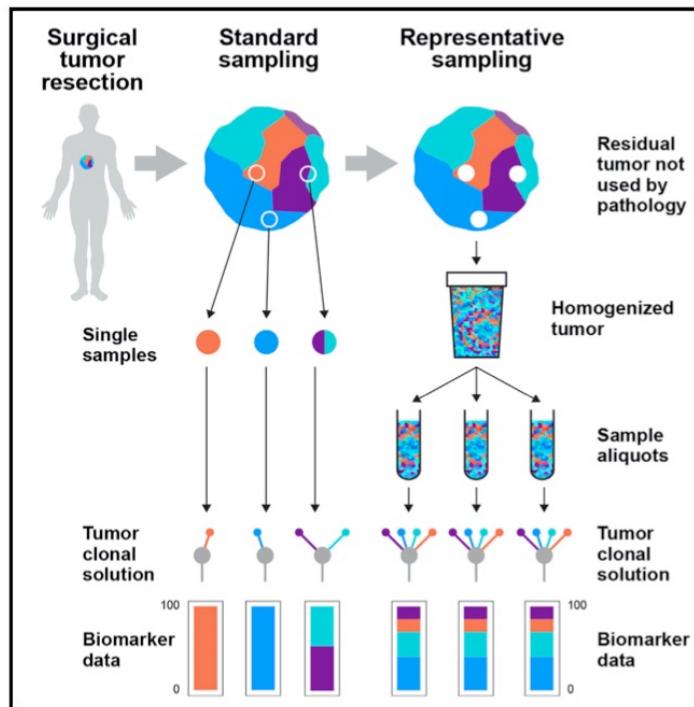
- mCRPC rapid autopsy cohort.
- WGS on multiple metastasis / case.
- Heterogeneity occurred mostly in AR, due to the evolutionary pressure of treatment.
- Supported by data from the Hartwig foundation.

The evolutionary history of lethal metastatic prostate cancer, Nature, 2015.

Limited evolution of the actionable metastatic cancer genome under therapeutic pressure, Nature Medicine, 2021.

Tumor heterogeneity – a practical suggestion

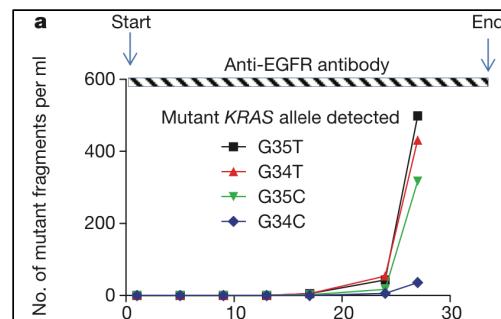
- Potential remedy – sequence a homogenized multiregional sample
- If not possible – only regarding "clonal" variants improves the situation
 - Difficult if cancer DNA fraction is low



Representative Sequencing: Unbiased Sampling of Solid Tumor Tissue, Cell Reports
2020

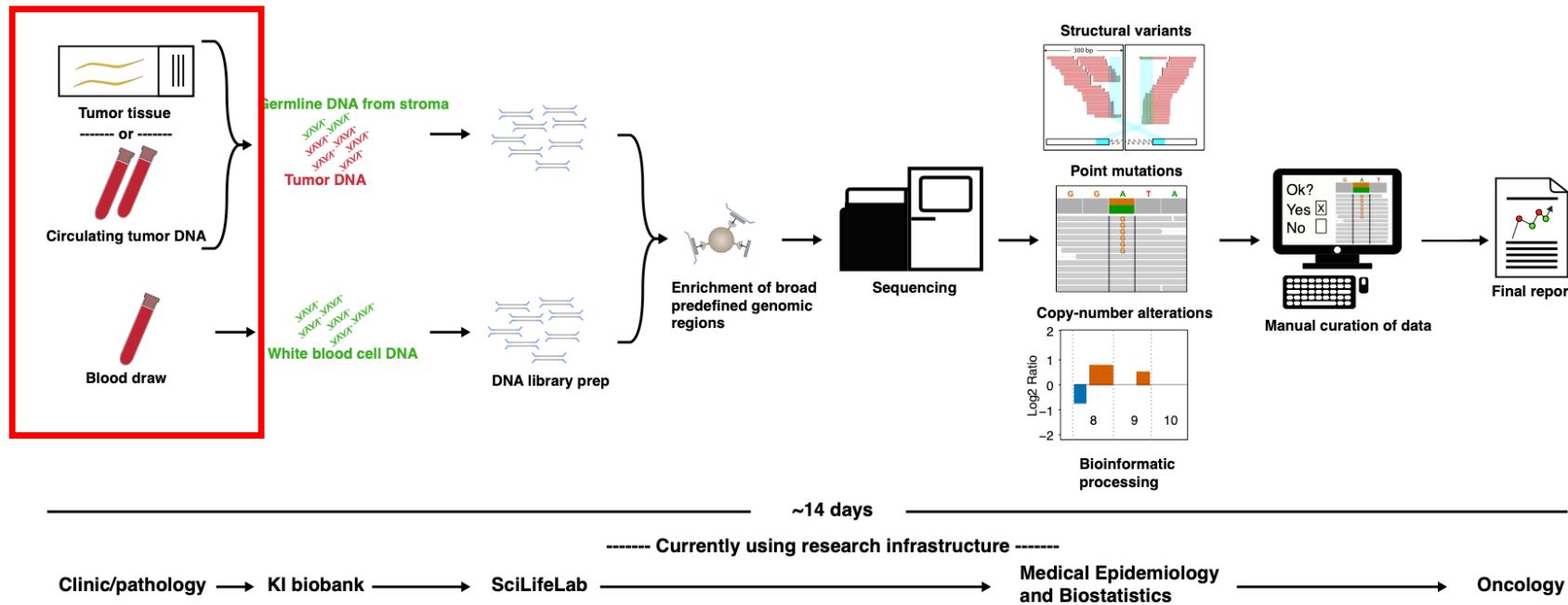
Summary

- Localised cancer
 - Pronounced heterogeneity
 - Potential remedy: sequenced homogenized multiregional samples
- Newly diagnosed advanced cancer
 - Limited heterogeneity
 - One metastatic sample likely provides an adequate representation
- Treated advanced cancer
 - As for newly diagnosed BUT clonal evolution will take place in the targeted genes/genes associated with resistance.



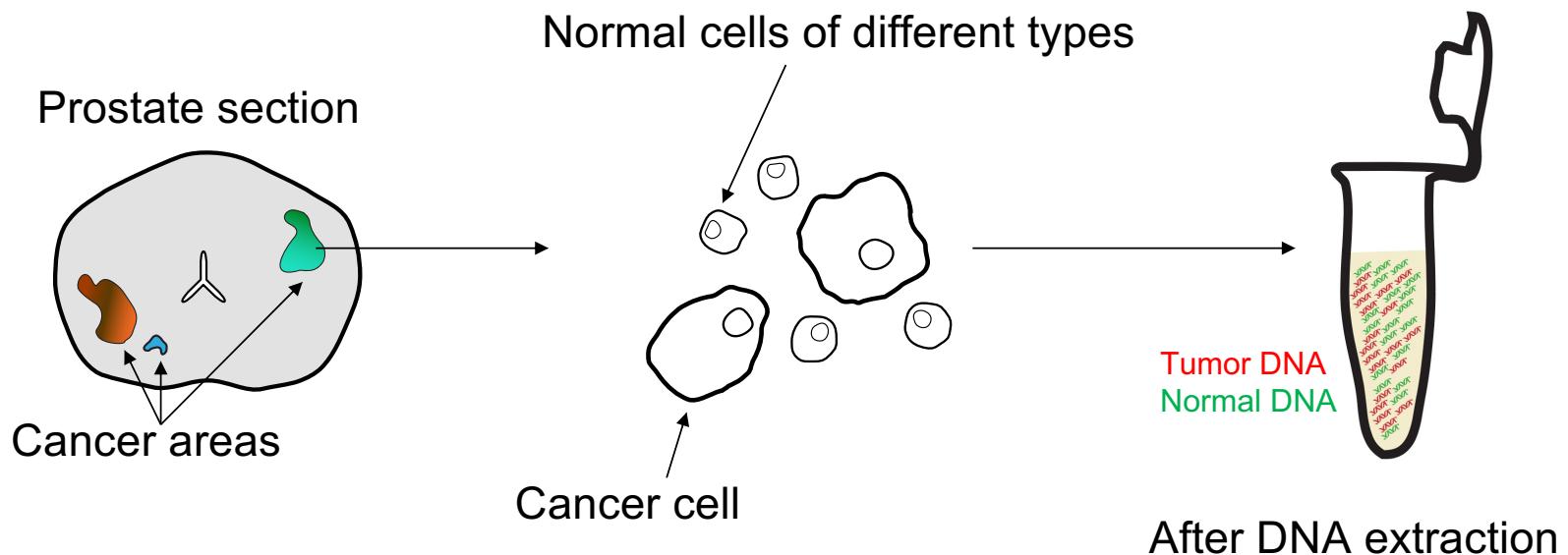
The molecular evolution of acquired resistance to targeted EGFR blockade in colorectal cancers, Nature, 2012

Practical considerations for performing cancer genomics

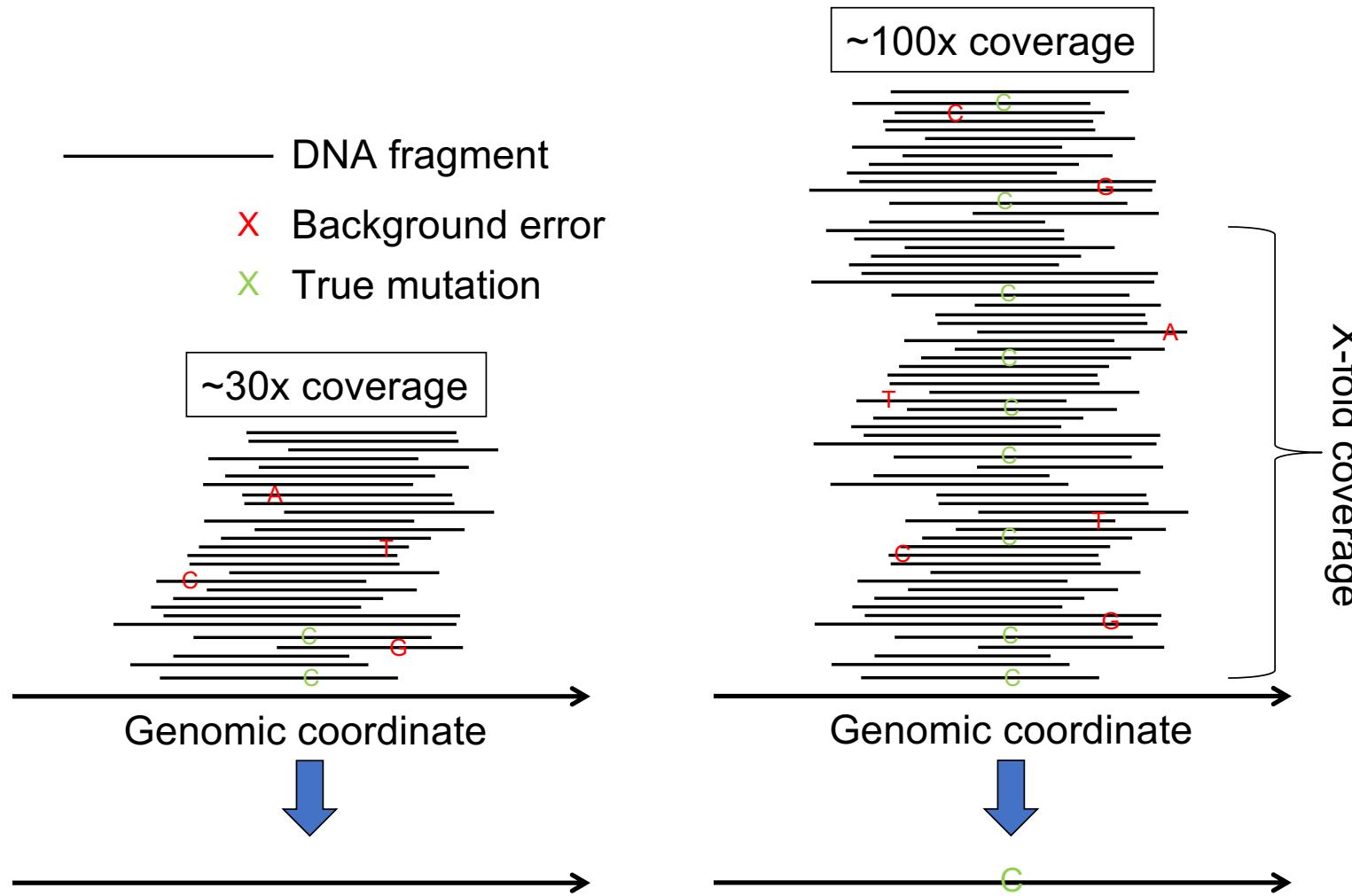


Starting material – heterogeneity and purity

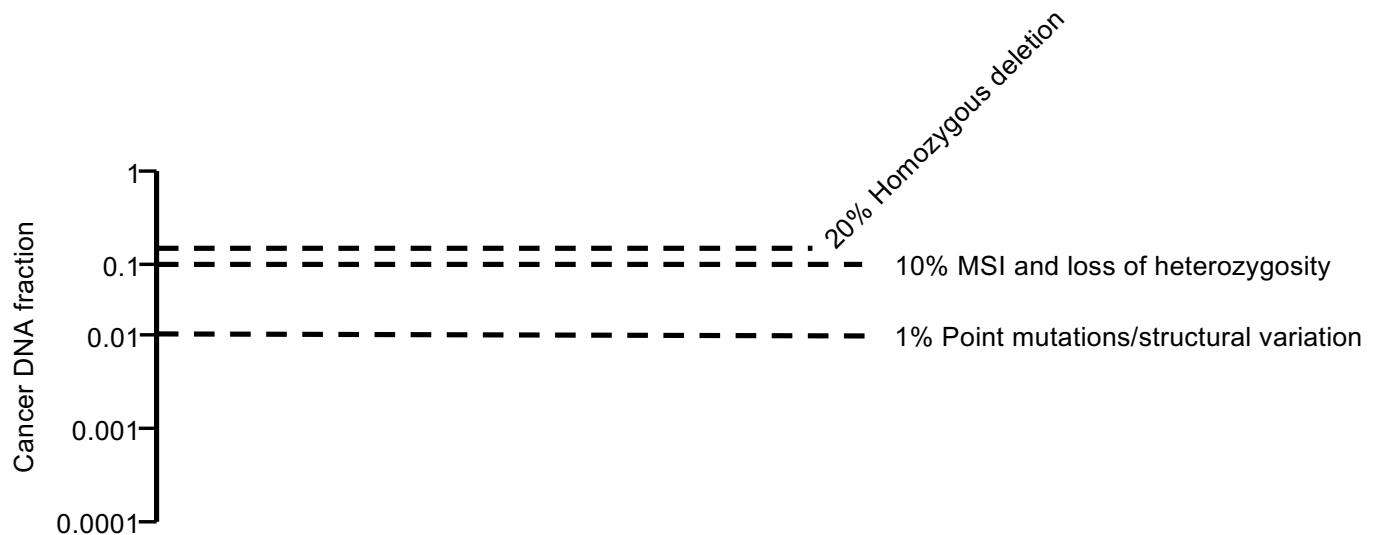
- Never 100% pure cancer cells
- Vary from 0.0001 – 0.7



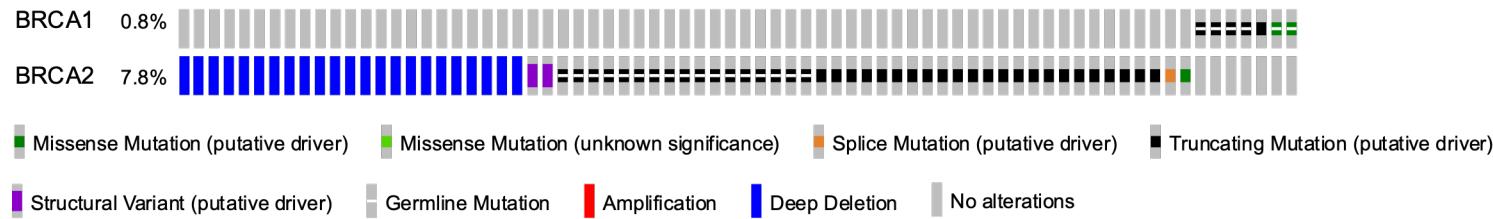
Coverage is king ... but not remedy for everything



Cancer DNA fraction and consequence for genomic profiling using sequencing



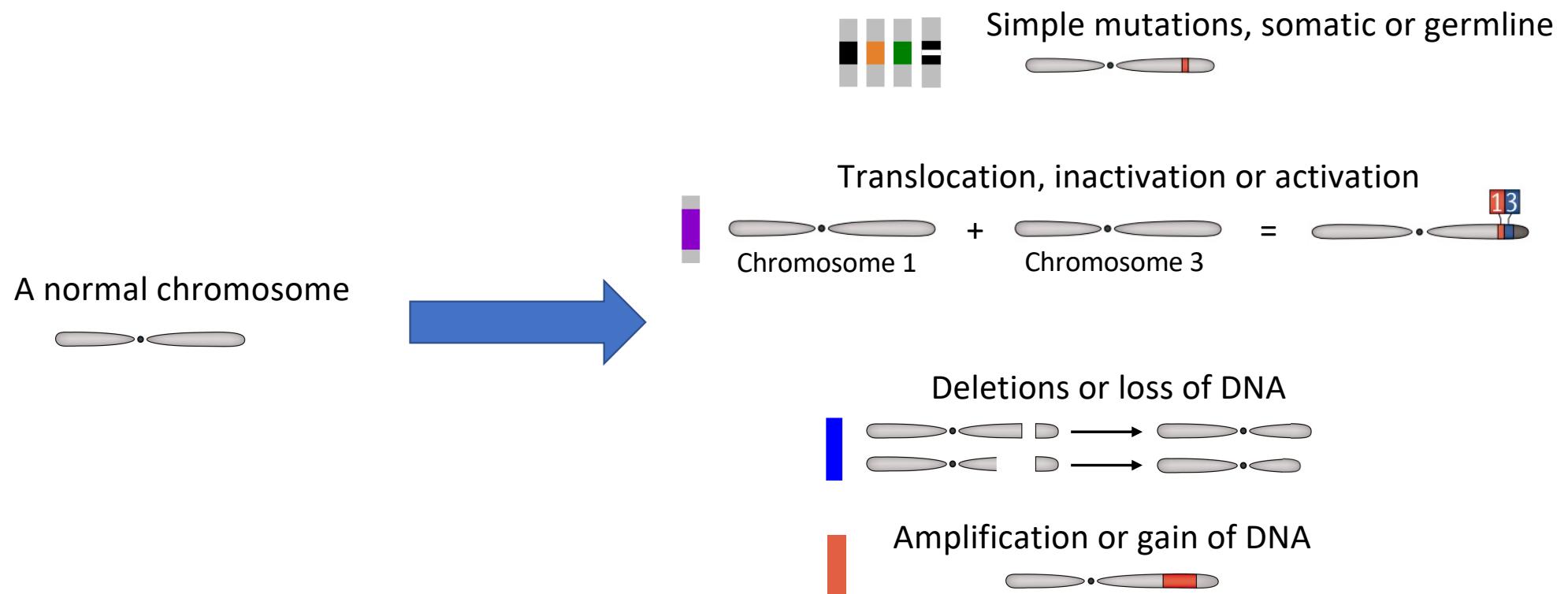
Frequency of BRCA alterations in metastatic prostate cancer



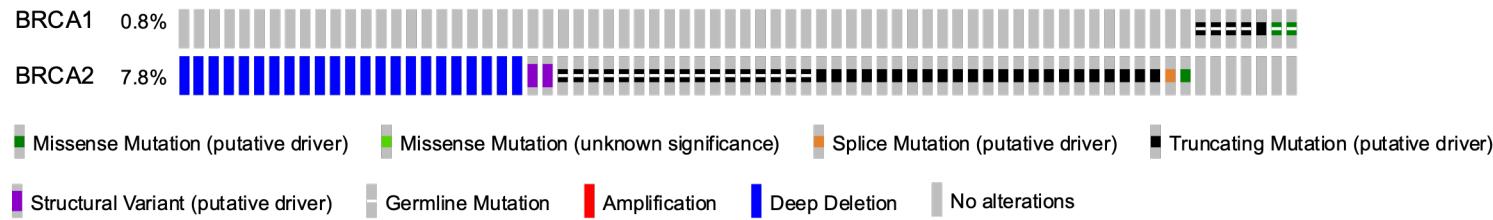
Metastatic castration-sensitive prostate cancer (MSK, Clin Cancer Res 2020)
N=424 cases, MSK-IMPACT panel sequencing.

Metastatic Prostate Adenocarcinoma (SU2C/PCF Dream Team, PNAS 2019)
N = 444 cases, whole-exome sequencing.

Type of somatic/germline alteration



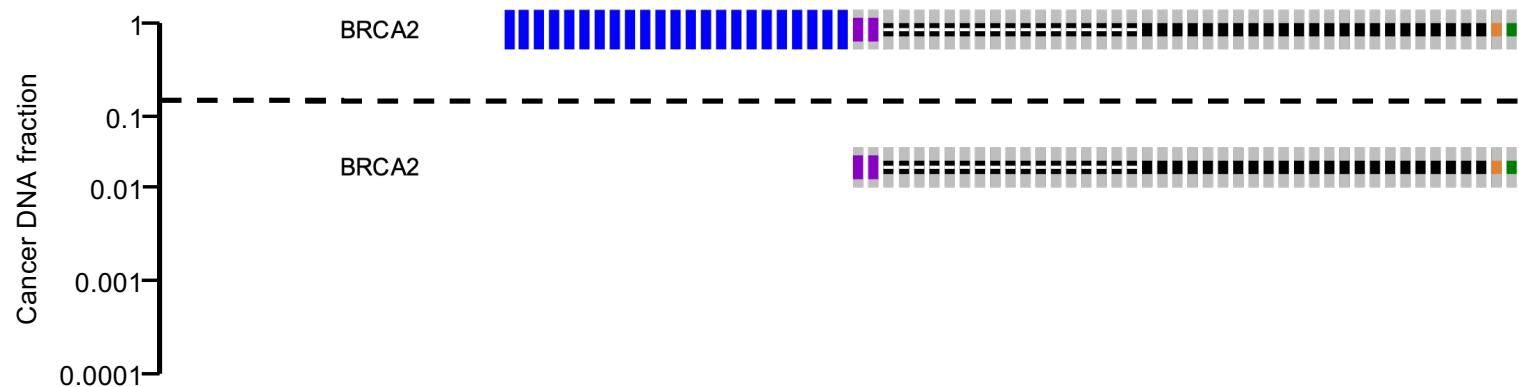
Frequency of BRCA alterations in metastatic prostate cancer



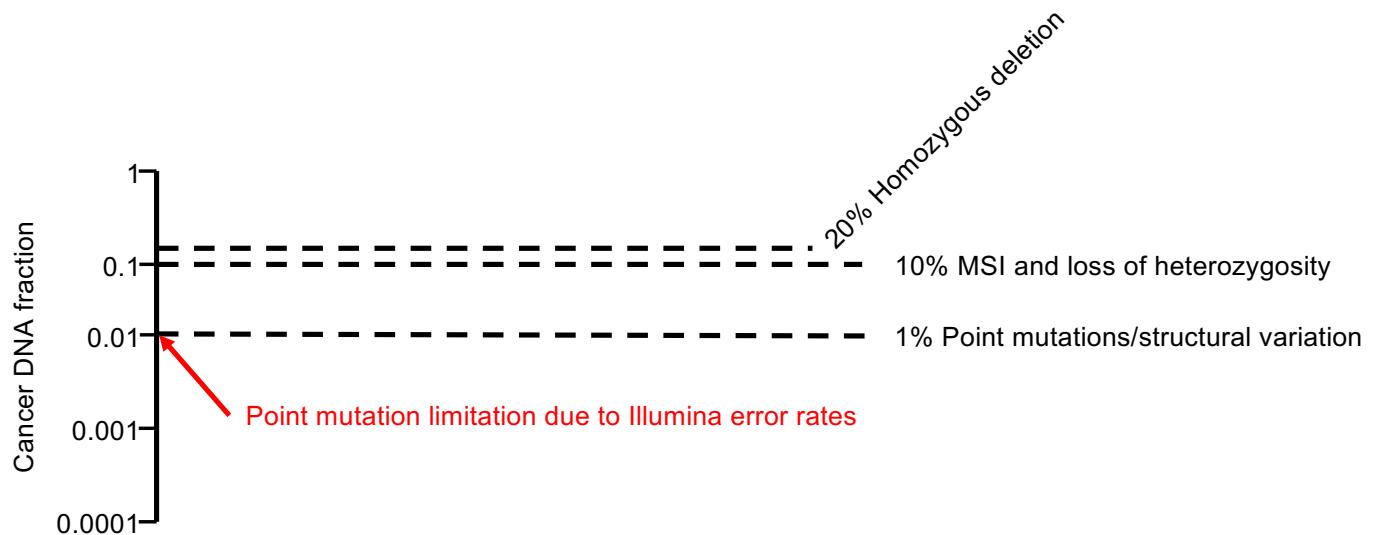
Metastatic castration-sensitive prostate cancer (MSK, Clin Cancer Res 2020)
N=424 cases, MSK-IMPACT panel sequencing.

Metastatic Prostate Adenocarcinoma (SU2C/PCF Dream Team, PNAS 2019)
N = 444 cases, whole-exome sequencing.

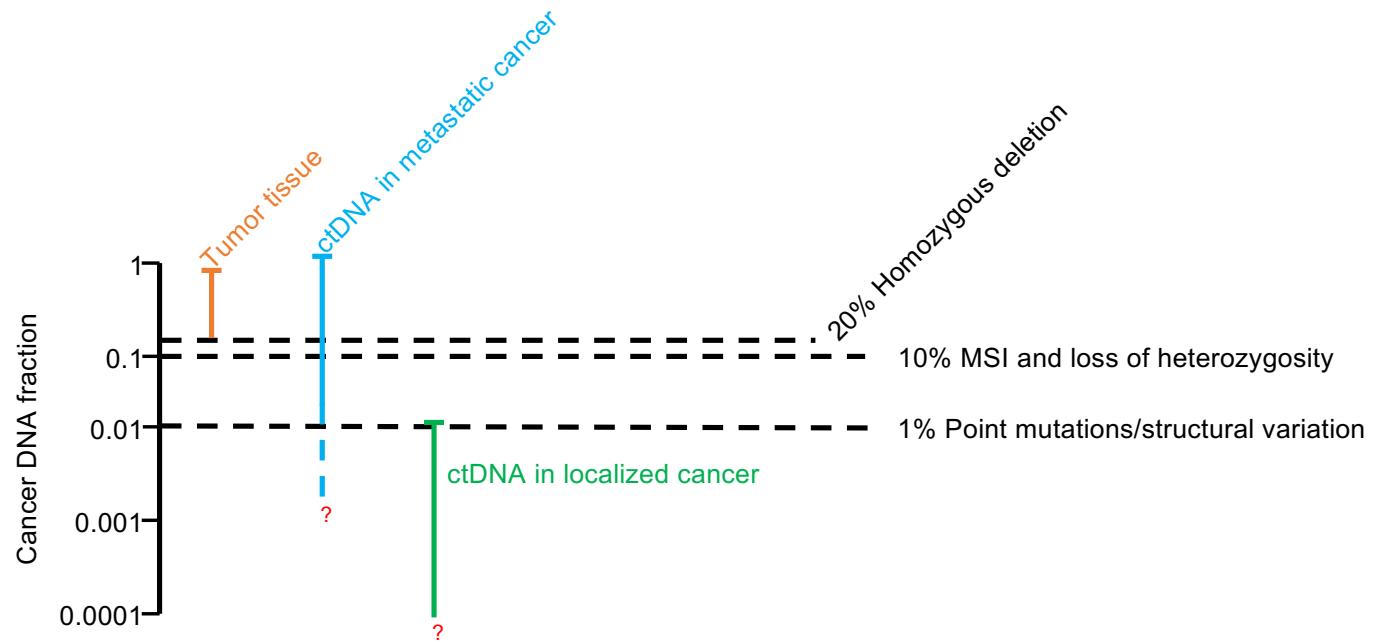
Cancer DNA fraction and consequence for genomic profiling using sequencing



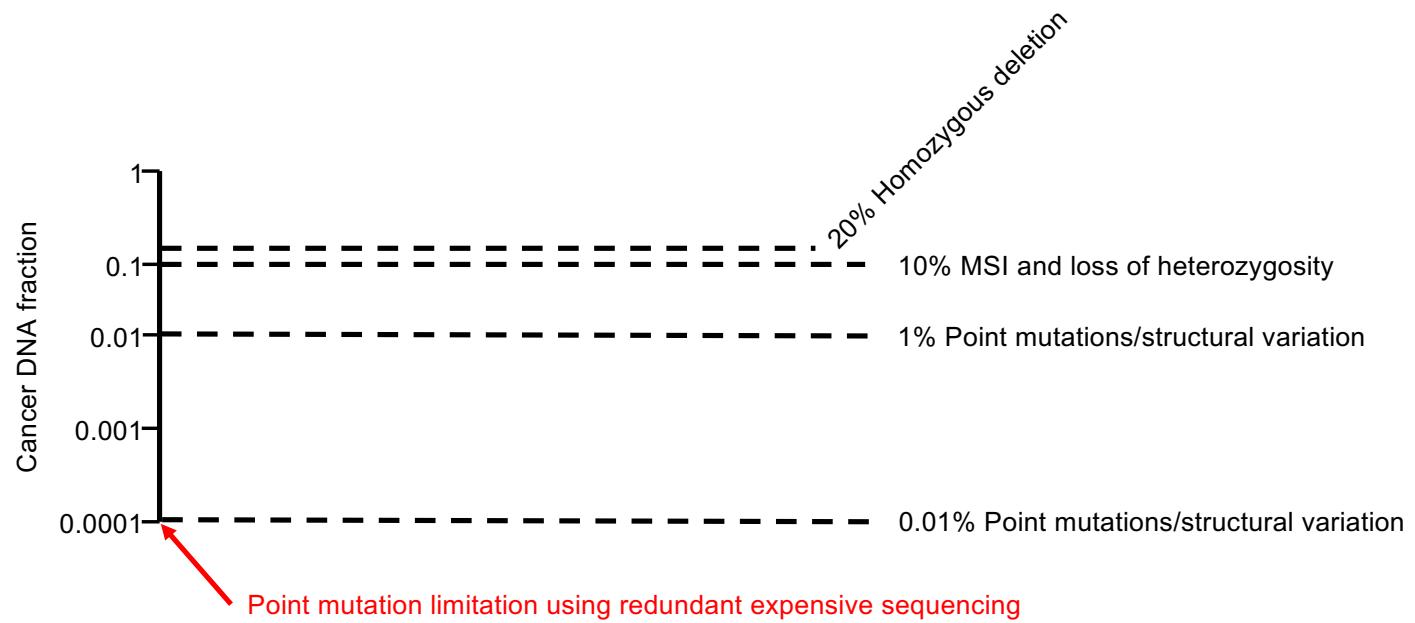
Cancer DNA fraction and consequence for genomic profiling using sequencing



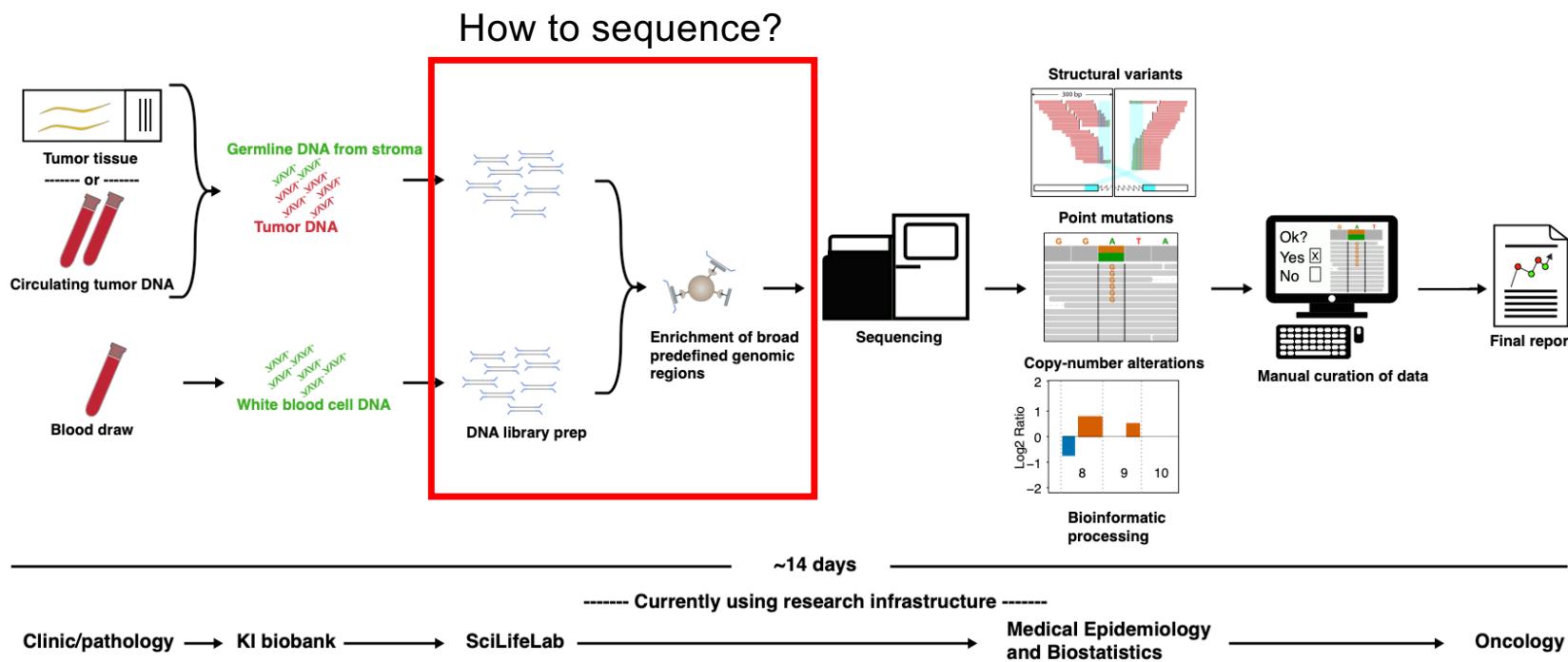
Cancer DNA fraction and consequence for genomic profiling using sequencing



Cancer DNA fraction and consequence for genomic profiling using sequencing

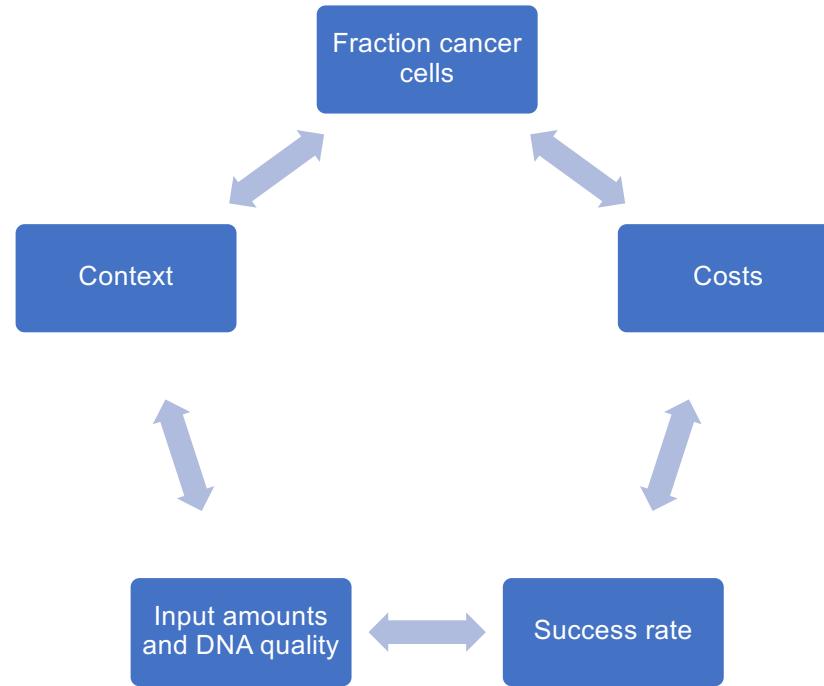


Practical considerations for performing cancer genomics



How to sequence depends on many things ..

- Research vs clinical.
- Clinical trial
 - High success rate
 - Fast turnover
 - Not acceptable with false positives
 - Allow for retrospective research

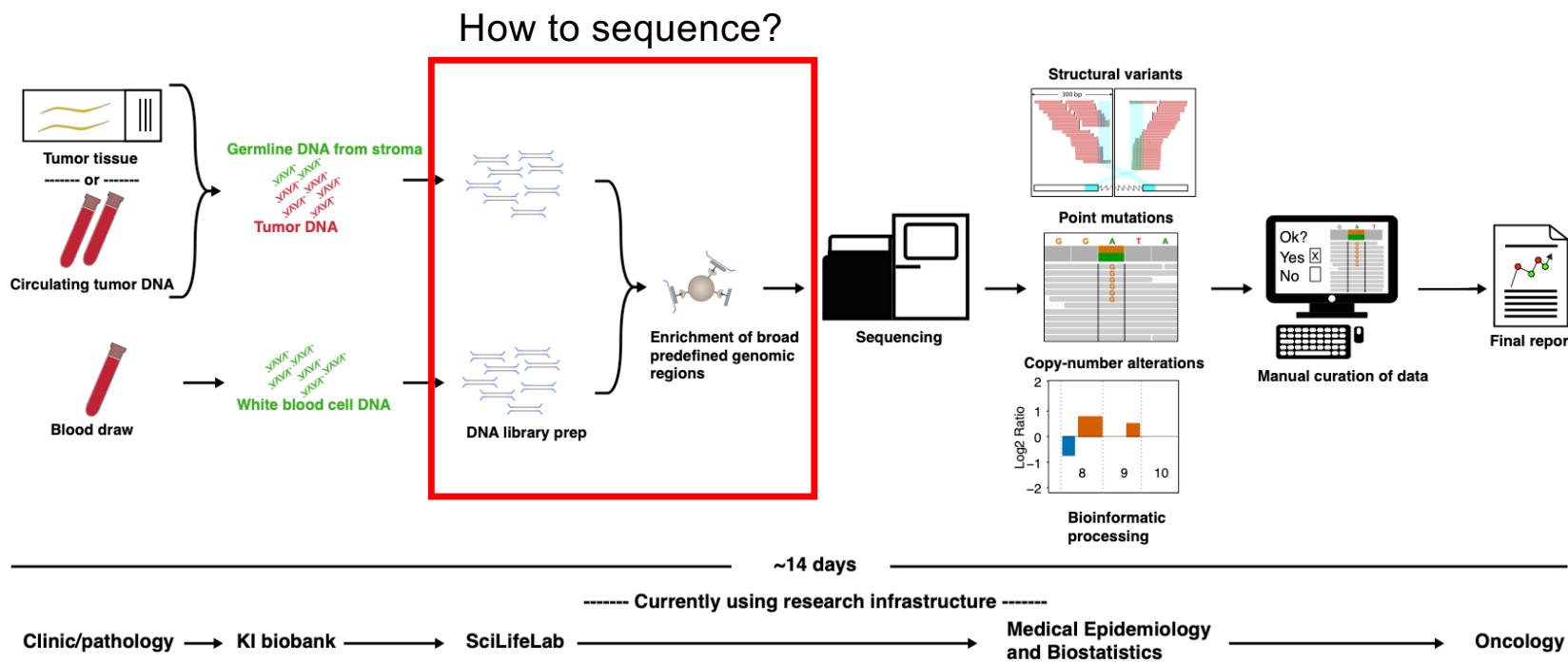


Why not deep whole genome sequencing?

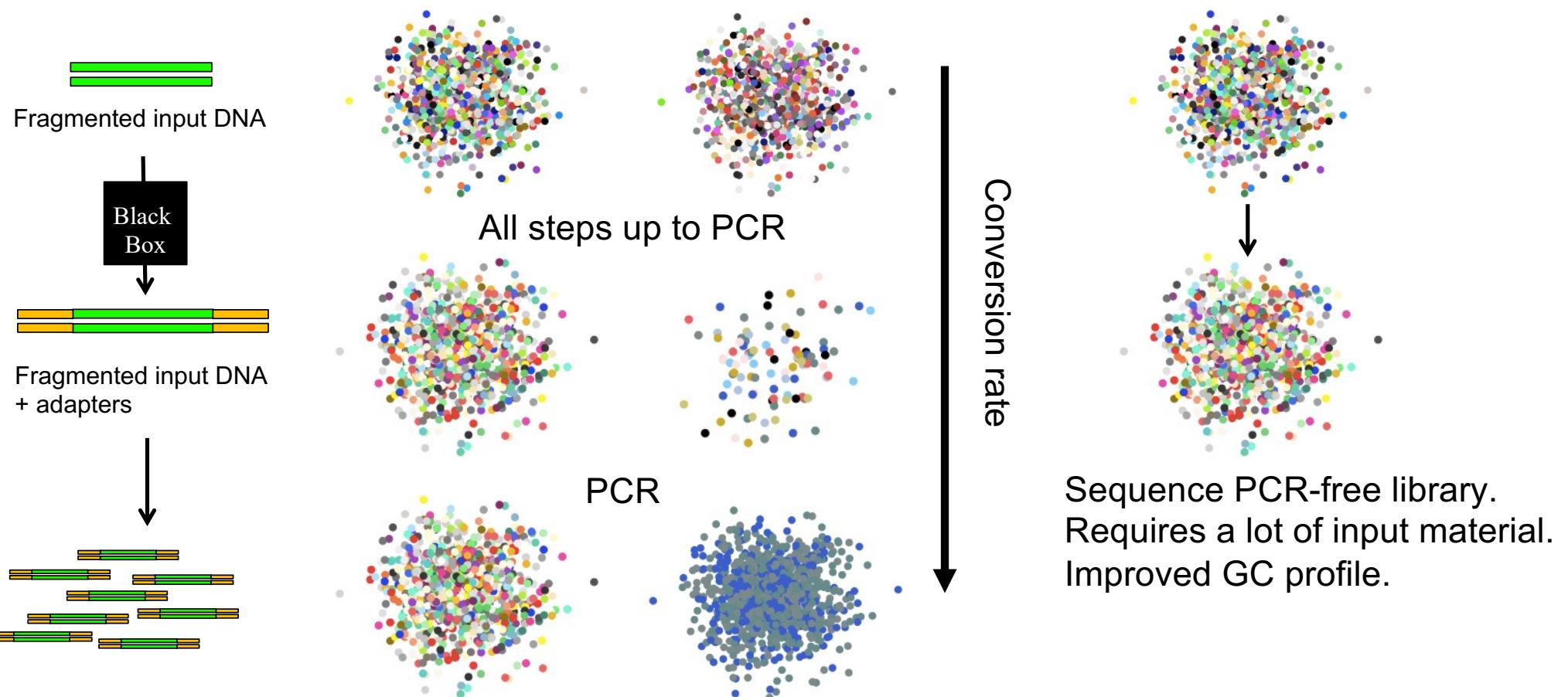
	Purity					
	100%	50%	20%	10%	1%	0.1%
Required coverage	100x	100x	500x	>1000x	>1500x	>5000x
Cost with WGS	€ 5 000	€ 5 000	-	-	-	-
Cost with targeted sequencing	€ 1 500	€ 1 500	€ 1 500	€ 1 500	€ 1 500	-
Minimal target and tailored bioinformatics	-	-	-	-	-	€1 500 - €2 000

Paired tumor and germline DNA analysis to enable identification of somatic- and germline alterations with good performance

Practical considerations for performing cancer genomics

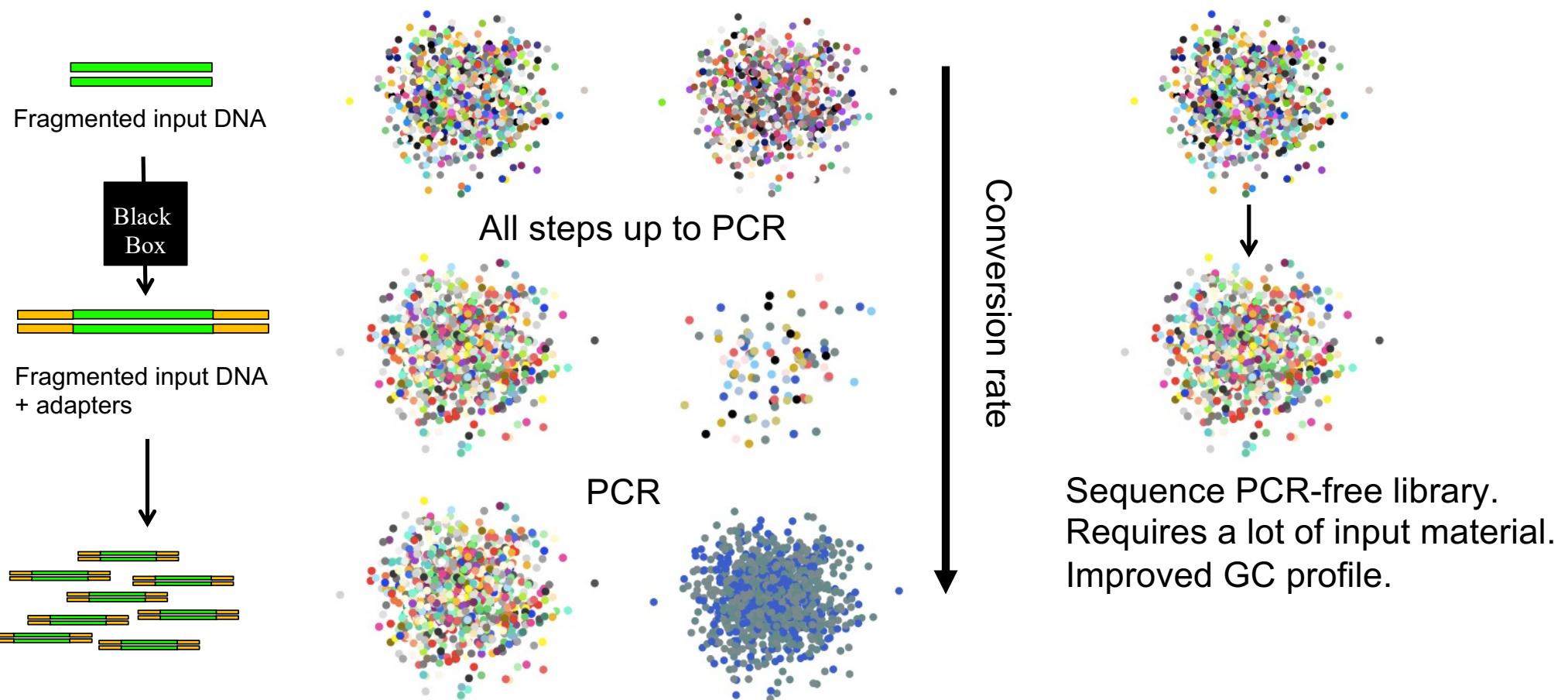


Library preparation in a nutshell

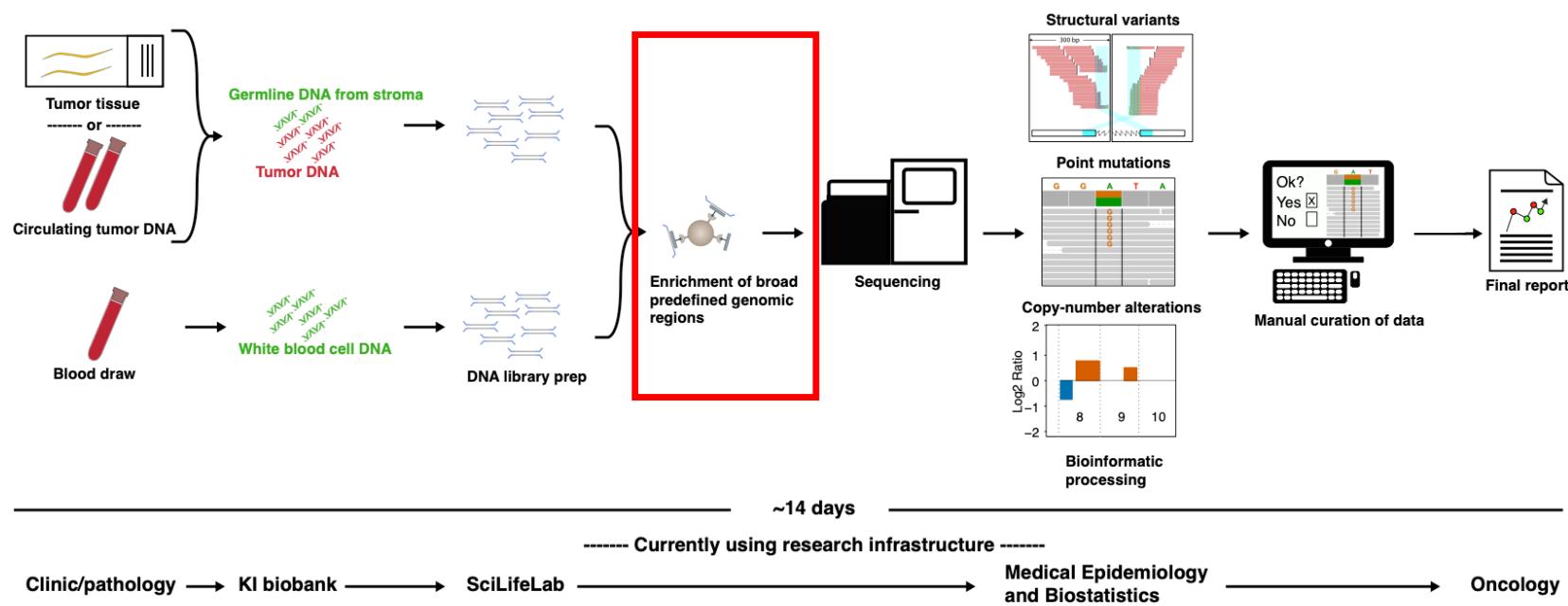


Menti - library prep efficiency

Library preparation in a nutshell

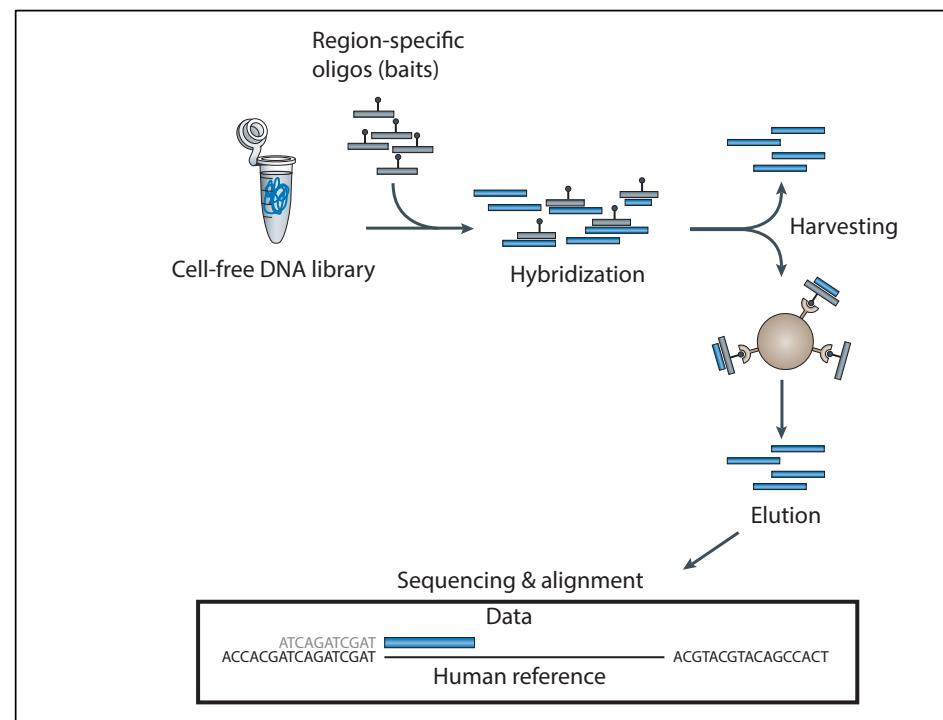


Key steps when performing DNA/RNA sequencing of cancer



Key steps when performing DNA/RNA sequencing of cancer

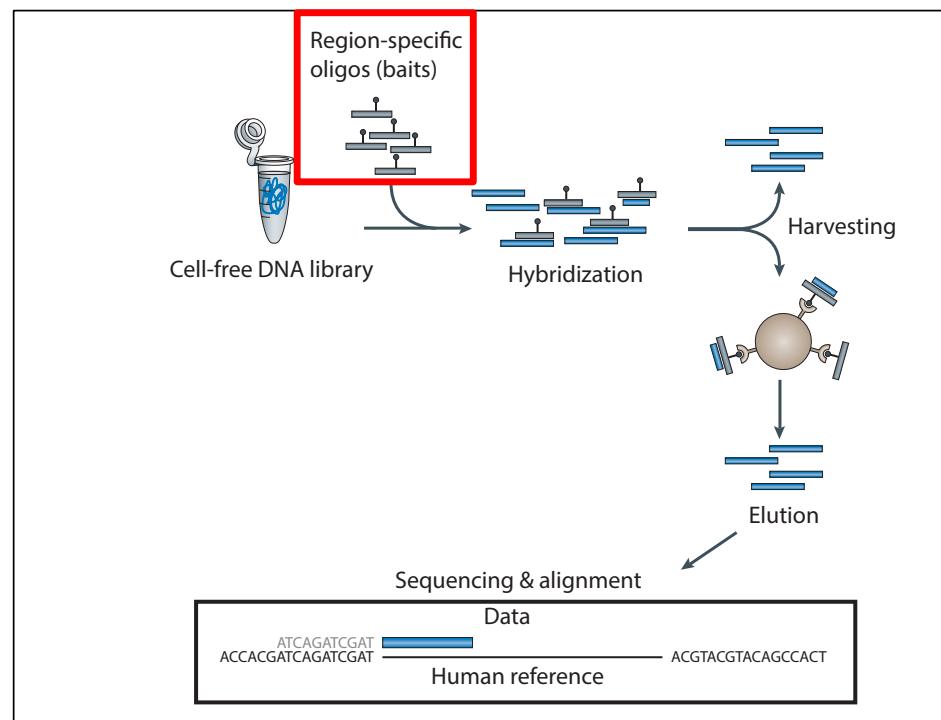
- In solution hybridisation based capture
- Flexibility in both breadth/depth



Adopted from: Advances in understanding cancer genomes through second-generation sequencing, Nat Rev Gen 2010

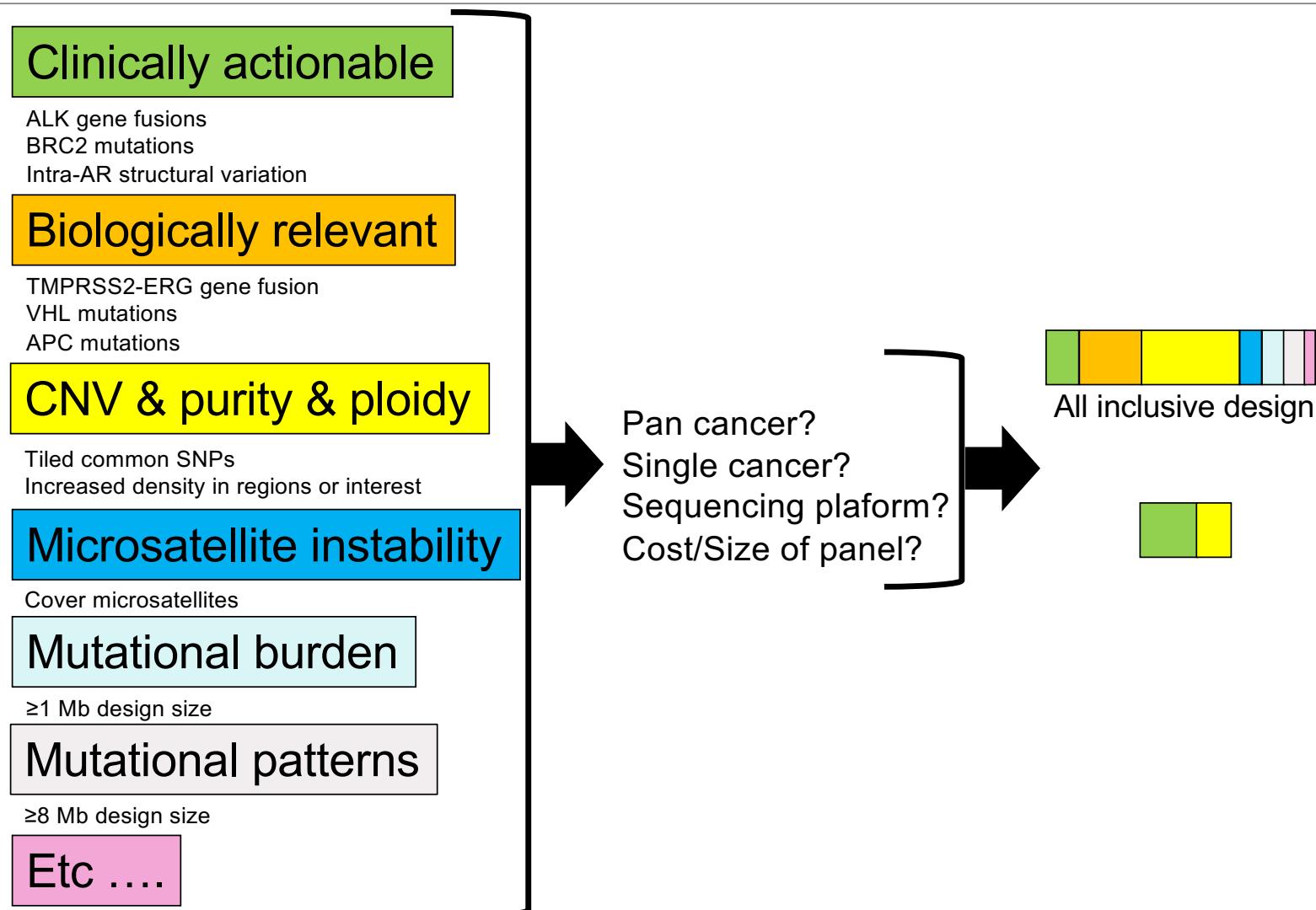
Key steps when performing DNA/RNA sequencing of cancer

- The baits determine the assay properties

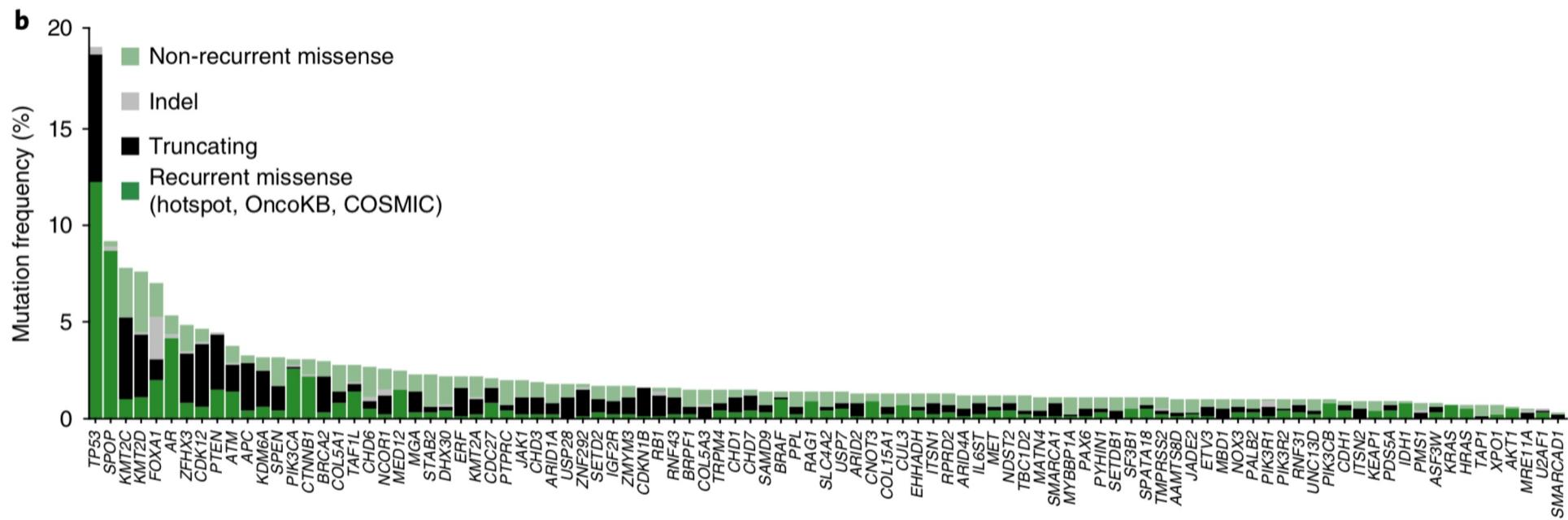
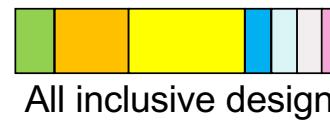


Adopted from: Advances in understanding cancer genomes through second-generation sequencing, Nat Rev Gen 2010

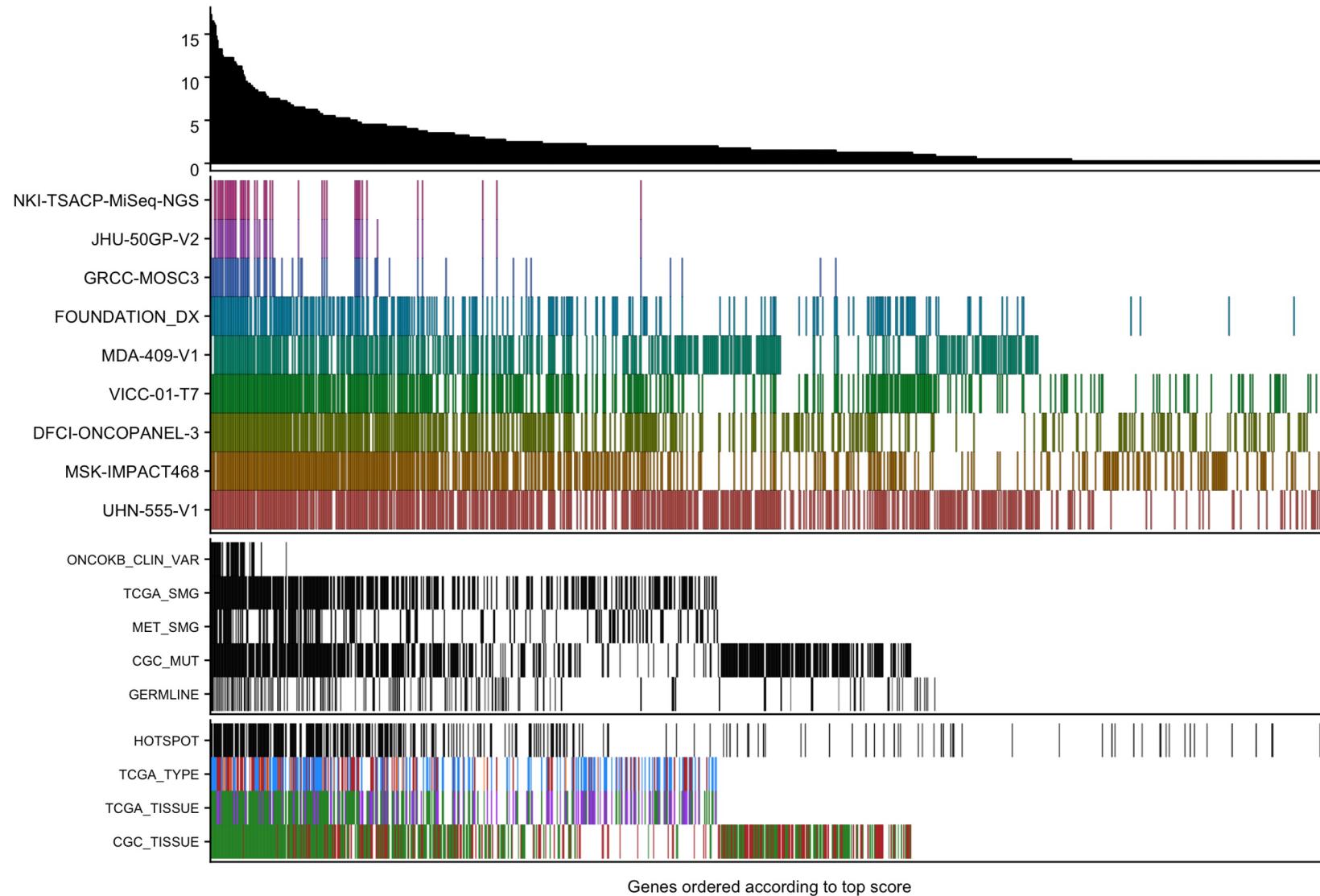
Defining a panel/bait set is like building lego ..



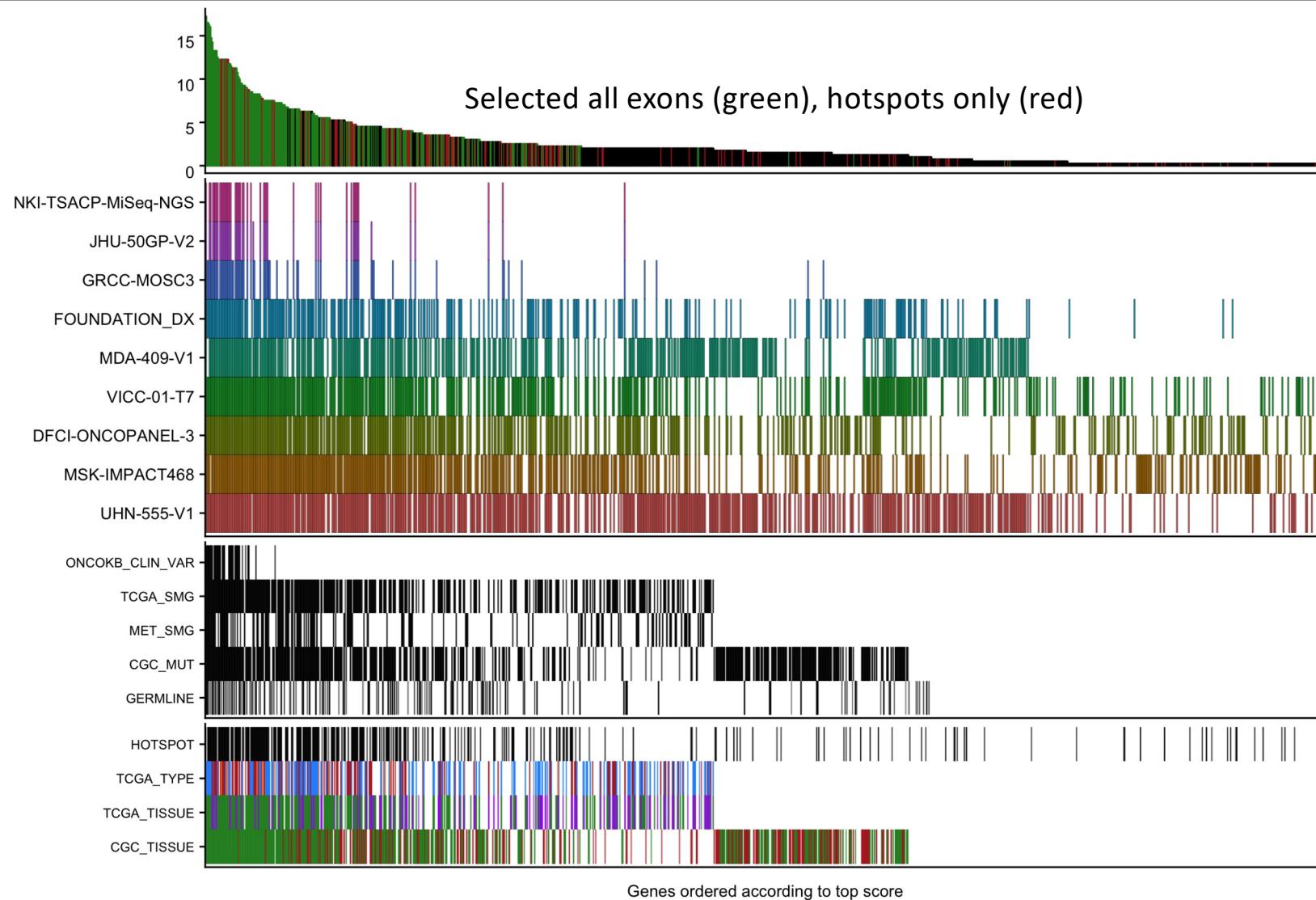
The long tail motivates broad genomic profiling



Overview heatmap, exonic regions



Overview heatmap, exonic regions



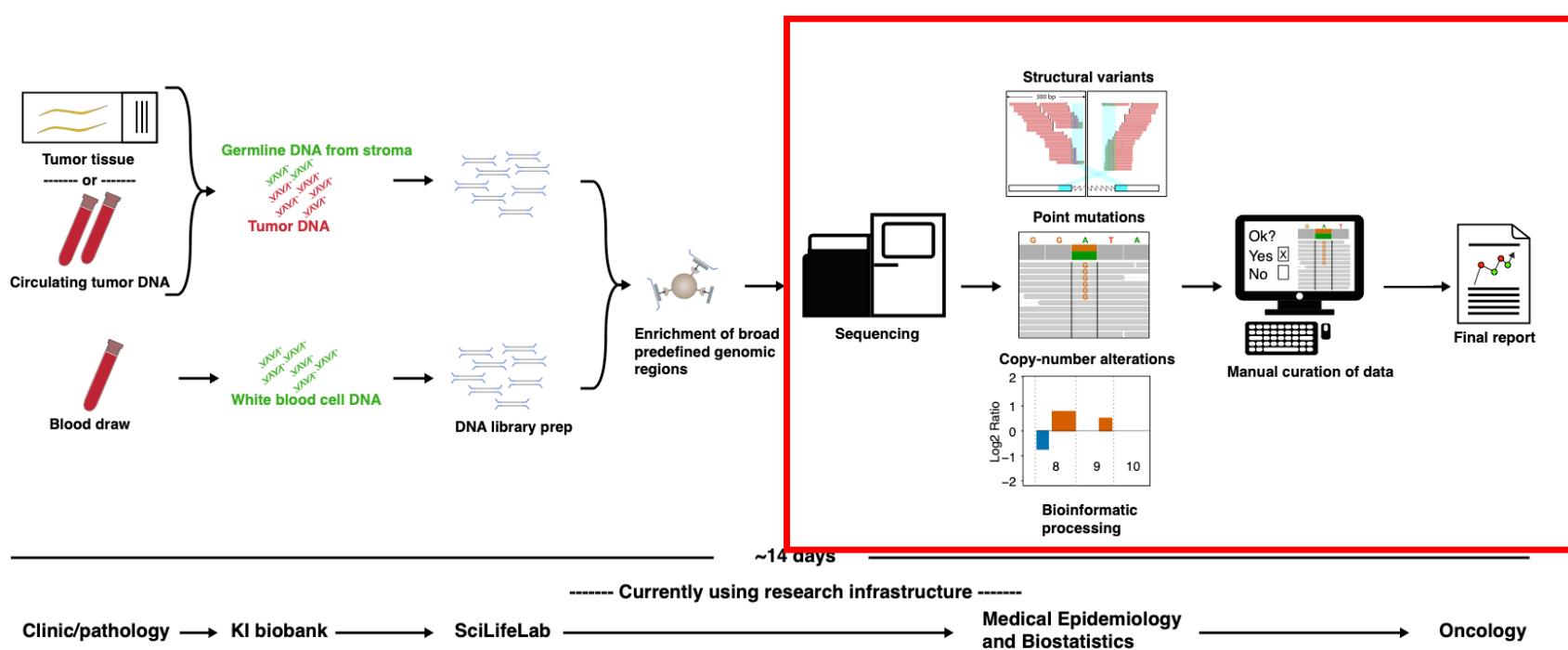
GMCK panel summary – soon to be updated

Mutations		OncoKB coverage
All coding exons	198 genes	26/31
	Hotspots	5/31
Pharmacogenetic variants		
	SNPs	9 genes
Copy-number alterations		
Tiled SNP for genome-wide CNV	2814 SNPs	
	Directed analysis to increase sensitivity	86 genes
Structural variation		
Gene fusions by intronic sequencing	19 genes	9/10
	Gene-body sequencing (e.g. BRCA1/2)	9 genes
Microsatellite instability & Hypermutation		
Microsatellites	63 in total	
	Hypermutation, entire design footprint	yes
	Associated genes	7
Total size (Mb)		2.4

The size of the panel was determined considering the sequencing price at Clinical Genomics @ SciLife

Practical considerations for performing cancer genomics

Covered in other lectures ...



Summary

- The fraction of cancer DNA in a sample determines which types of somatic alterations that can be detected
 - Increased sequencing depth can improve detection but only to a certain limit
- Approximately 1/3 of starting DNA molecules may be interrogated

Questions?
