PATHOLOGY AND MOLECULAR MEDICINE

Laboratory Aspects of Biomarker Studies

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Disclosures

- CSO with Indoc Research
- Research partner with Life Technologies (ThermoFisher)

Objectives

- Understand the different types of biomarkers and some of their major characteristics
- Appreciate the general approaches for measuring common biomarkers
- Understand the limitations and challenges of developing biomarkers

bi·o·mark·er

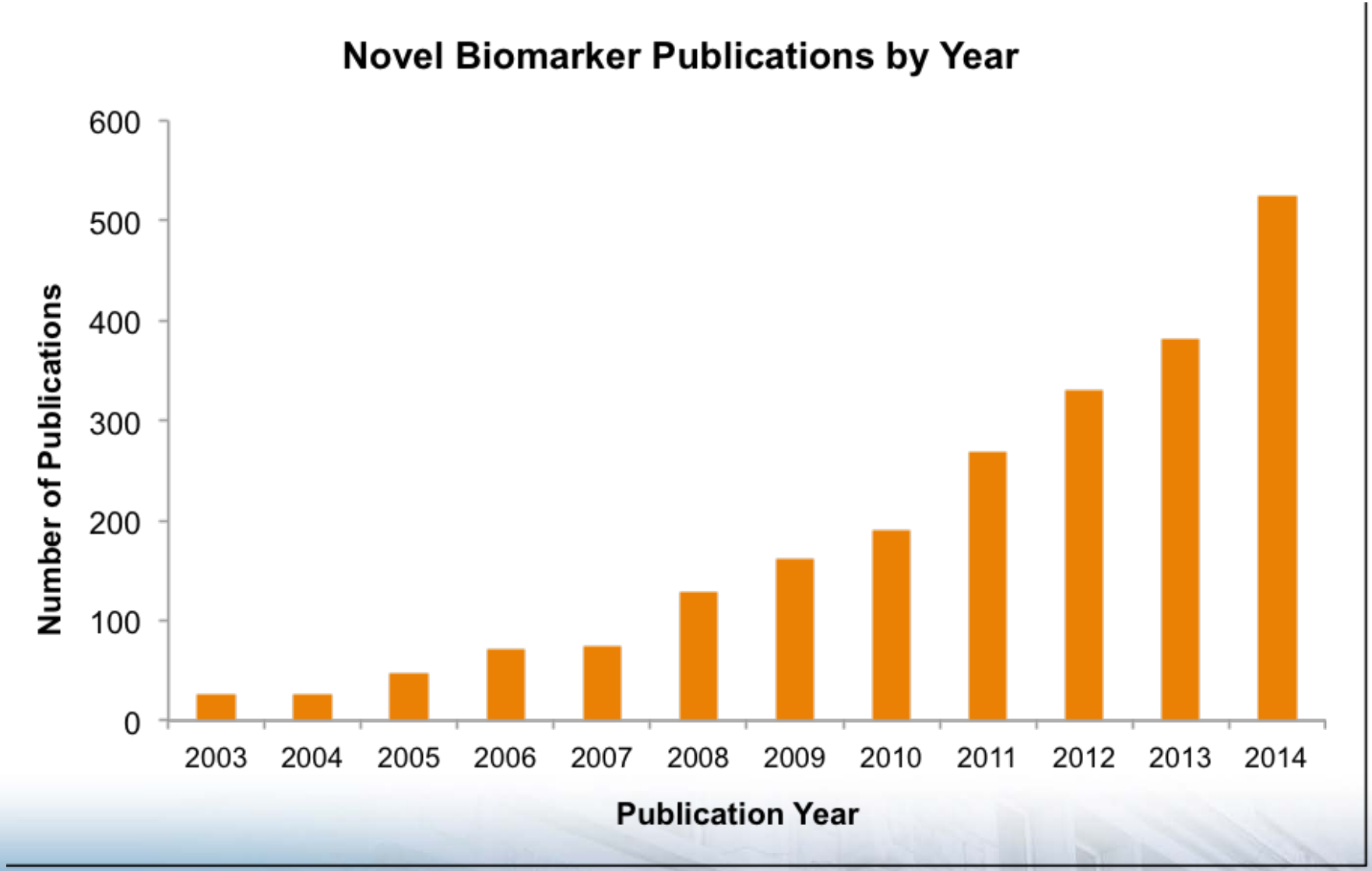
/ˈbīōˌmärkər/

noun

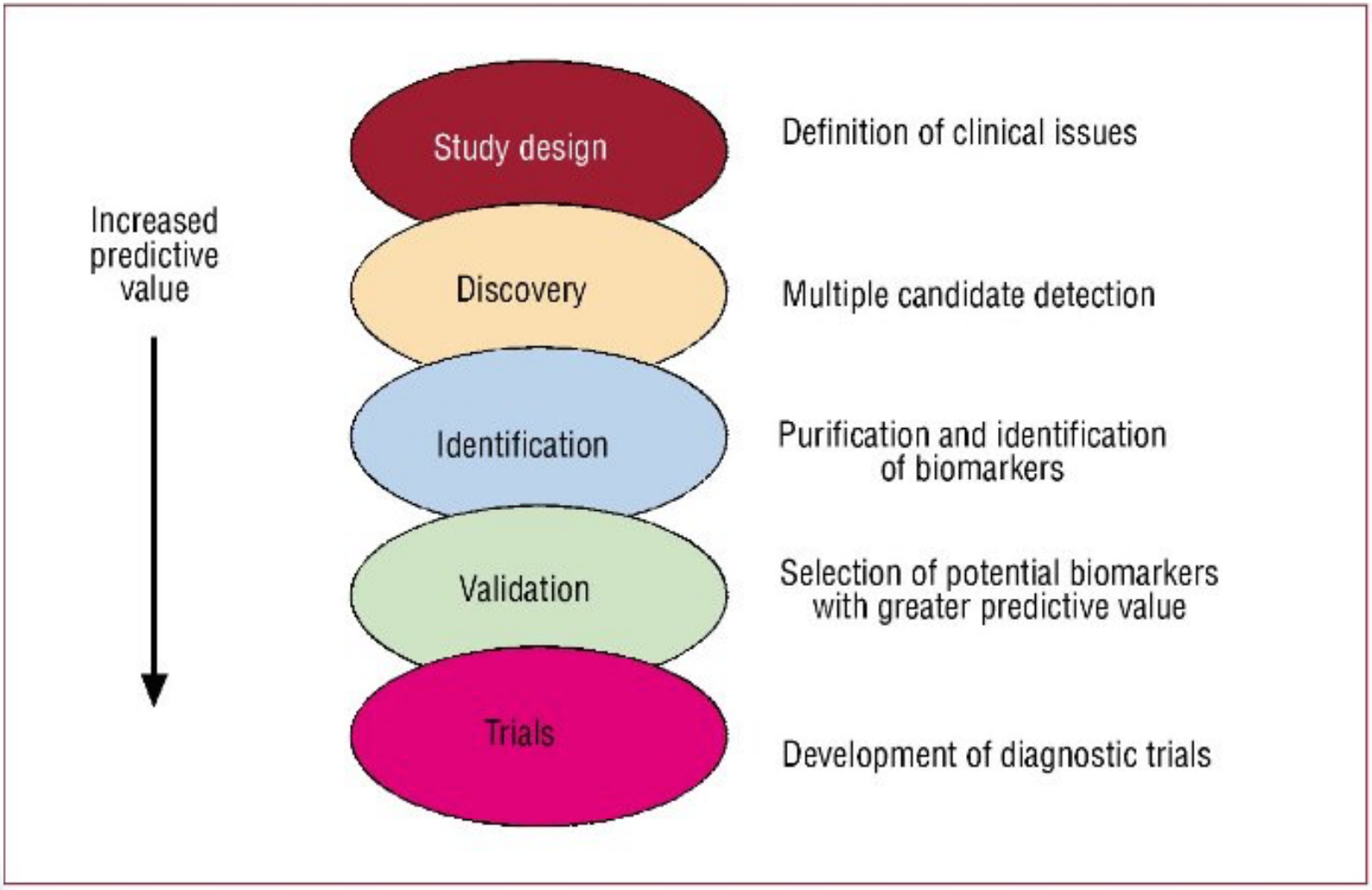
a measurable substance in an organism whose presence is indicative of some phenomenon such as disease, infection, or environmental exposure.
"a biomarker that may predict aggressive disease recurrence in liver transplant recipients"

Molecular Biomarkers. A **biomarker** is a characteristic that can be objectively measured as an indicator of normal biological processes, pathogenic processes or a pharmacological response to a therapeutic intervention.

Journal of Molecular Biomarkers & Diagnosis - OMICS Group
www.omicsonline.org/**molecular-biomarkers**-diagnosis.php



http://www.biomarker-trends.com/tag/emerging-biomarkers/

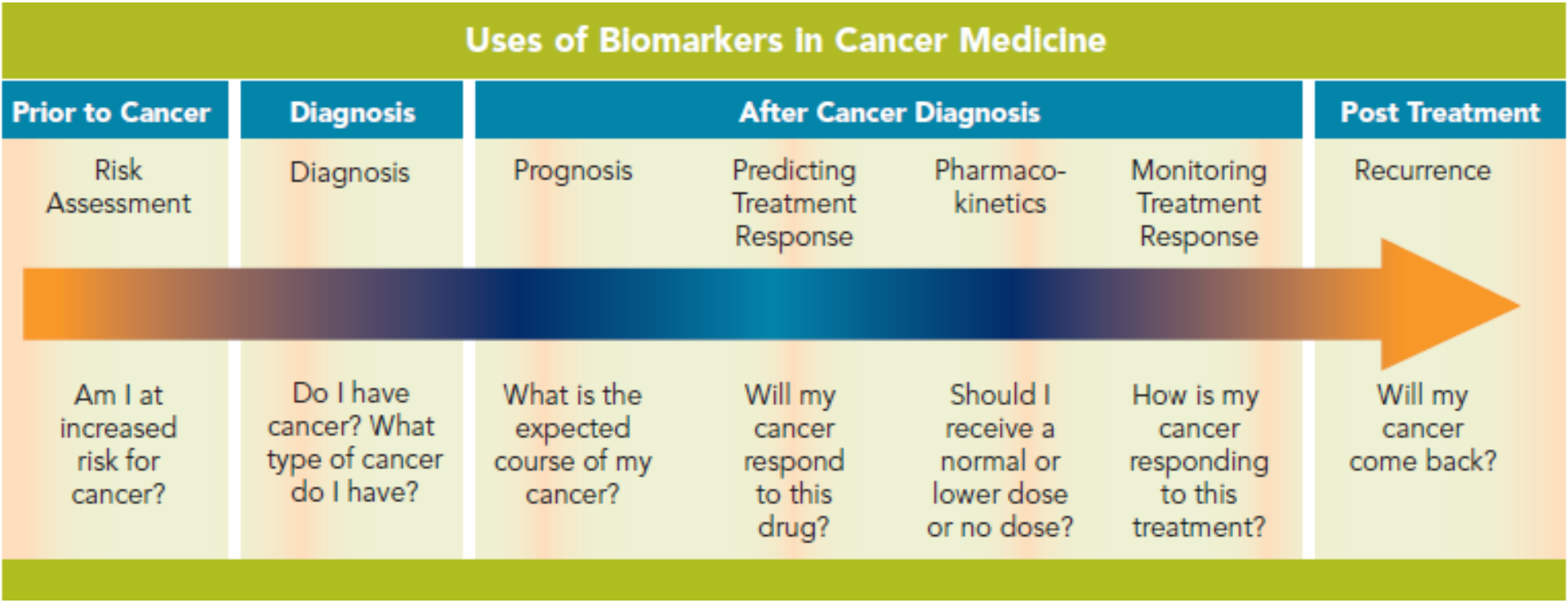


Rev Esp Cardiol. 2009;62(06):677-88 - Vol. 62

Considerations

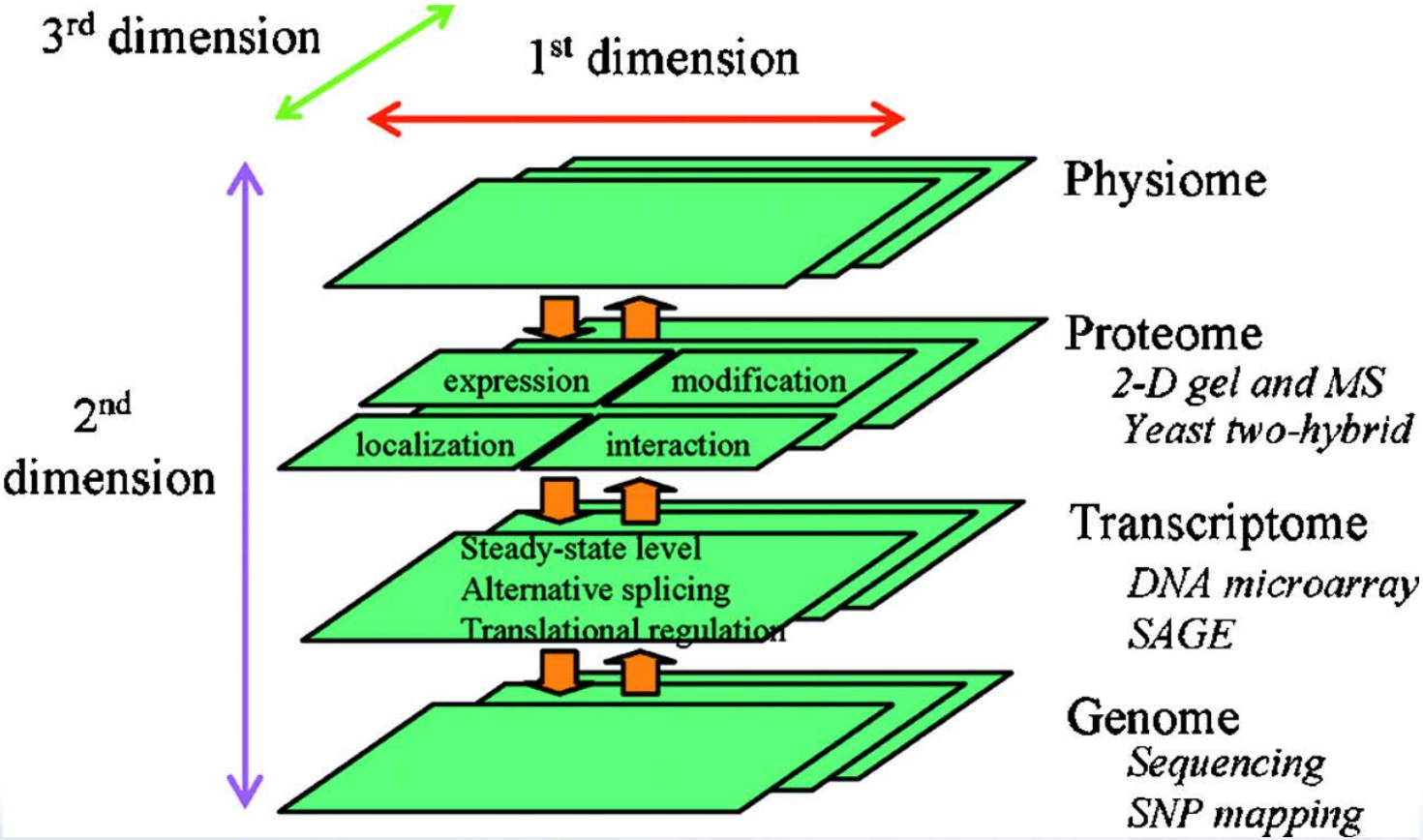
- What kind of question are you asking?
- What kind of biomarker will you measure?
- What type of sample will you have access to?
- How will your sample have been handled?
- How will your measurements be made?
- How reliable is your biomarker measurement?

What kind of question?

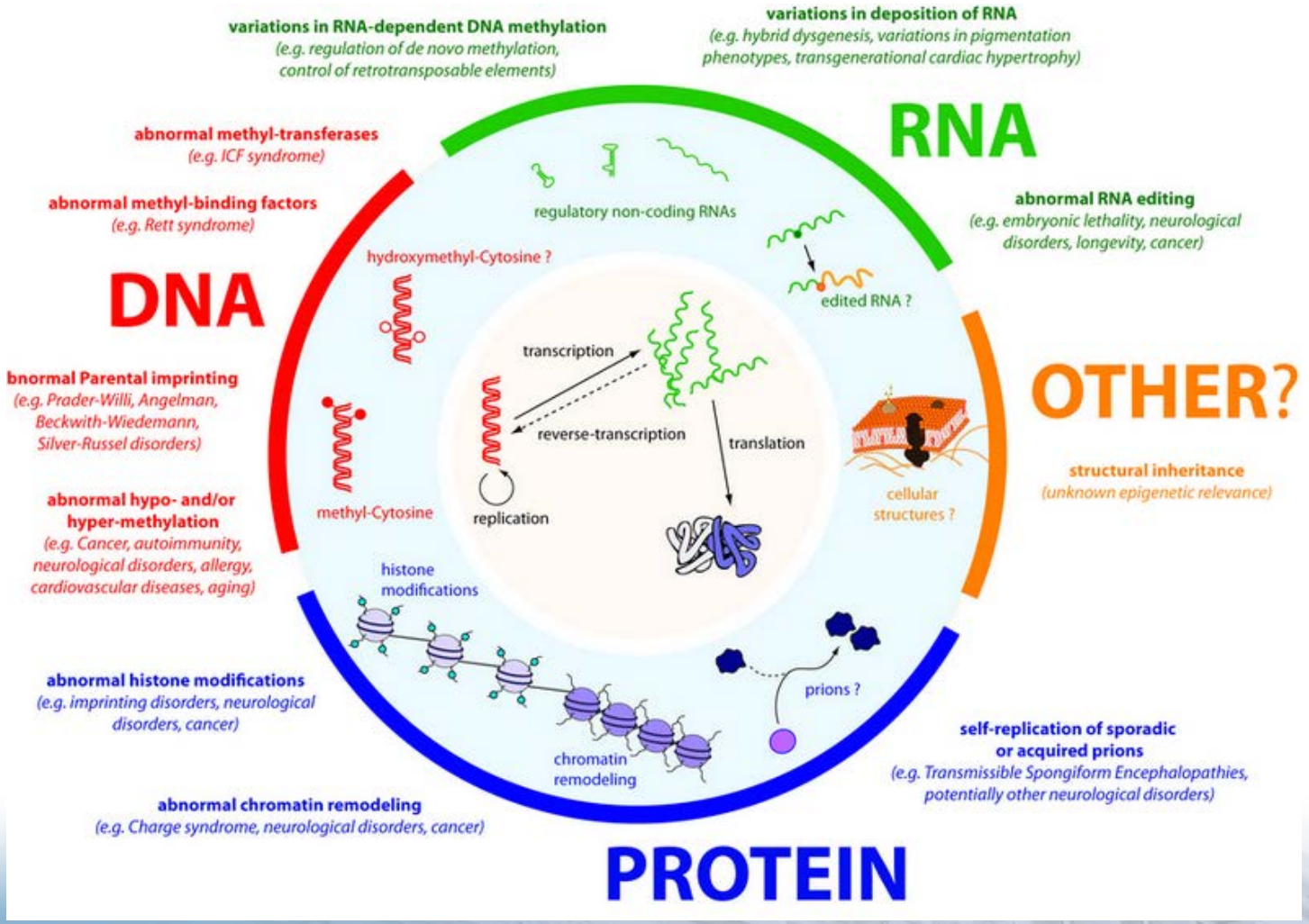


Source: Biomarkers in Cancer- An Introductory Guide for Advocates, Research Advocacy Network, 2010.

What kind of biomarker?



Properties of common biomarkers



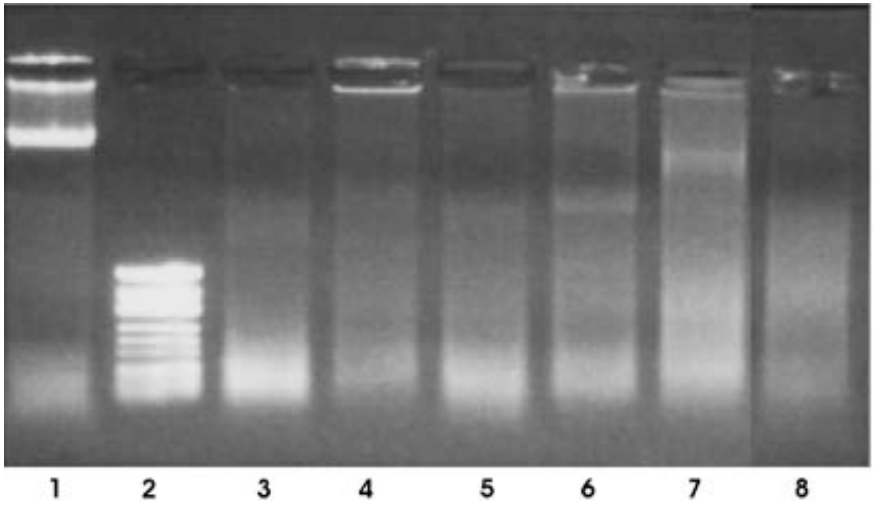
Richard Chahwan

[The Multidimensional Nature of Epigenetic Information and Its Role in Disease]

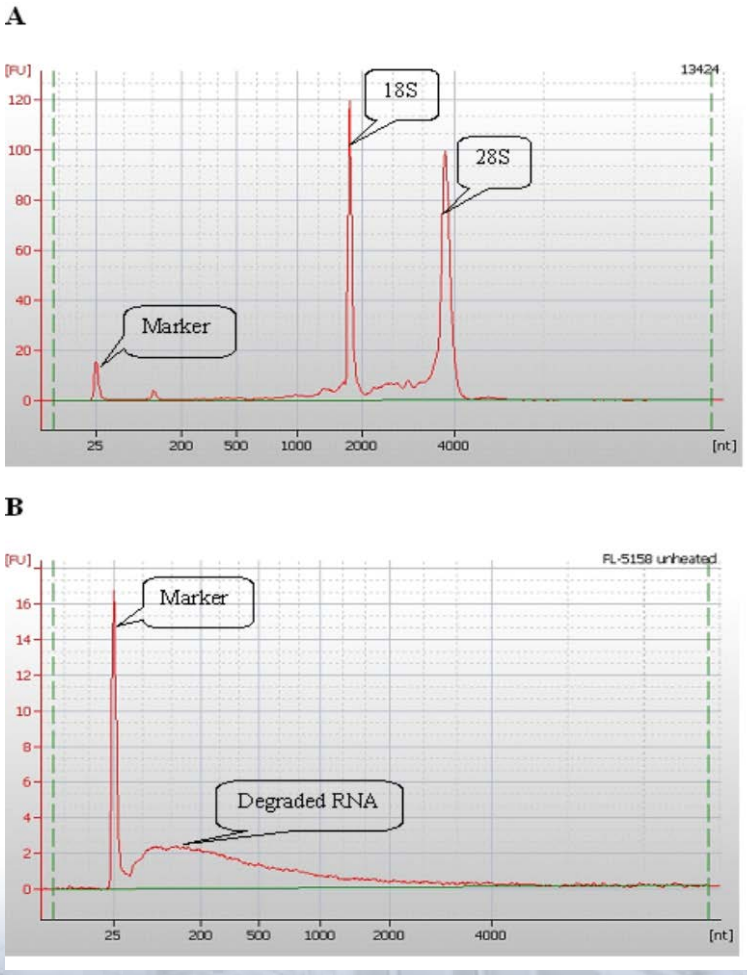
What type of sample?

- Need to consider the source
- Need to consider the pre-analytic preparation
- Need to consider the timing
- Need to consider the gender
- Other factors?

Quality of macromolecules



Source: Aude Lamy, et al (2011) Mod Pathol 24: 1090-1100;



Effect of fixation on biomarkers

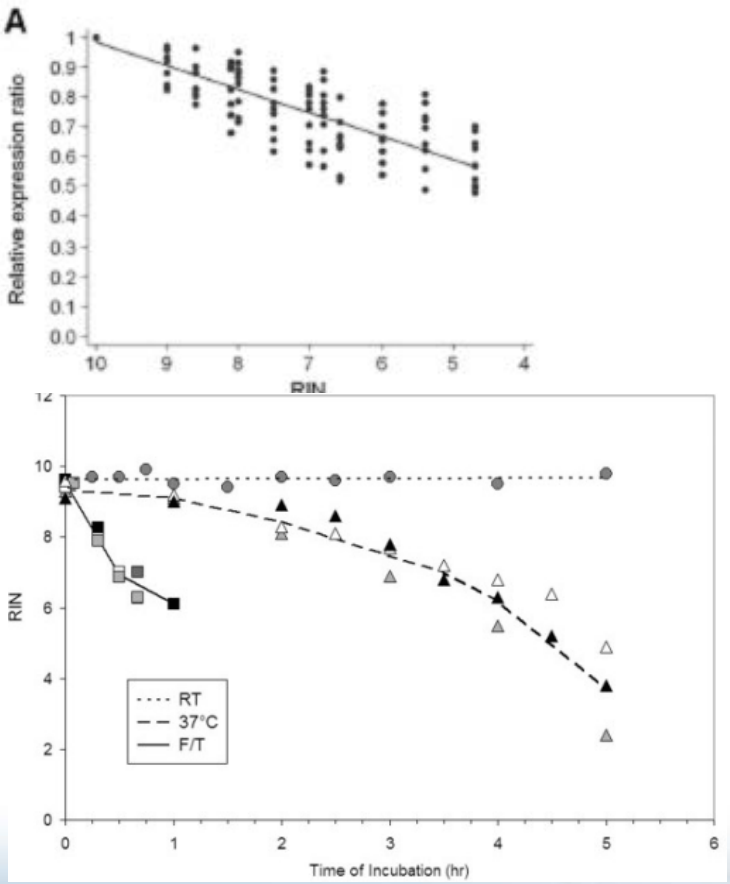
Table 1. FFPE-Related Preanalytical Factors Categorized by the Extent Each Has Been Investigated in the Literature for Potential Effects on DNA, RNA, Protein, and Morphology Analytes

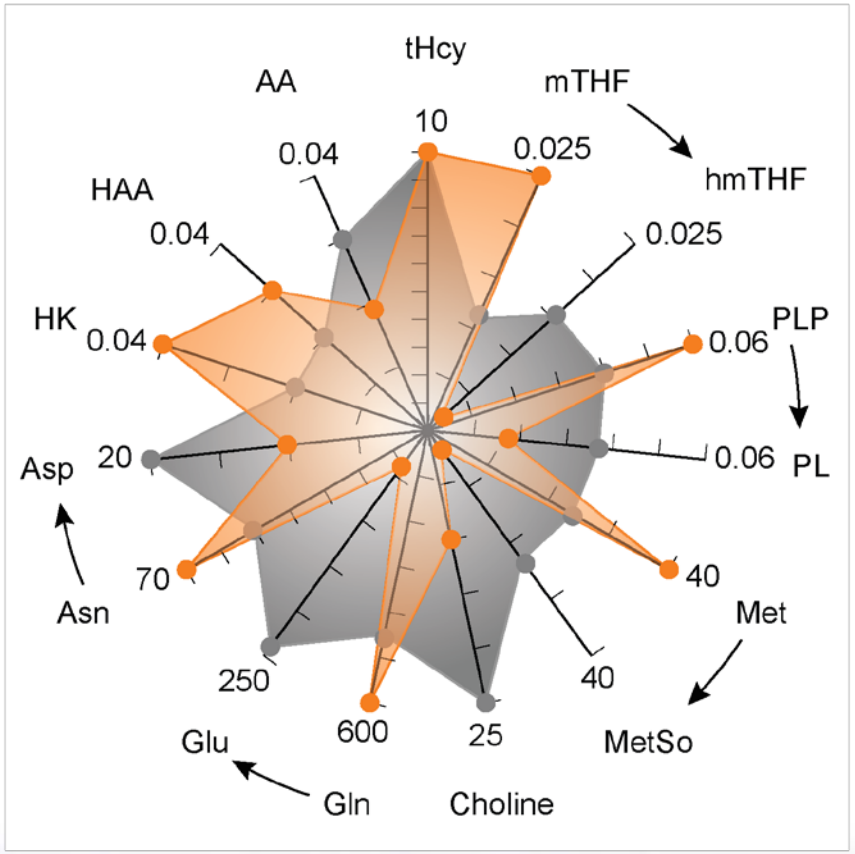
Comprehensive (All 4 Analytes Have Been Evaluated)	Incomplete (Some but Not All Analytes Have Been Evaluated)	Unexplored (No Analytes Have Been Evaluated)
Cold ischemia	Postmortem interval	Pathology ink
Decalcification	Warm ischemia time	Fixative age
Fixation duration	Specimen size	Commercial versus in-house fixative
Duration of paraffin block storage	Prefixation handling	Use of recycled formalin
	Fixative buffer	Movement during fixation
	Tissue to fixative ratio	Light exposure during fixation
	Fixation temperature	Fixation container
	Fixative delivery method	Fixation alone or with other biospecimens
	Dehydration reagent and conditions	Postfixation wash solution and conditions
	Clearing reagent and conditions	Reagents and conditions of interim alcohol storage
	Paraffin embedding reagent and conditions	Use of recycled dehydration and clearing reagents
	FFPE block size or section thickness	Automated versus manual processing
	Type of slide or adhesive	Use of recycled paraffin for impregnation and embedding
	Slide drying duration and temperature	Embedding conditions
	Storage duration of slide-mounted FFPE sections	Slide pretreatment
		Equipment and conditions of sectioning and section transfer

Taken from Bass et al, (2014)Archives of Pathology & Laboratory Medicine: Vol. 138, No. 11, pp. 1520-1530.

Stability of dynamic markers

BMC Molecular Biology 2009, **10**:31



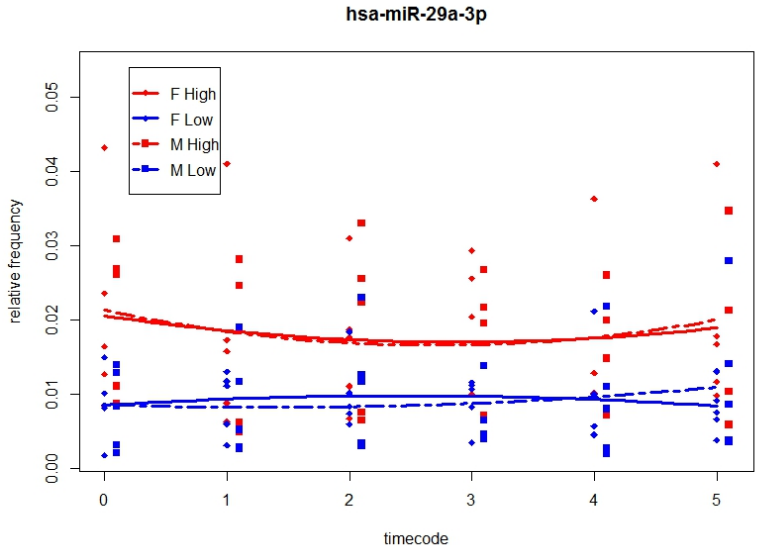


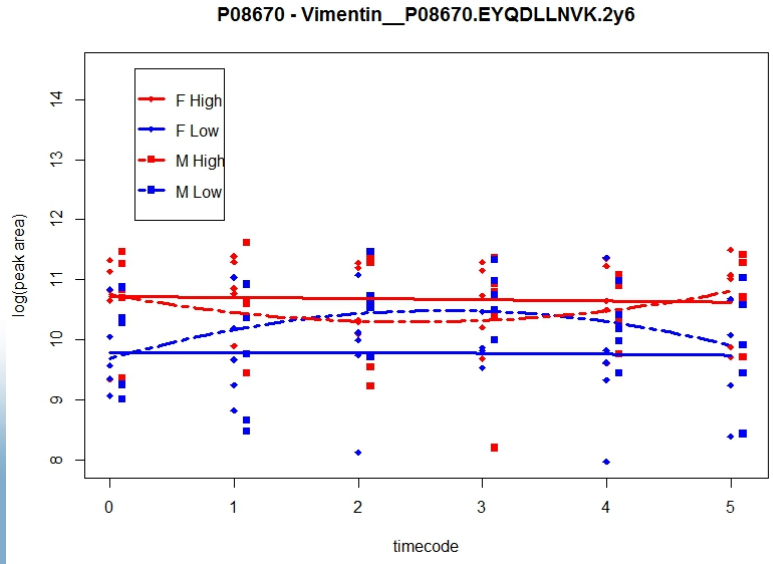
Biomarker profile of selected metabolites in fresh (orange) and improperly stored (grey) serum/plasma. Arrows indicate conversions, and concentrations are given in µmol/L.

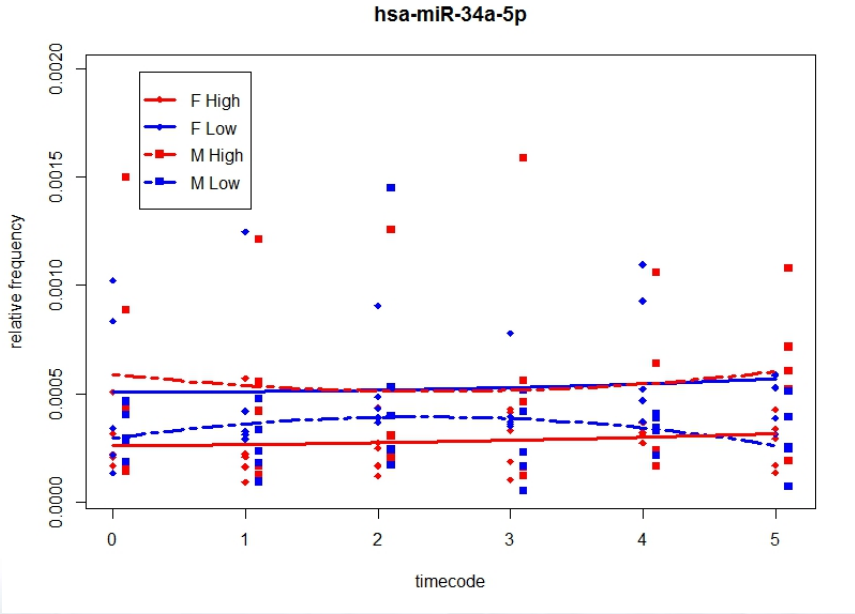
http://www.gene-quantification.de/rna-integrity2.html
Thompson KL, Pine PS, Rosenzweig BA, Turpaz Y, Retief J - (2007)
http://folk.uib.no/mfapu/Pages/BV/BVSite/stability.html

Queen's UNIVERSITY

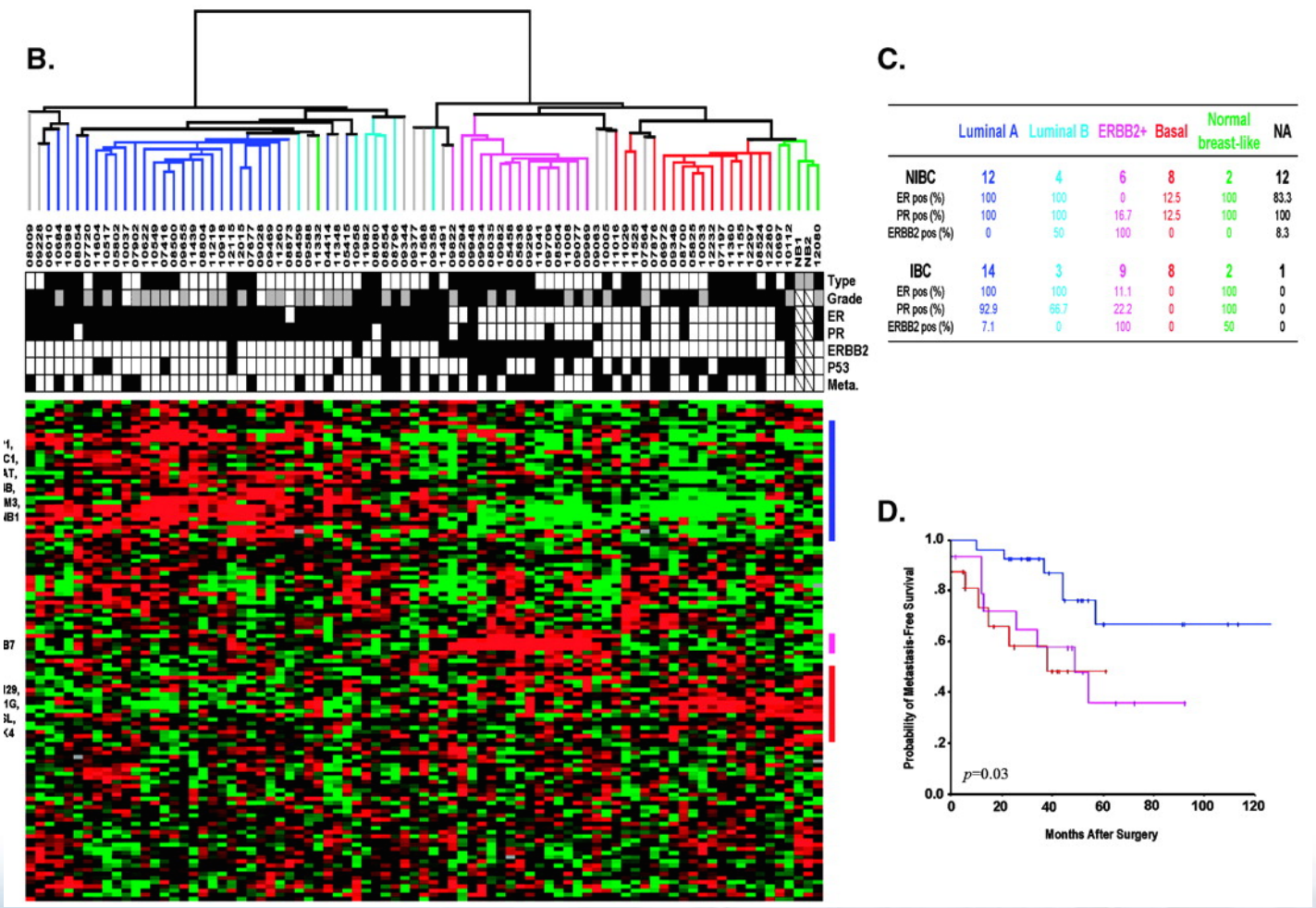
CLL: miRNA and protein profiling over time





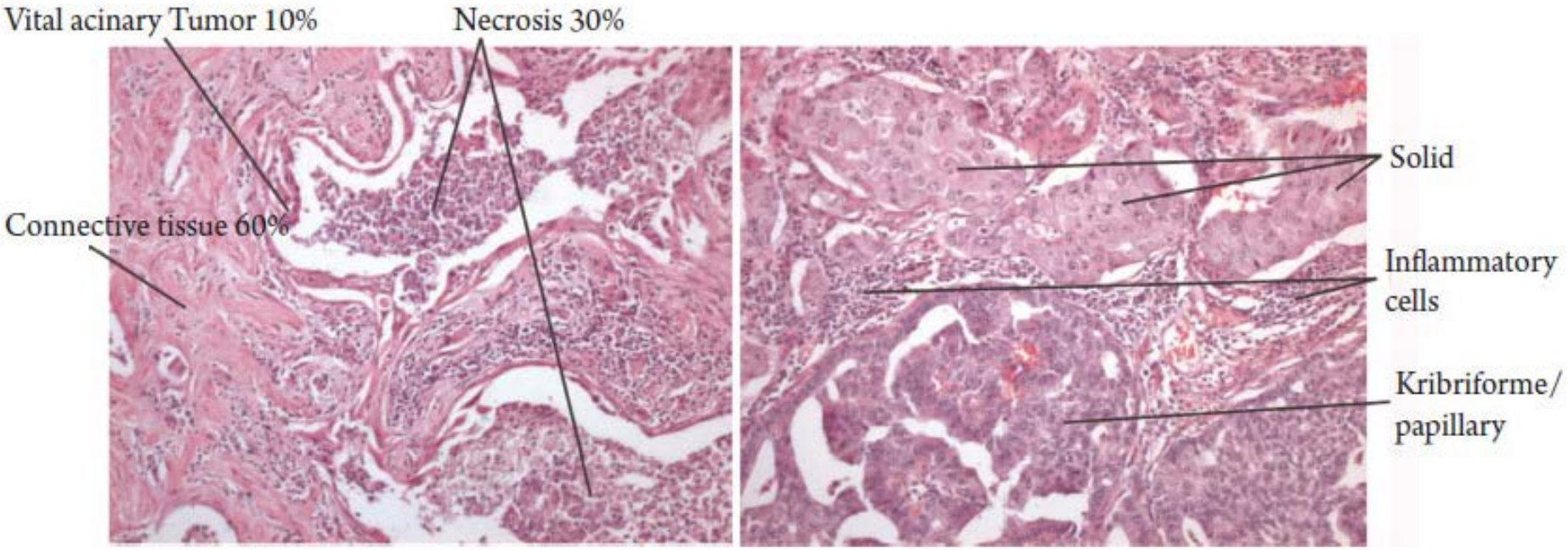


Inter-tumour heterogeneity



Cancer Res March 15, 2005 vol. 65 no. 6 2170-2178)

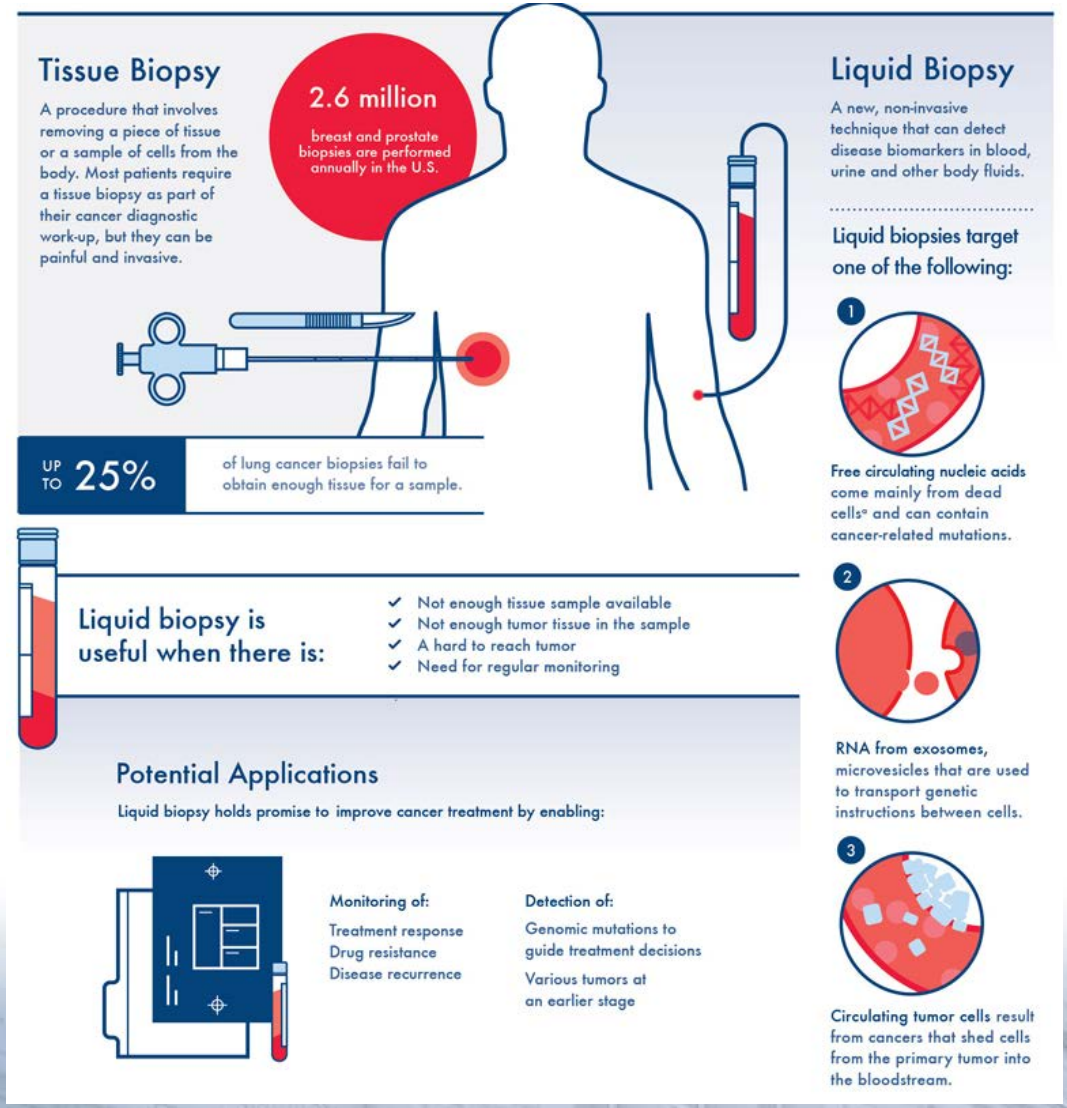
Sample or intratumour heterogeneity



Muley T, Herth FJF, Schnabel P, Dienemann H, Meister M.
From tissue to molecular phenotyping: Pre-analytical requirements Heidelberg Experience.
Transl Lung Cancer Res 2012;1(2):111-121.

Liquid biopsy

- Use of blood, plasma, urine, csf
- May measure CTCs, cfDNA, protein, miRNA, mRNA, metabolites



http://www.clpmag.com/article/liquid-biopsies-less/

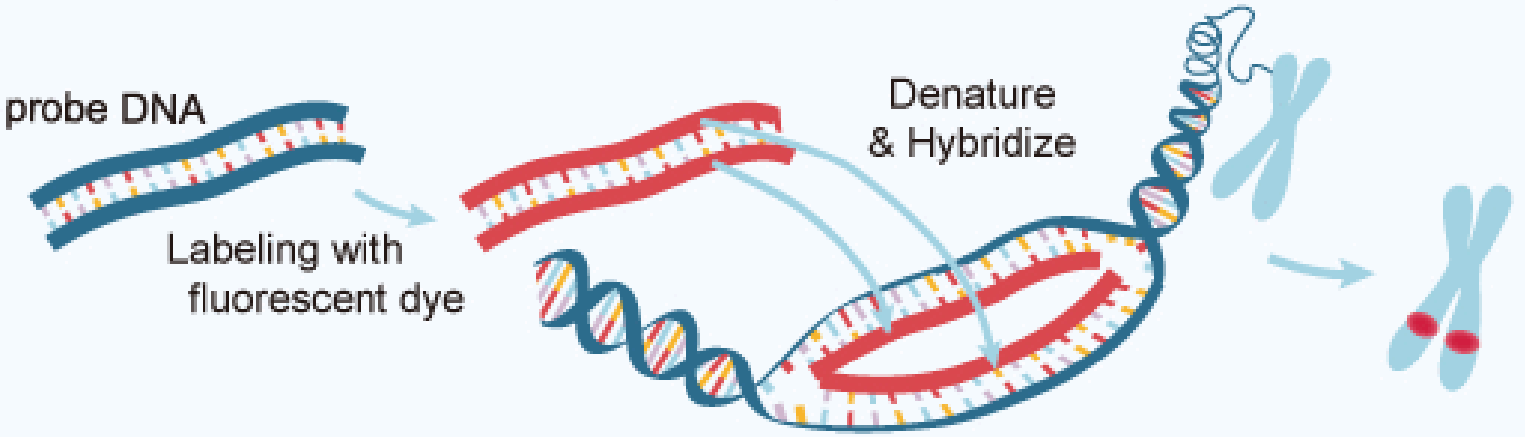
Sample considerations

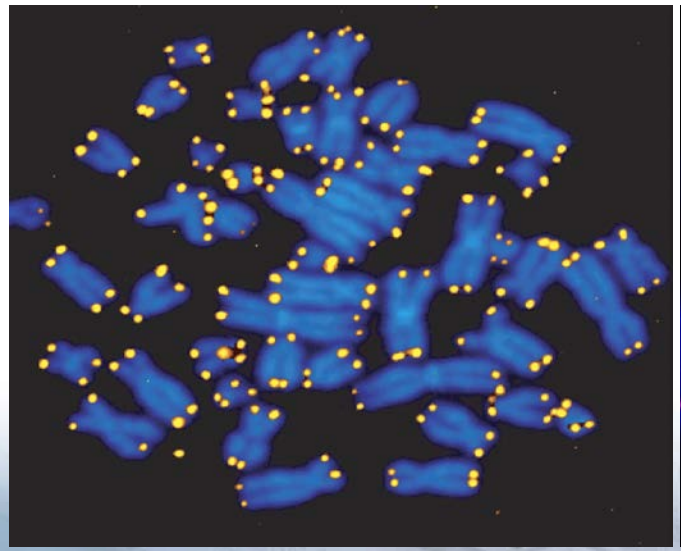
- The type of sample you have access to makes a difference in what you can measure
- The collection of the sample can influence the behaviour of a biomarker
- The processing of a sample will influence the behaviour of a biomarker
- Biomarkers don't necessarily behave the same in different sample types

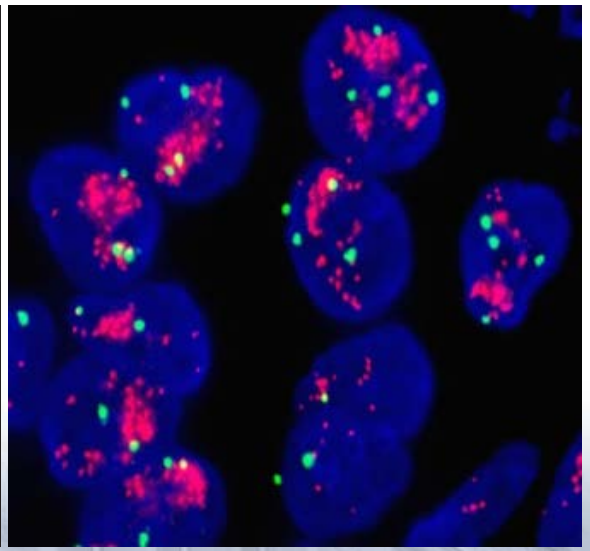
Common methods to measure

- FISH- Fluorescence in situ hybridization
- Arrays
- Sequencing – Sanger and NGS
- Nanostring

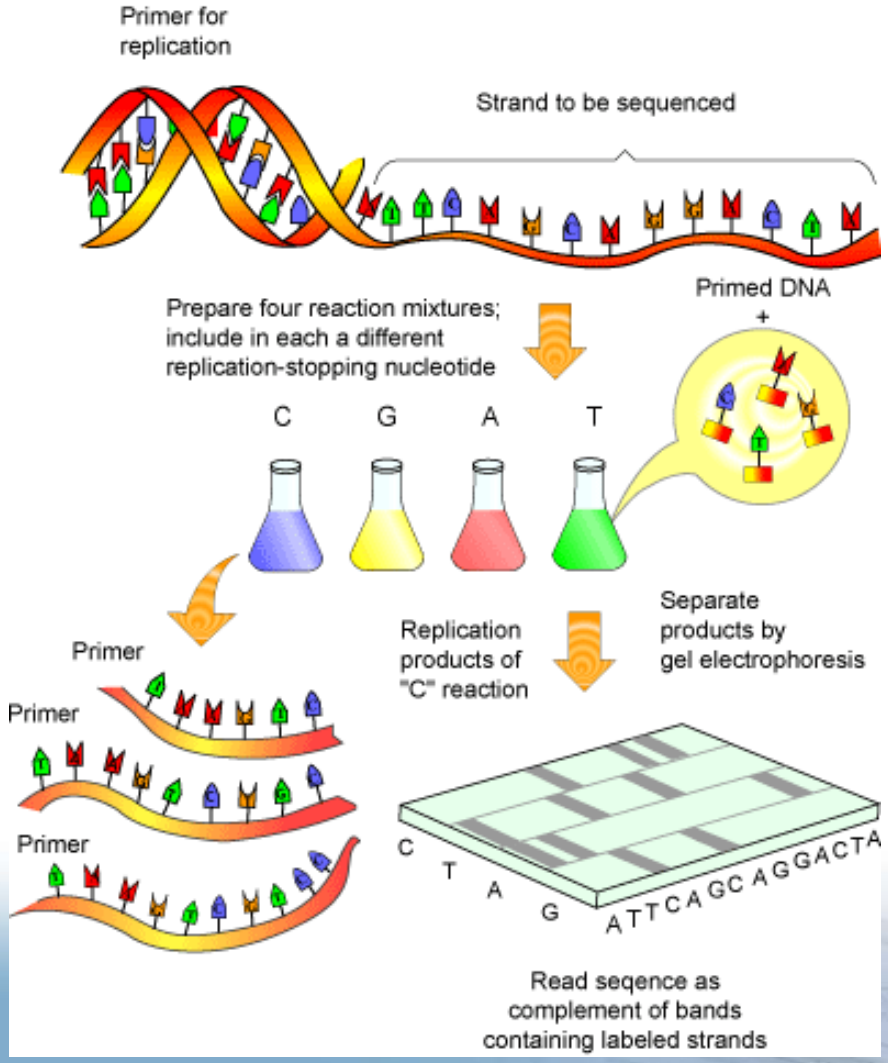
Fluoresence In Situ Hybridization

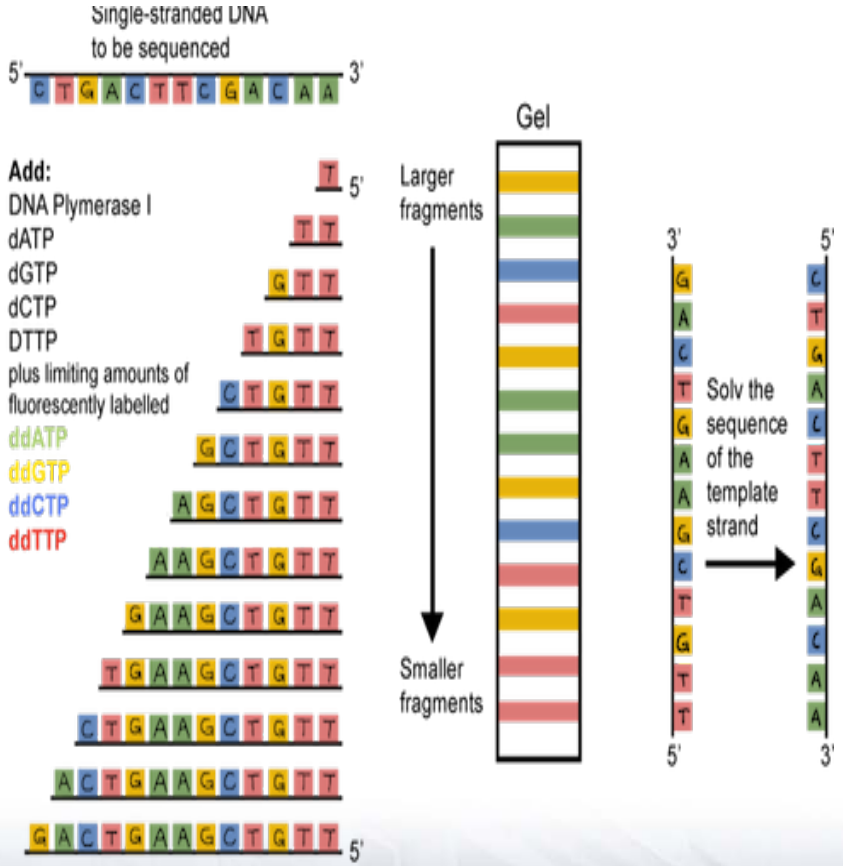






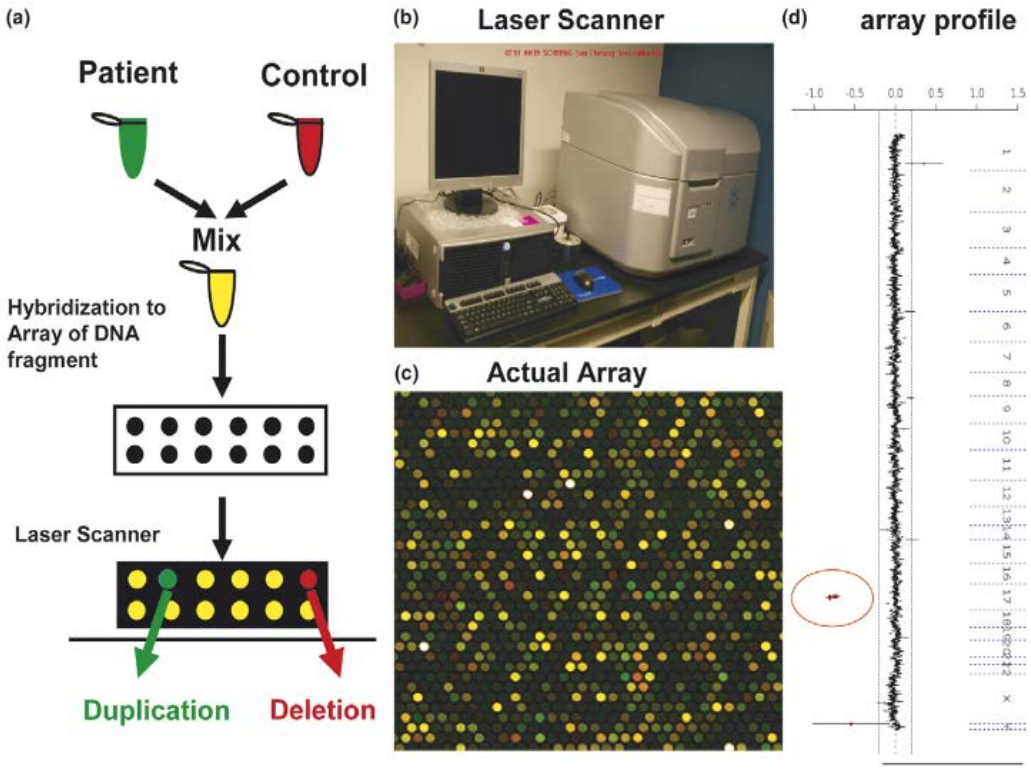
Sanger Sequencing





Microarray Approach

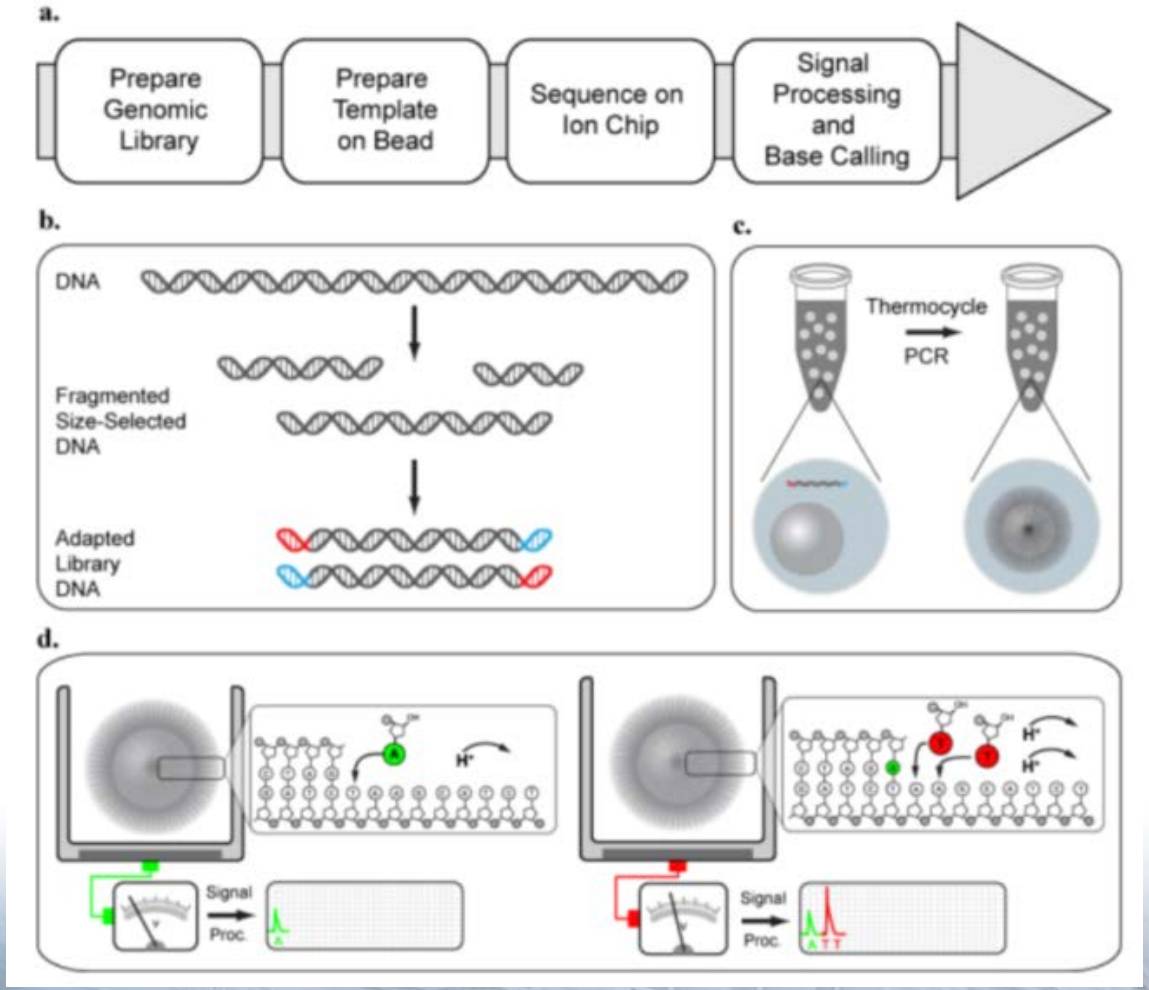
- Global or targeted
- DNA, RNA, methylation, SNPs
- Semi quantitative
- Not great for degraded material



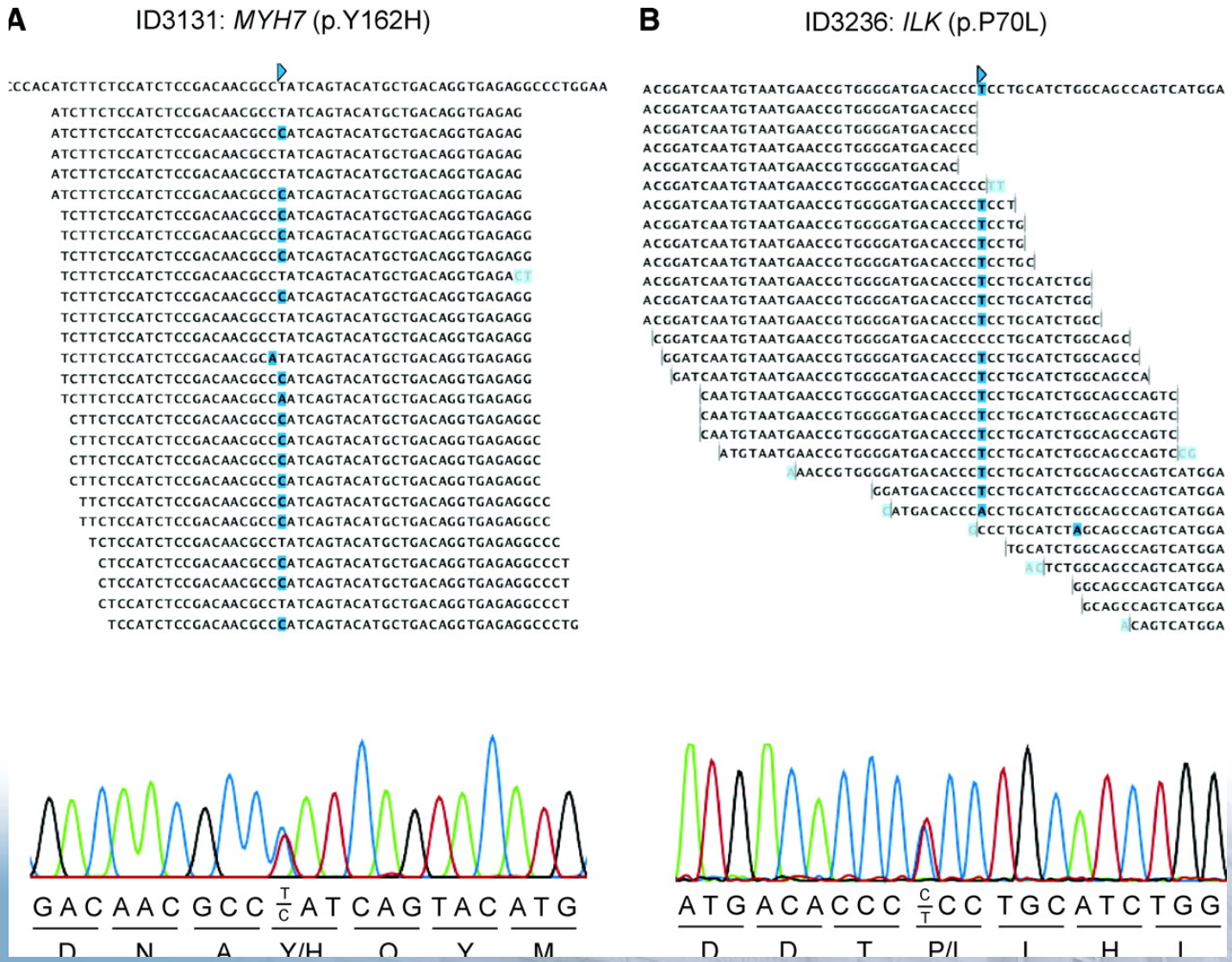
http://www.intechopen.com/books/recent-advances-in-autism-spectrum-disorders-volume-i/discovering-the-genetics-of-autism

Next Generation Sequencing

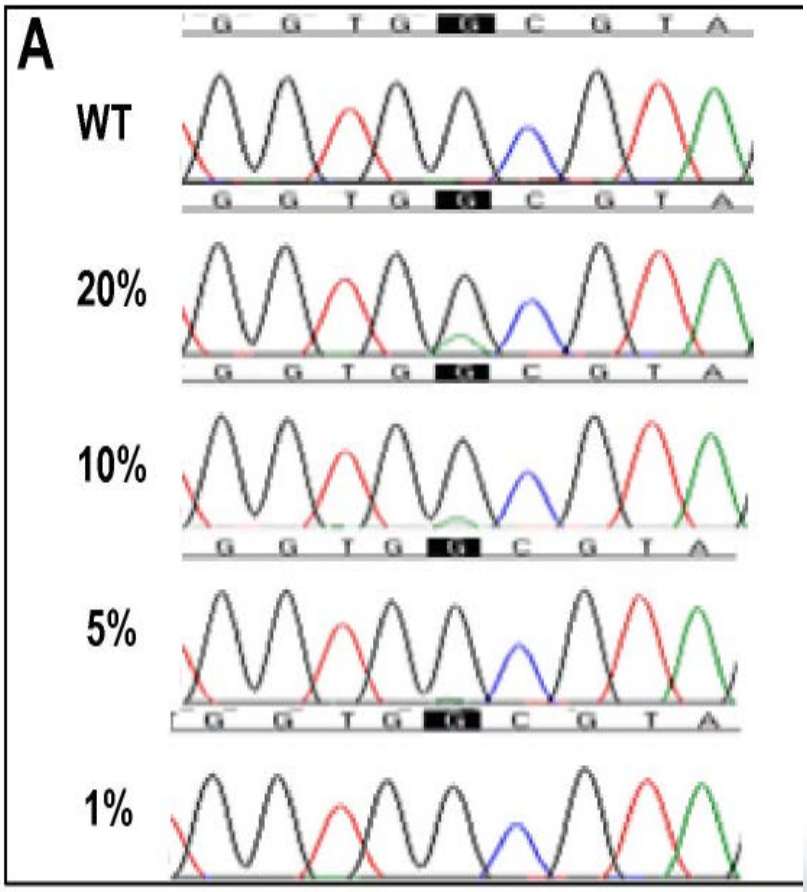
- Global or targeted
- High throughput
- Library to sequence
- Depth controls sensitivity
- Short fragments

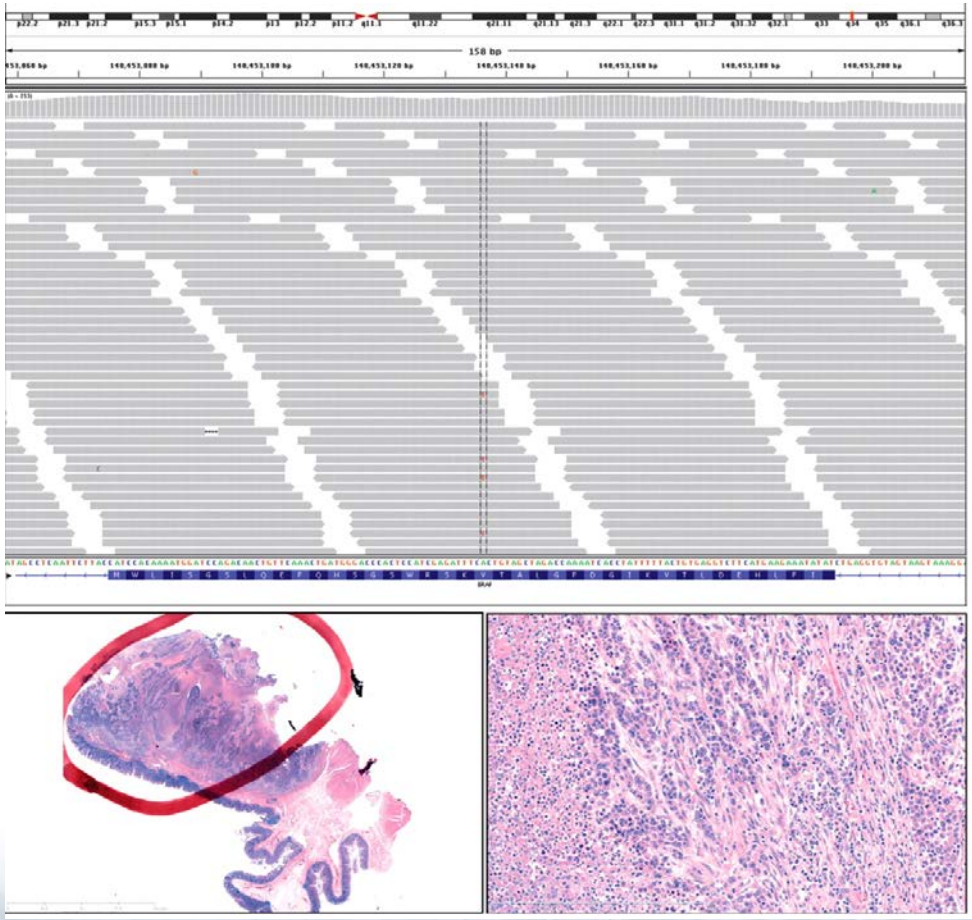


Next Gen Sequencing Data



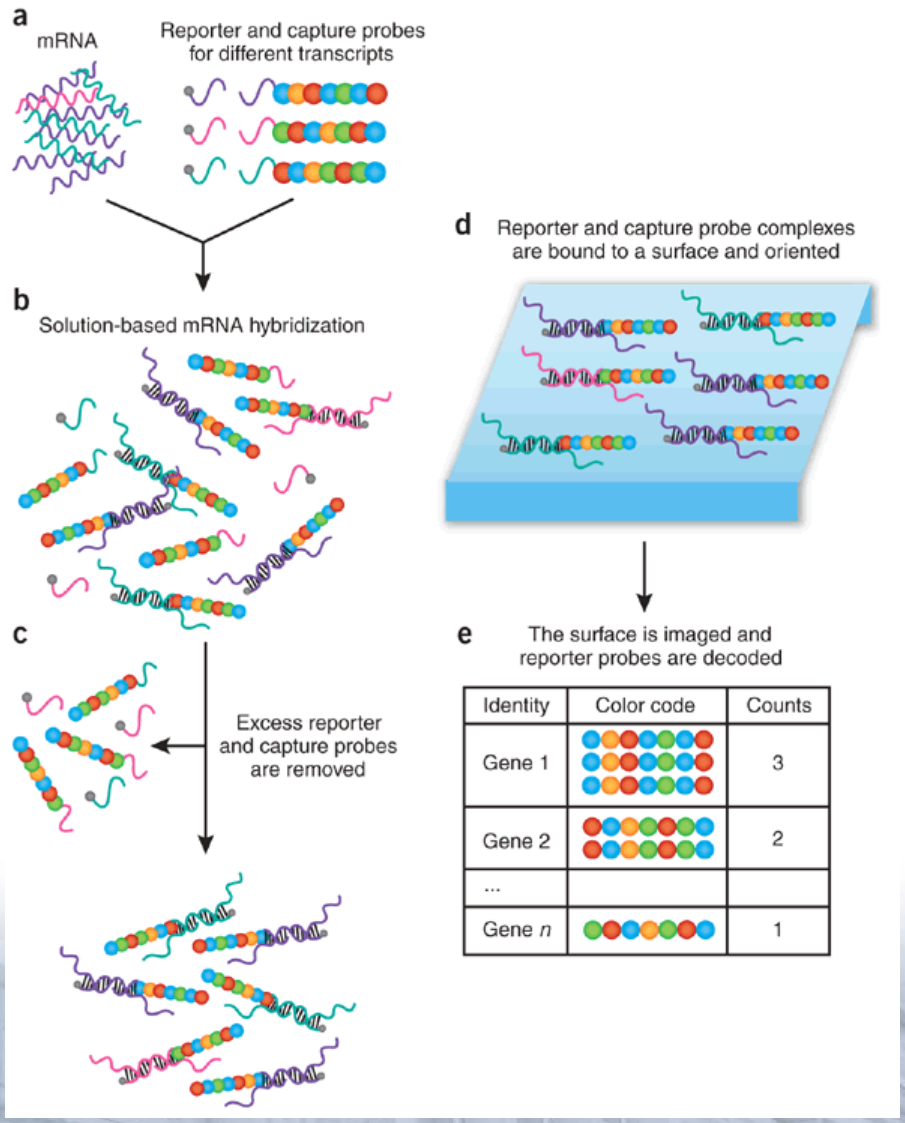
Sensitivity of NGS vs Sanger





Nanostring

- Targeted
- RNA or DNA
- Quantitative
- Short fragments



Some further considerations

- Sensitivity and specificity
- Precision and accuracy
- Analytical validity versus clinical validity
- Data preprocessing
- Data analysis

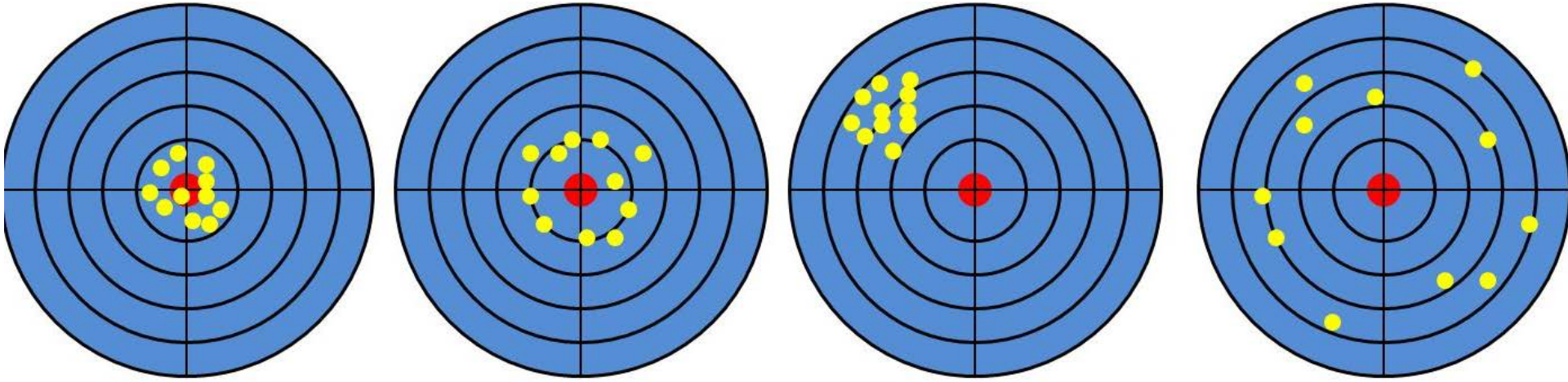
Analytic sensitivity and specificity

	Genotype			
	Present	Absent	A: True positives	C: False negative
			B: False positives	D: True negative
Test				
Positive	A	B	Sensitivity:	A/(A+C)
			Specificity:	D/(D+B)
Negative	C	D	Positive predictive value:	A/(A+B)
			Negative predictive value:	D/(C+D)

If you are sequencing a tumour looking for KRAS mutations, ***analytic sensitivity*** refers to the proportion of time that the mutation is there and you can find it. ***Analytic specificity*** refers to how often the mutation is not there and you think you see it.

It has no bearing on the clinical utility of whether or not there is a KRAS mutation

Accuracy, precision



Accurate & Precise

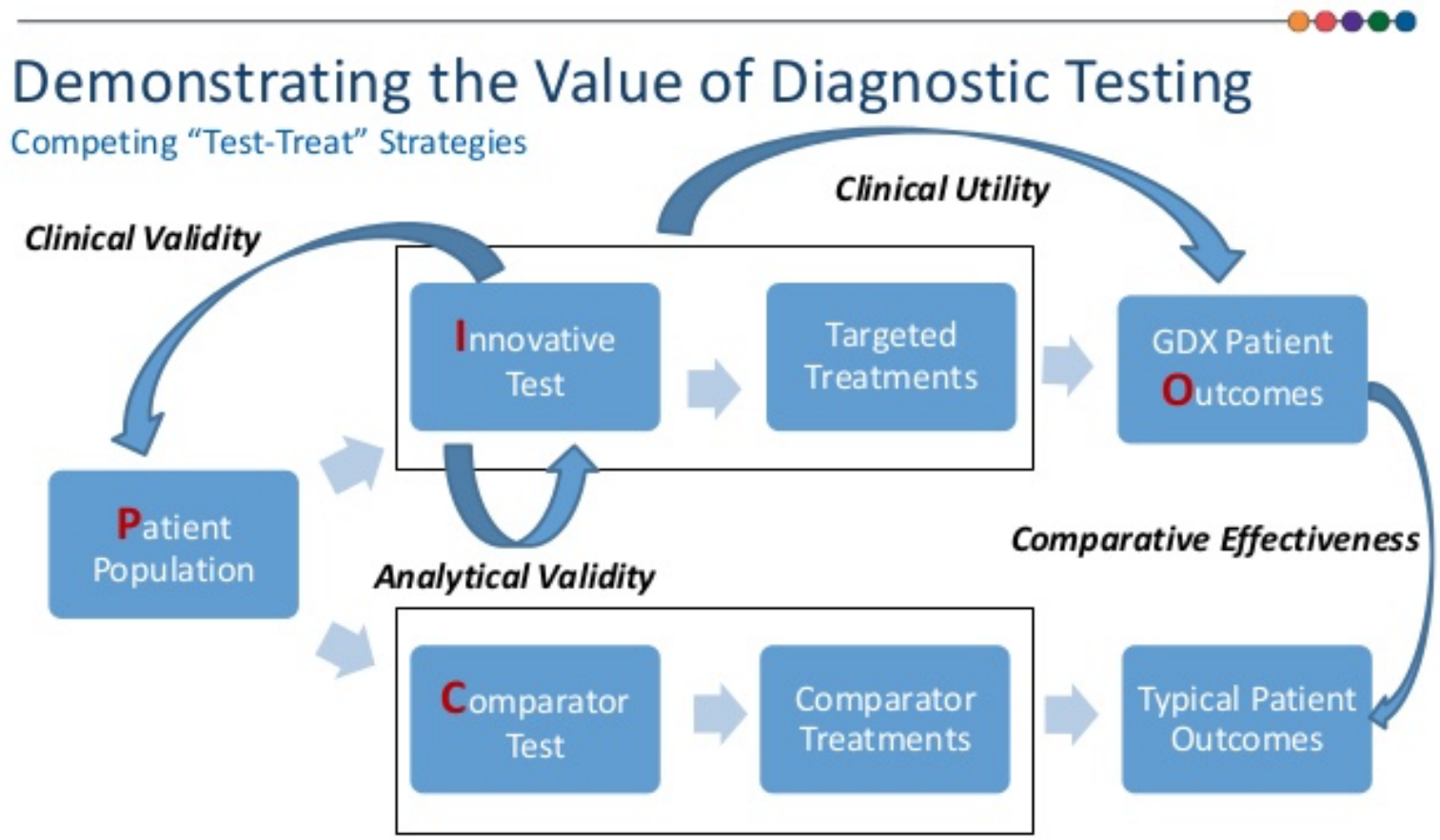
Accurate & Imprecise

Inaccurate & Precise

Inaccurate & Imprecise

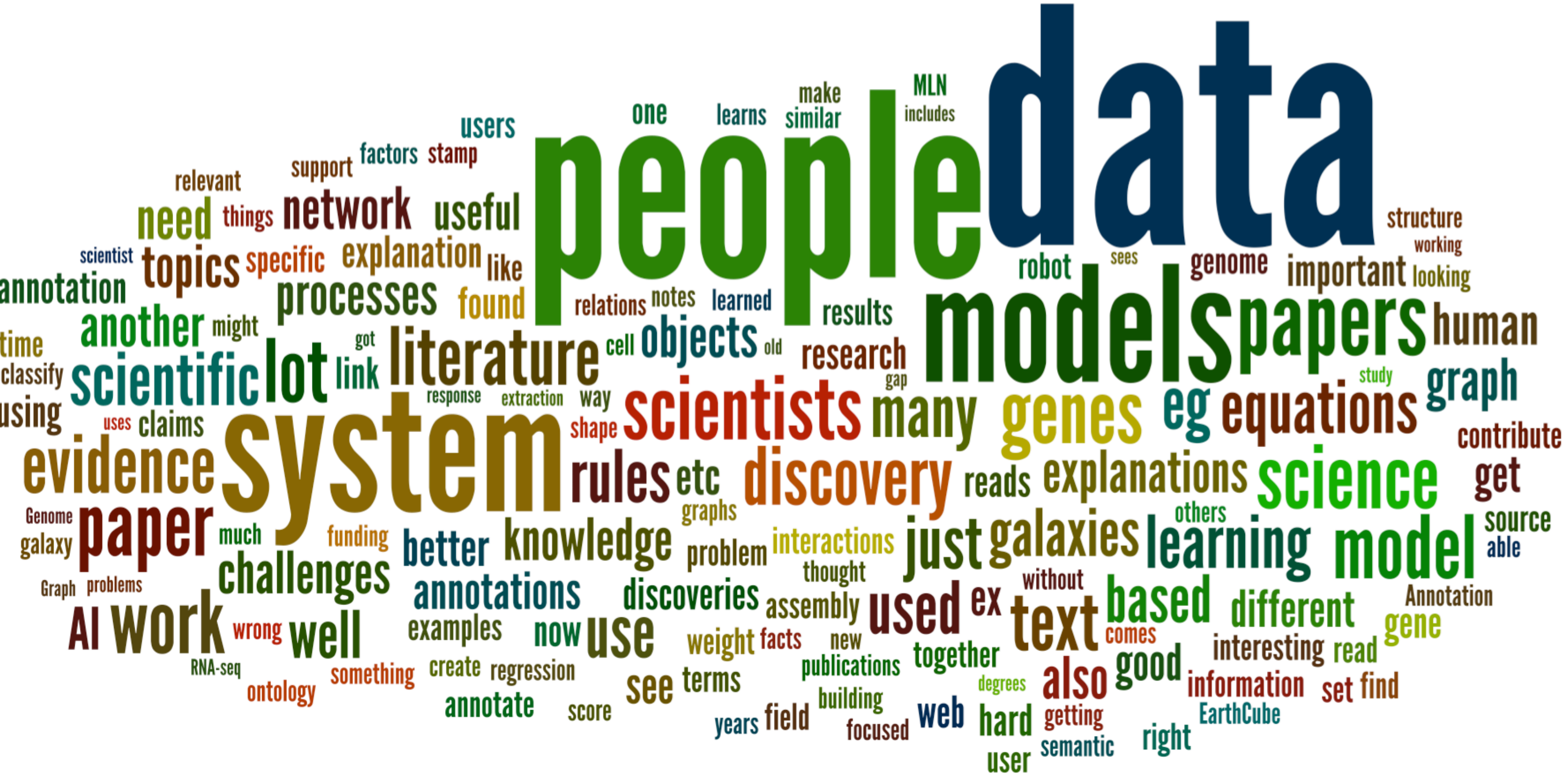
Do all biomarkers need to be accurate and precise?

Clinical versus Analytical Validity



GENOVA DIAGNOSTICS

Data



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others
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galaxy
much
funding
better
problem
interactions
thought
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Annotation
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problems
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create
regression
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terms
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EarthCube
years
field
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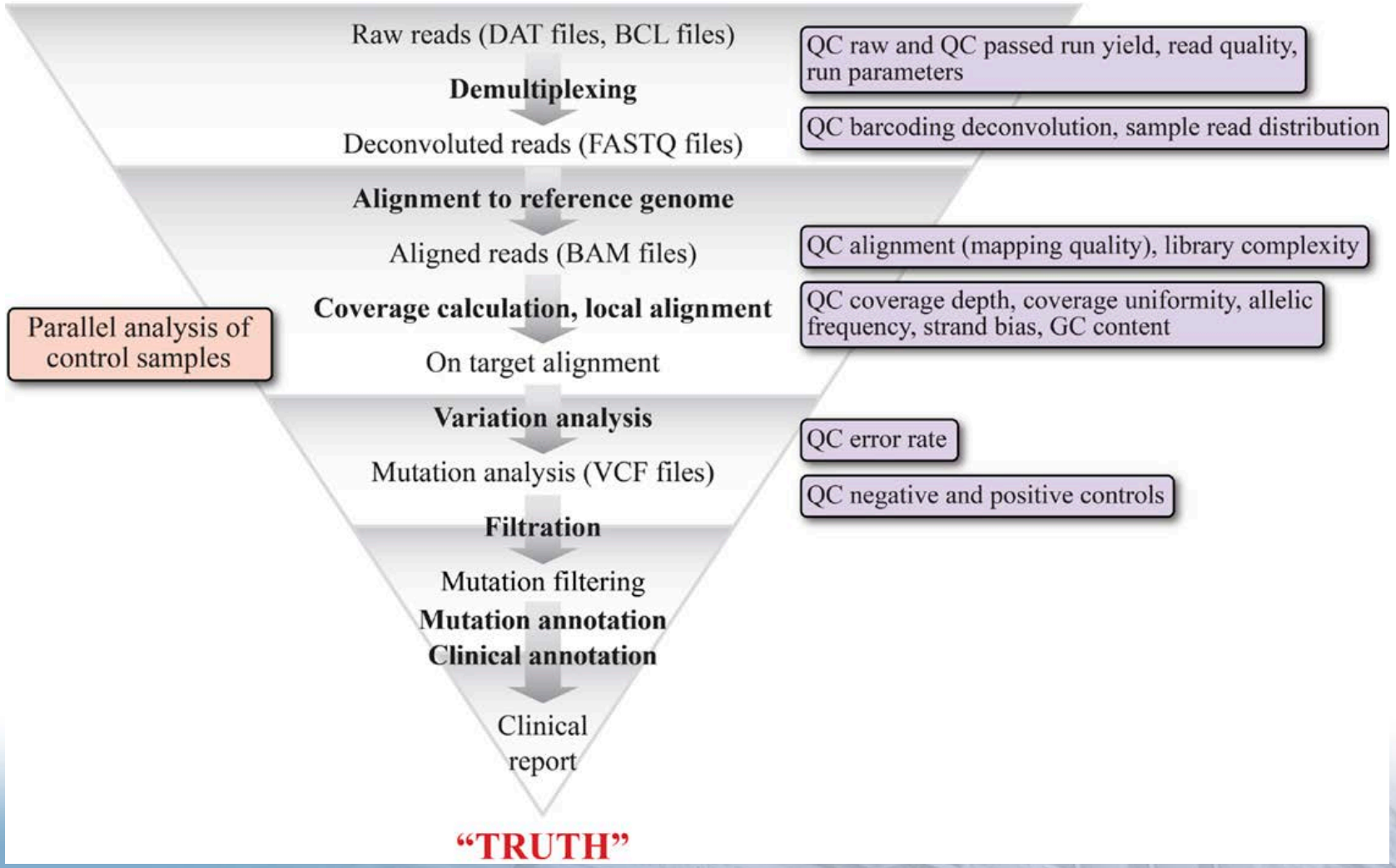
user

Data Handling

- Feature extraction
- Processing, cleaning, filtering
- Alignment
- Analysis
- Pipeline for these events must be locked down prior to using biomarker

Before you get it…



Front. Oncol., 17 April 2014 | http://dx.doi.org/10.3389/fonc.2014.00078

Conclusions

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