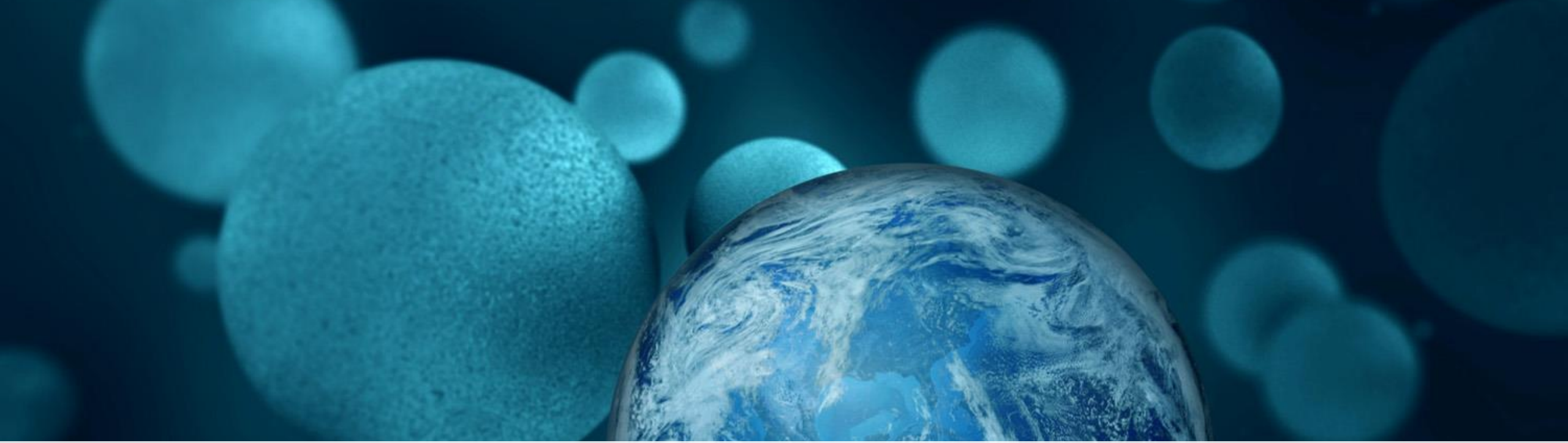

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Why Do You Need Quality Controls For Oncology Assays?

Kara Norman, Ph.D.

Kara Norman Introduction

Ph.D. in Molecular Oncology & Virology at the University of Calgary, Canada

- Cancer-killing reovirus

Postdoctoral Fellowship at Stanford University

- Hepatitis C virus and microRNA-122
- RNA regulation

Life Technologies / Thermo Fisher Scientific

- Quality Controls for Research and Diagnostics

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Thermo Fisher Scientific Quality Controls: MAS And AcroMetrix

MAS Brand: Clinical Chemistry Controls

AcroMetrix Brand: Molecular Controls

Clinical Diagnostics Labs						Blood Screening Labs	IVD Manufacturers
Clinical Chemistry				Molecular Lab			
General Chemistry	Urinalysis	Specialty + Toxicology	Immuno-assay + Cardiac	Infectious Disease Testing	Oncology Research Testing	Infectious Disease NAT / Serology	Custom Controls
Alcohol, CSF, Bilirubin, Urichem-TRAK	UA DipTube, Sentry Urine Diptstick	Immunology, Diabetes, DOA, TOX	Liquimmune, T-marker, Cardio-Immune	HIV, HCV, Transplant Viruses, MRSA etc.	NGS and PCR Controls	Yellow,Purple, HIV/HCV/HBV Syphilis etc.	Oncology, Serology, Infectious Diseases Assay Feasibility, LOD, Specificity V&V Panels, Implementation Packages

Omni Controls
Integrated controls: Omni•CORE, Omni•Immune, Omni•Immune Pro, Omni•Cardio

Quality Assurance
Lab Link xL 2.0 Cloud-based, all-in-one quality assurance software with real time peer QC data comparison

- Mimics patient samples
- Traceable to higher order standard eg. WHO

simplerQC

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Agenda

1. How do you choose a quality control for NGS?
2. How do you choose a *good* quality control for NGS?
3. Why should you perform regular QC for NGS?

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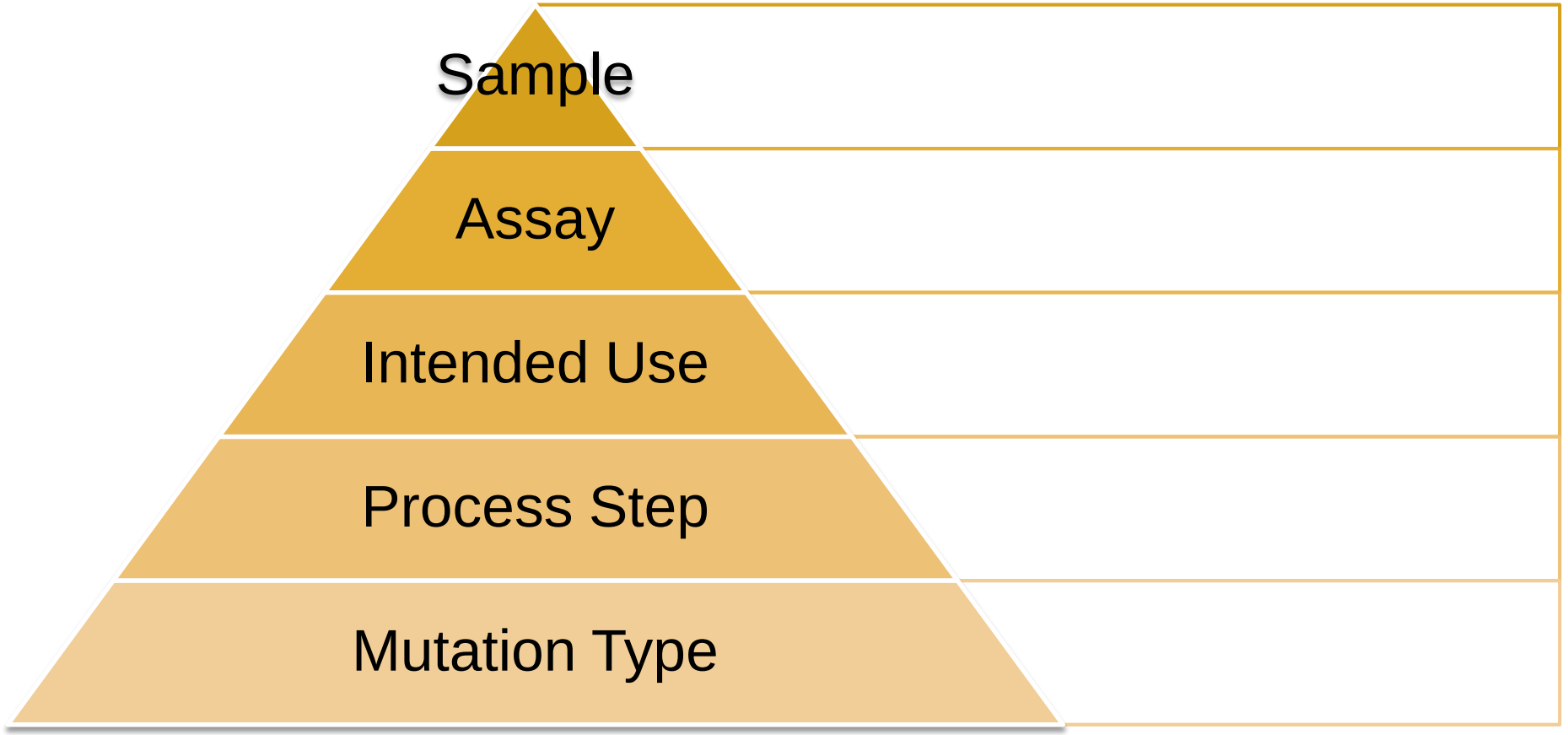
How Do You Choose The Right Quality Control Material?



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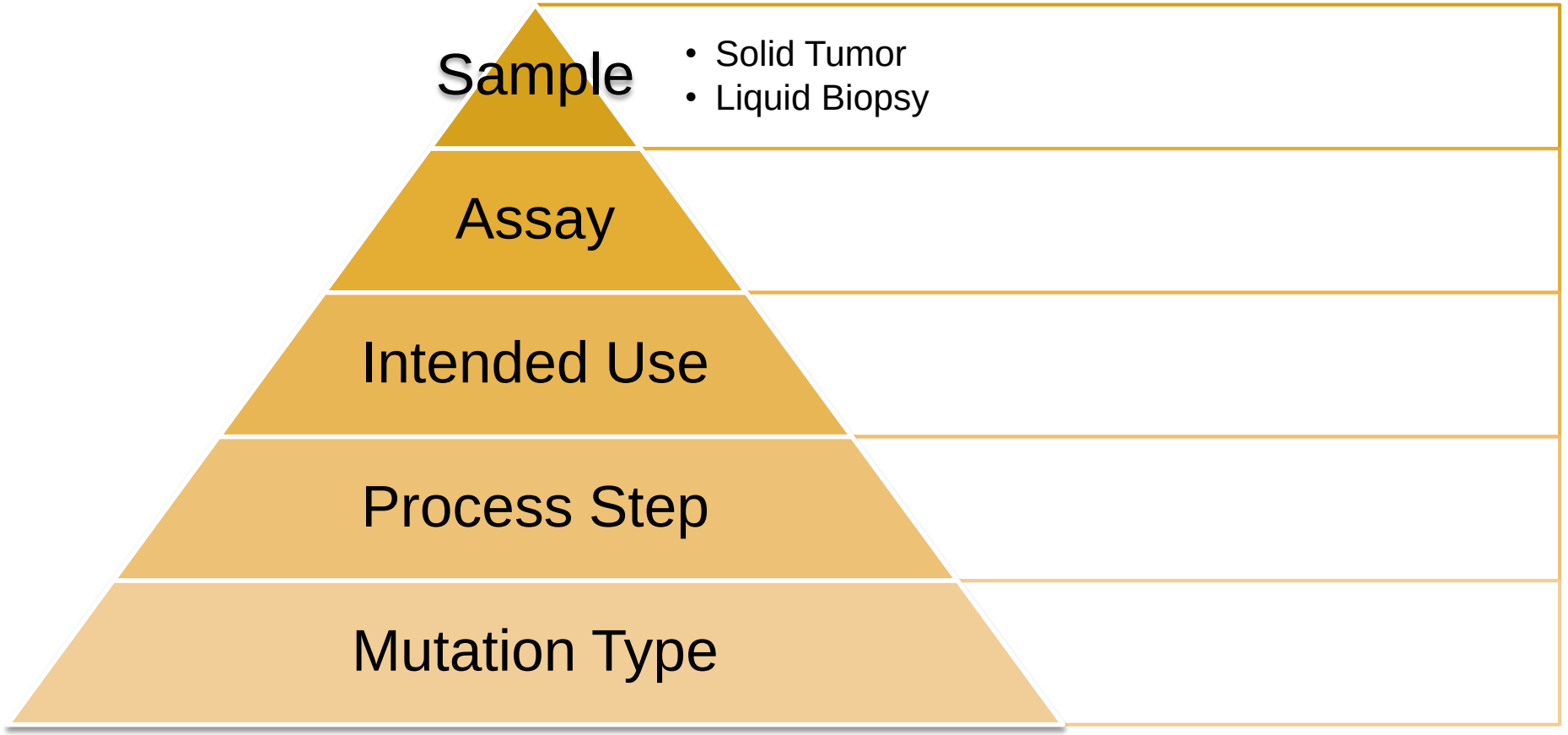

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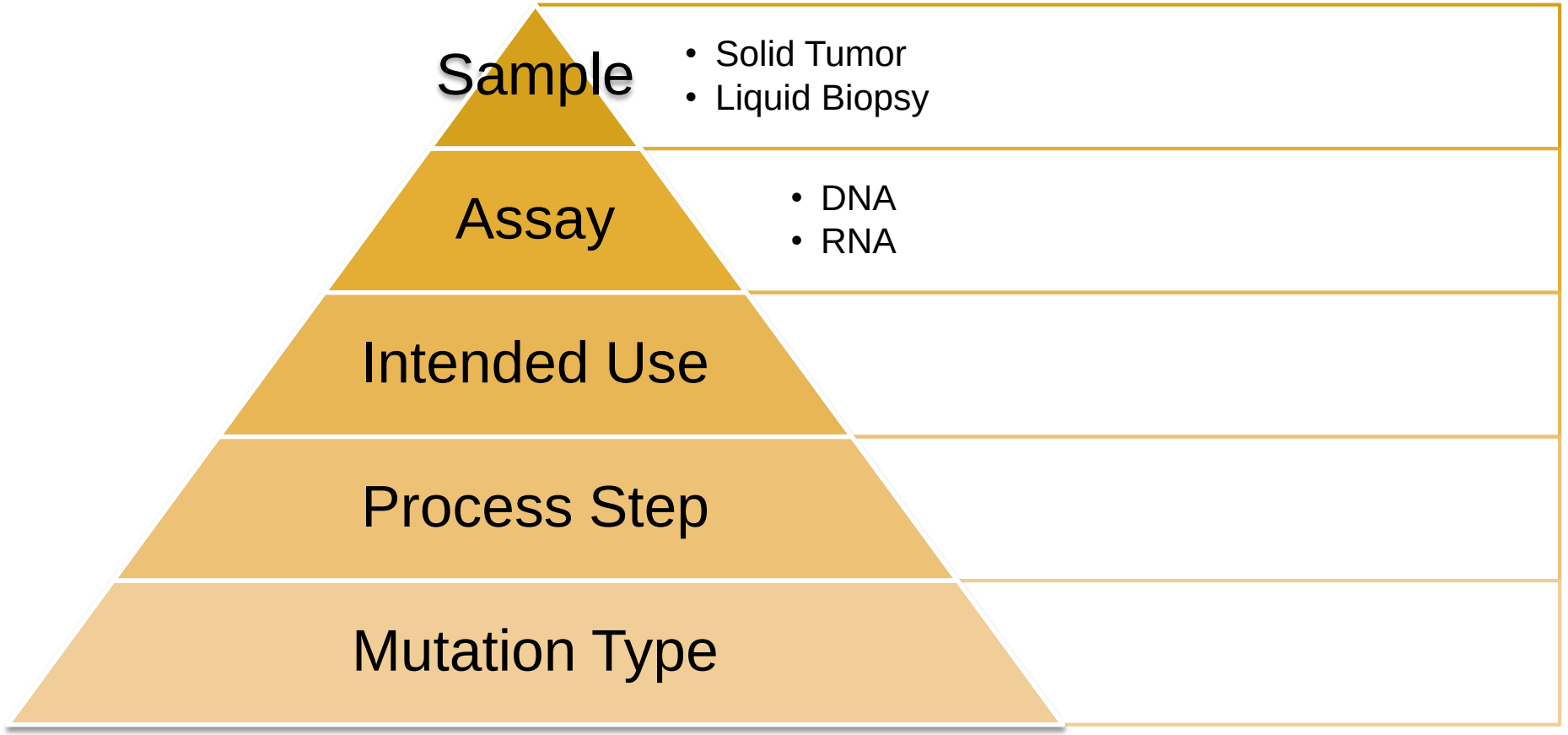

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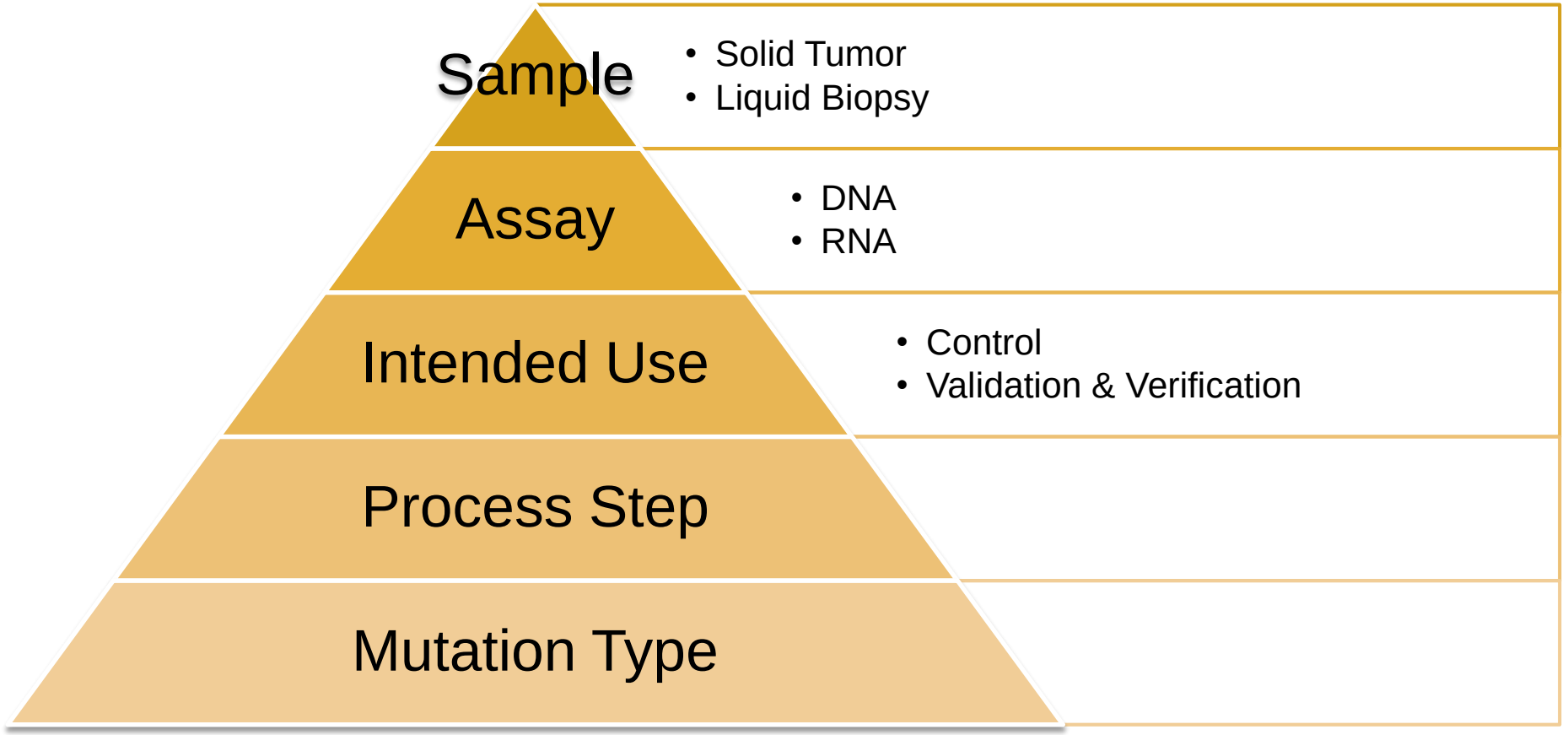

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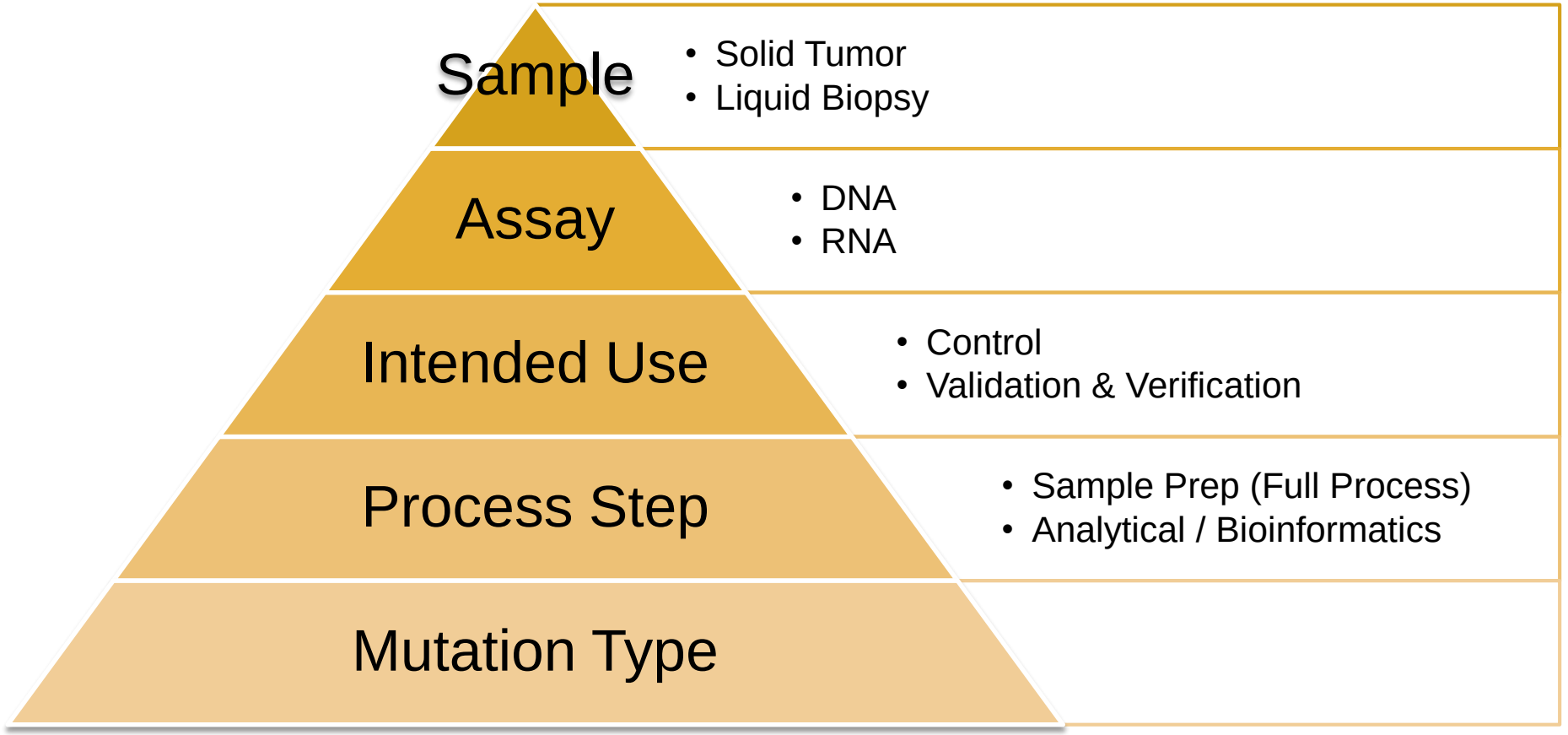

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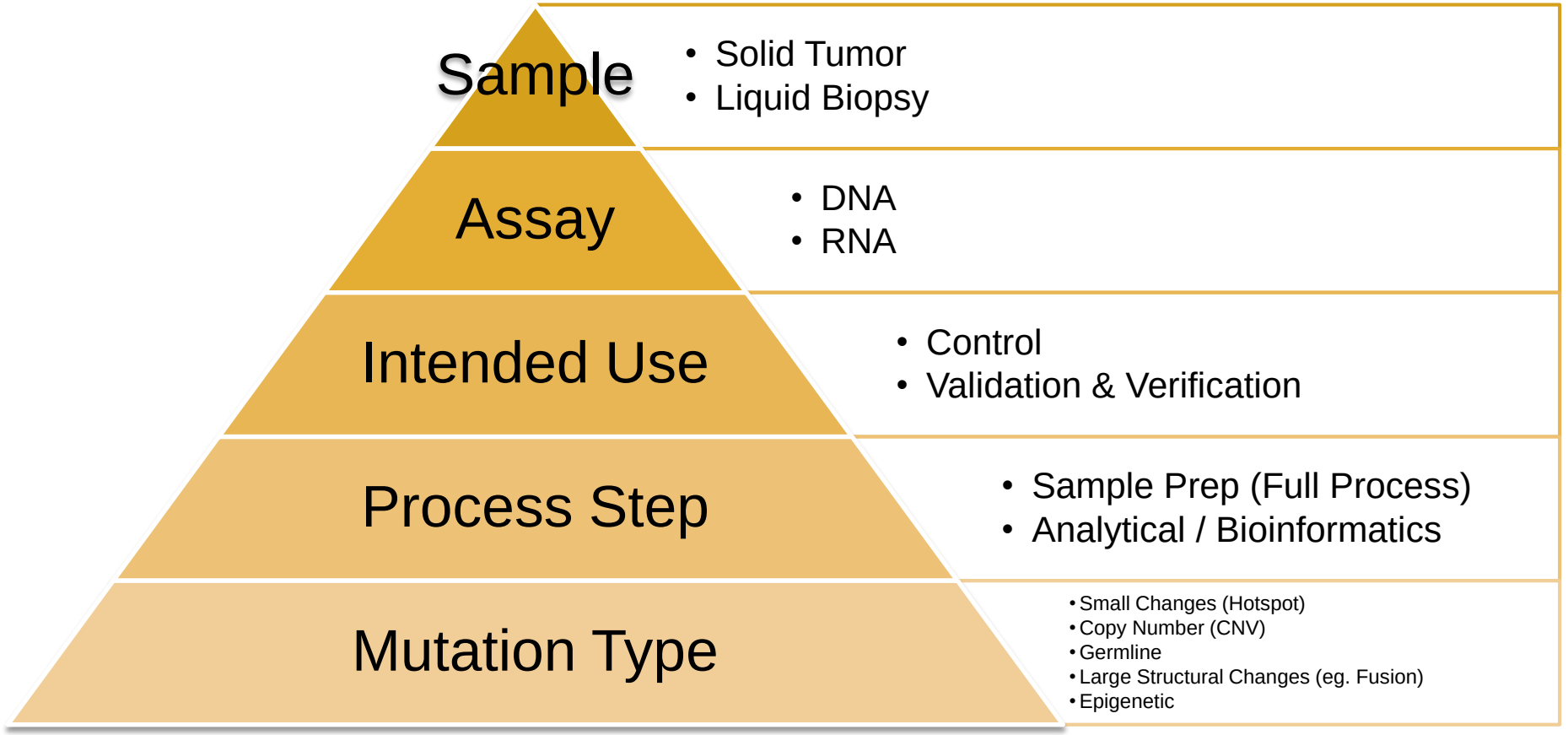

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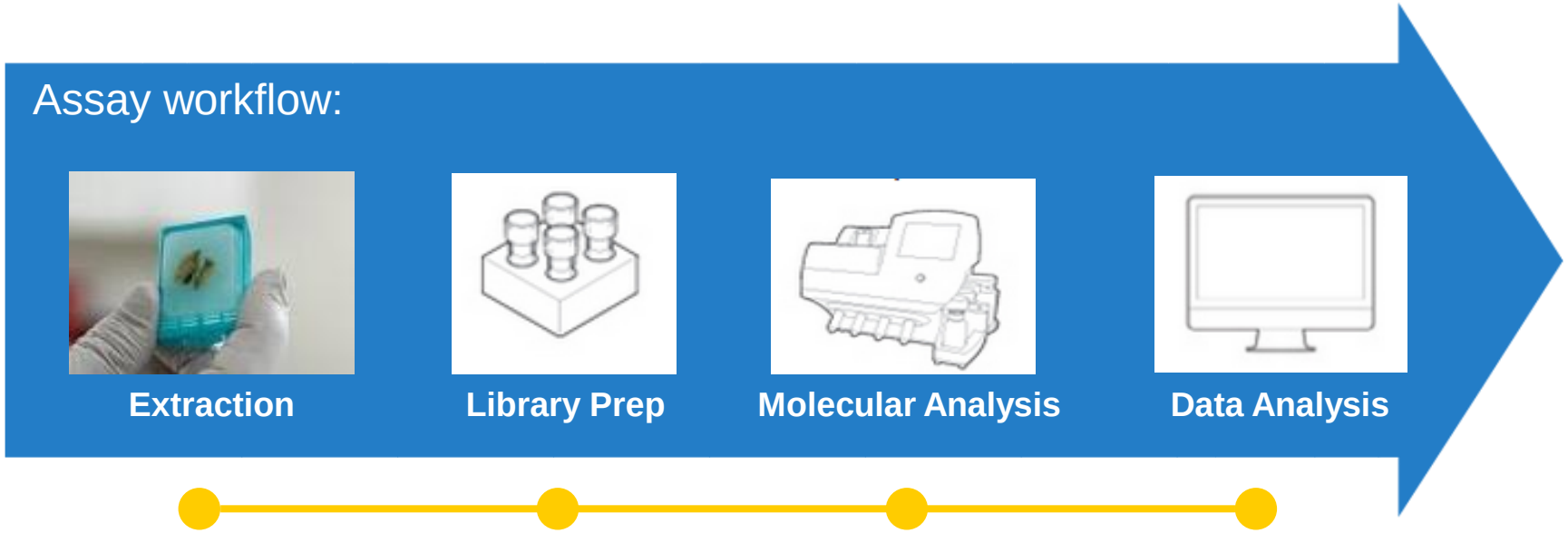
Choosing The Right Material For Your Application



Full Process FFPE ctDNA in plasma	Downstream QC Pure DNA or RNA

Quality controls and reference materials are used for assay development and V&V, implementation, and regular QC monitoring

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Agenda

1. How do you choose a quality control for NGS?
2. How do you choose a *good* quality control for NGS?
3. Why should you perform regular QC for NGS?

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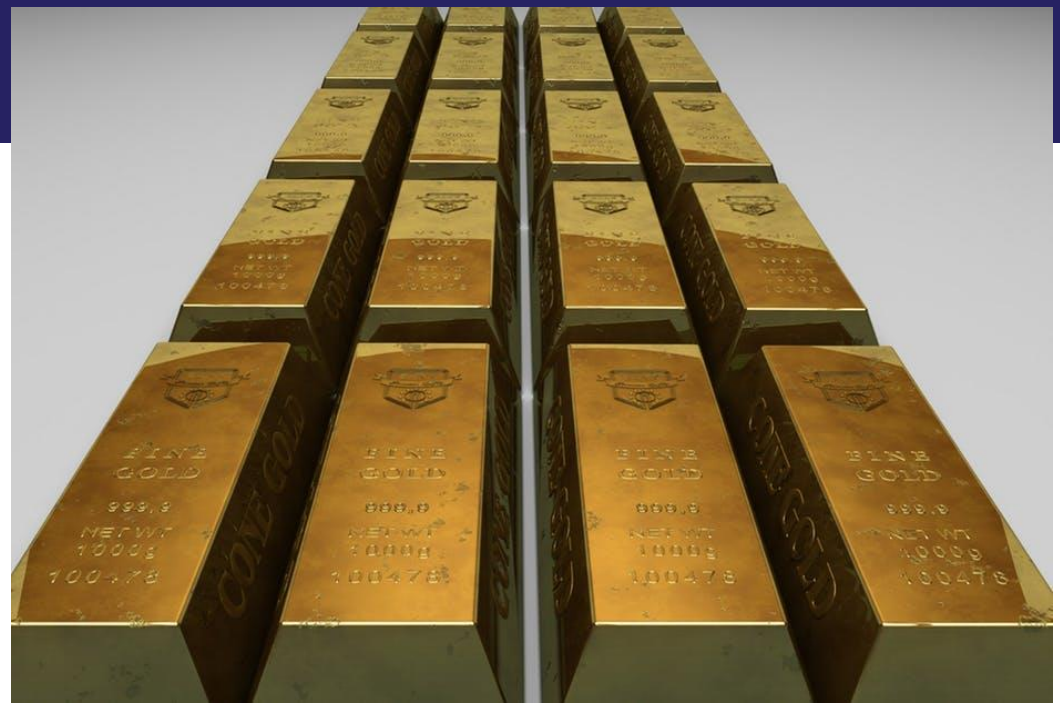
How do you choose a *good* quality control for NGS?

- Gold Standard Truth
- Traceable
- Commutable
- Streamlined
- Consistent

- Truth is the foundation of controls & standards field
- Need a Gold Standard
- Assay development is dependent on the availability of well characterized samples



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False positives and false negatives are clearer when you know the truth

- Cell lines / lab tissue culture
  - Reference sequence unknown: NGS on one platform only is not sufficient – every platform has limitations and only Sanger is generally accepted as the gold standard method
  - Effects of cell passage: unstable genomes / ploidy changes, cross-contamination (727 problematic cell lines identified[1])
  - Scars from genetic engineering / off-target modifications
  - *Has the donor consented?*

[1]Cellosaurus: https://web.expasy.org/cgi-bin/cellosaurus/search?input=%27problematic%20cell%20line%27

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How do you choose a *good* quality control for NGS?

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  - Scars from genetic engineering / off-target modifications
  - *Has the donor consented?*
- So what do we do?

[1]Cellosaurus: https://web.expasy.org/cgi-bin/cellosaurus/search?input=%27problematic%20cell%20line%27

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The NIST Genome In A Bottle

The Genome in a Bottle Consortium
(www.genomeinabottle.org) is hosted by
NIST: National Institute of Standards and Technology

Source	**Human, Male, 45yr**
Cell Type	**B-Lymphocyte**
Cell ID	**GM24385**

- Academic, industry, regulatory, and measurement science institutions with a goal to promote standardisation in whole genome sequencing.
- Incorporate sequences from as many platforms as possible to get to the truth sequence
  - The human genome is 3 billion base pairs
  - Every platform has strengths and weaknesses, and some regions are impossible for all platforms
  - GIAB enables IVD manufacturers to benchmark performance against a Gold Standard, eg. GM24385
- From the Personal Genome Project (http://www.personalgenomes.org)
  - Ensures appropriate donor consent is obtained

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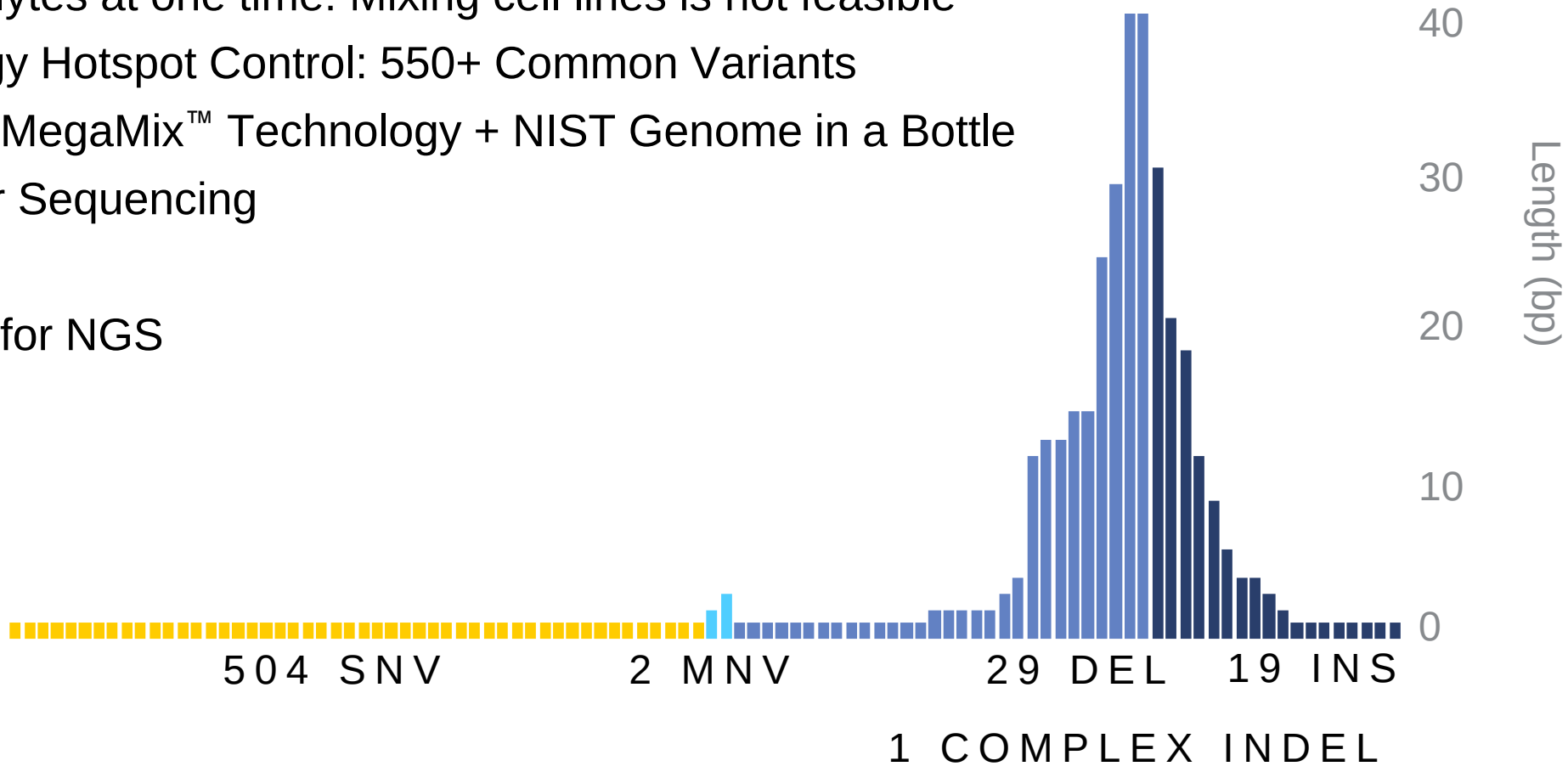
Knowing The Truth

- NGS tests many analytes at one time: Mixing cell lines is not feasible
- AcroMetrix™ Oncology Hotspot Control: 550+ Common Variants
- Built on AcroMetrix™ MegaMix™ Technology + NIST Genome in a Bottle
- Confirmed by Sanger Sequencing
- Platform Agnostic
- The only IVD control for NGS



simplerQC

For In Vitro Diagnostic Use

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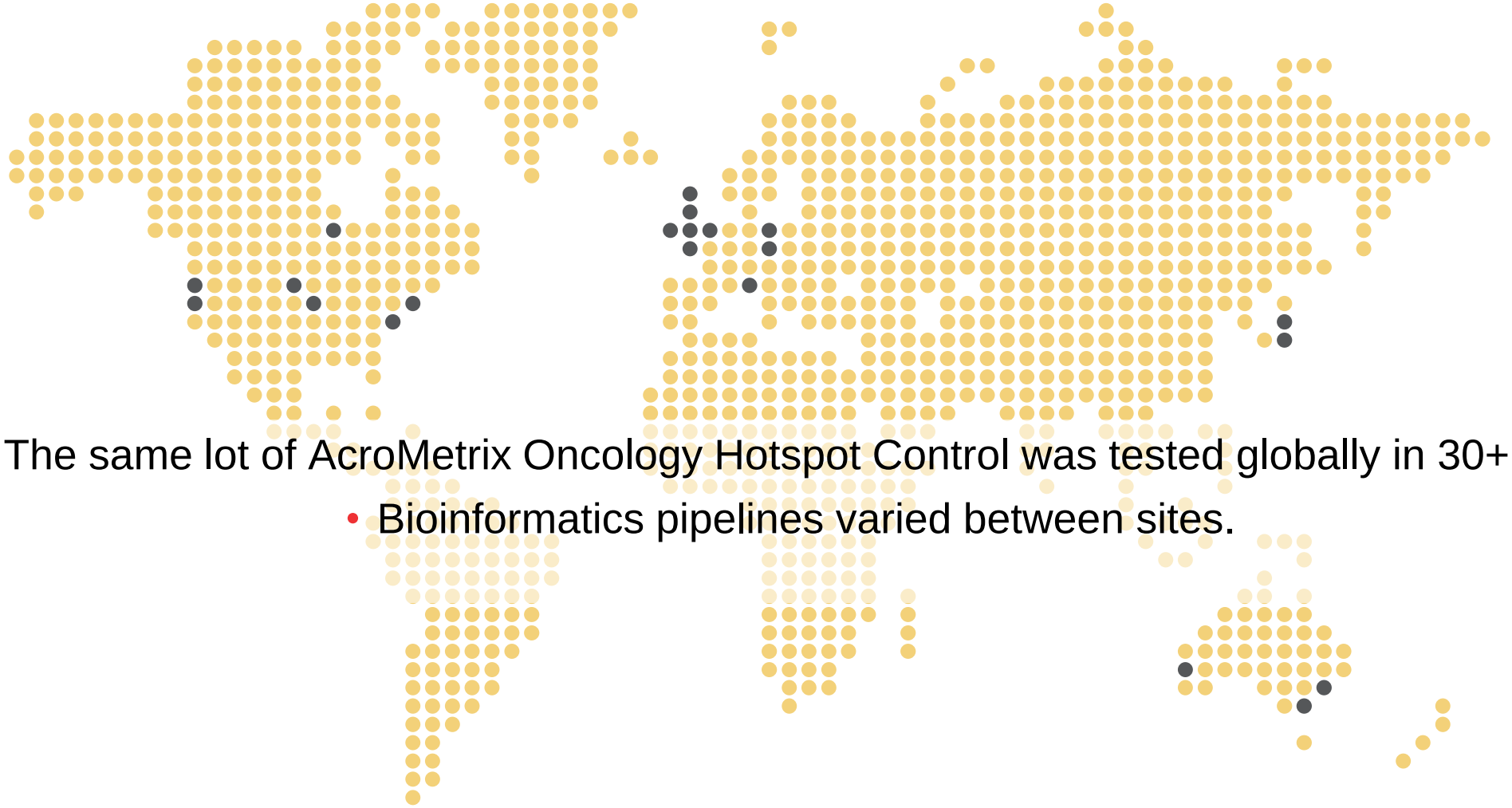
Knowing The Truth



- The same lot of AcroMetrix Oncology Hotspot Control was tested globally in 30+ sites
  - Bioinformatics pipelines varied between sites.

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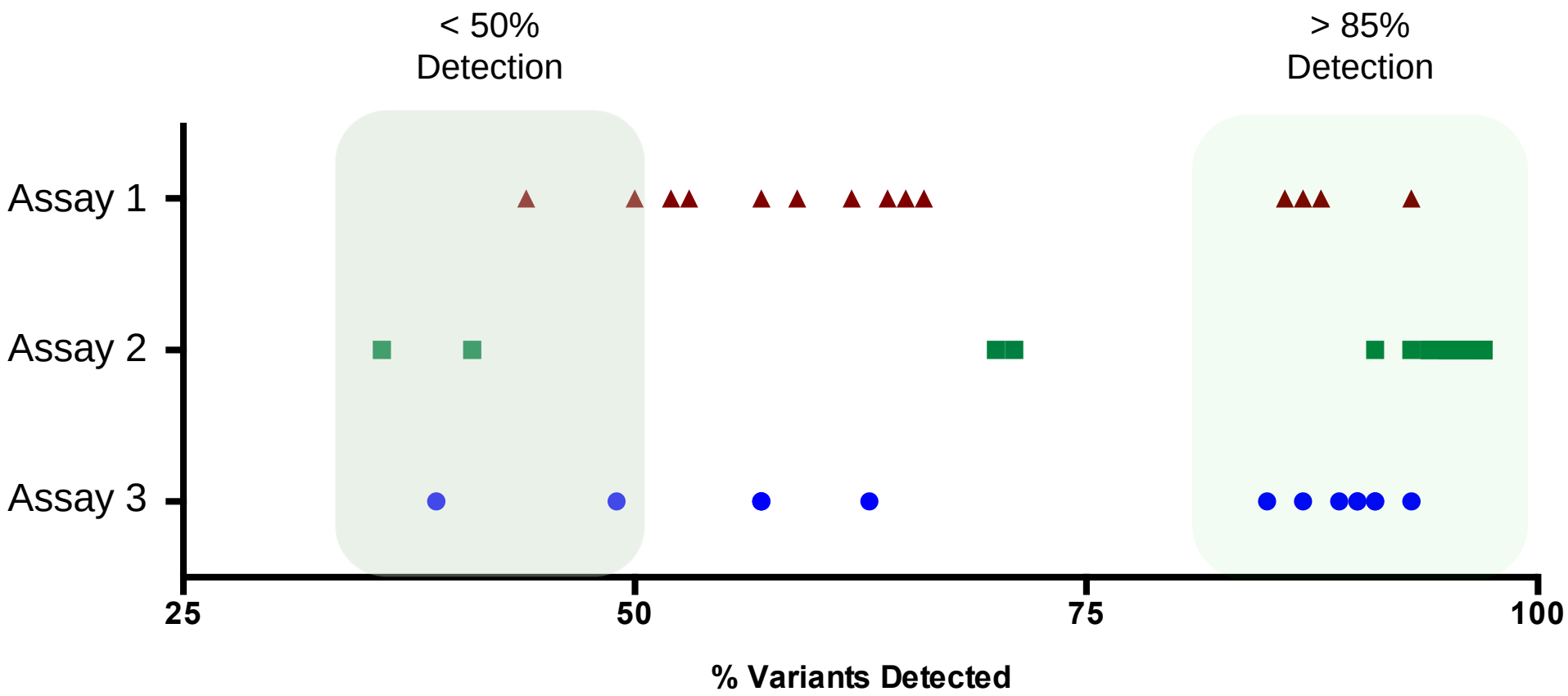
Knowing The Truth Enables Labs To Test Sensitivity

Genome in a Bottle Background + 555 Variants
Most labs had > 85 % Sensitivity



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How Do You Choose A *Good* Quality Control For NGS?

False positives and false negatives are clearer when you know the truth

- The human genome is extremely large and complex
- Using well-characterized controls enables simpler performance assessment
  - Fewer questionable results

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How Do You Choose A *Good* Quality Control For NGS?

- Gold Standard Truth
- Traceable
- Commutable
- Streamlined
- Consistent

- Why is traceability important in measurement?
- It is easy to take measurement for granted
- How long is a meter?
  - Two planes flying by each other from two different countries – they need to agree on how far is 50 kilometers

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Traceable Reference Materials Promote Measurement Standardization

- Gold Standard Truth
- Traceable
- Commutable
- Streamlined
- Consistent

- Standardization and traceability improve with technology
- Some older definitions for the meter
  - the length of a pendulum having a half-period of one second (this was a problem because, gravity)
  - one ten-millionth of the length of the earth's meridian through Paris from pole to the equator (this was also a problem, because the earth has flat parts)
- Now: The meter is the length of the path travelled by light in vacuum over 1/299,792,458 th of a second

https://physics.nist.gov/cuu/Units/meter.html

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Traceable Reference Materials Promote Measurement Standardization

- Gold Standard Truth
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- Traceability in NGS is still evolving
- Calibrate all measurements to an international standard
- Anchor needs to be unchanging
- Anchor value assignment can be arbitrary (eg. International Unit) or established by a reference method
- Given that there is no anchor for most alleles, how much variability do we see in frequency across platforms and runs?

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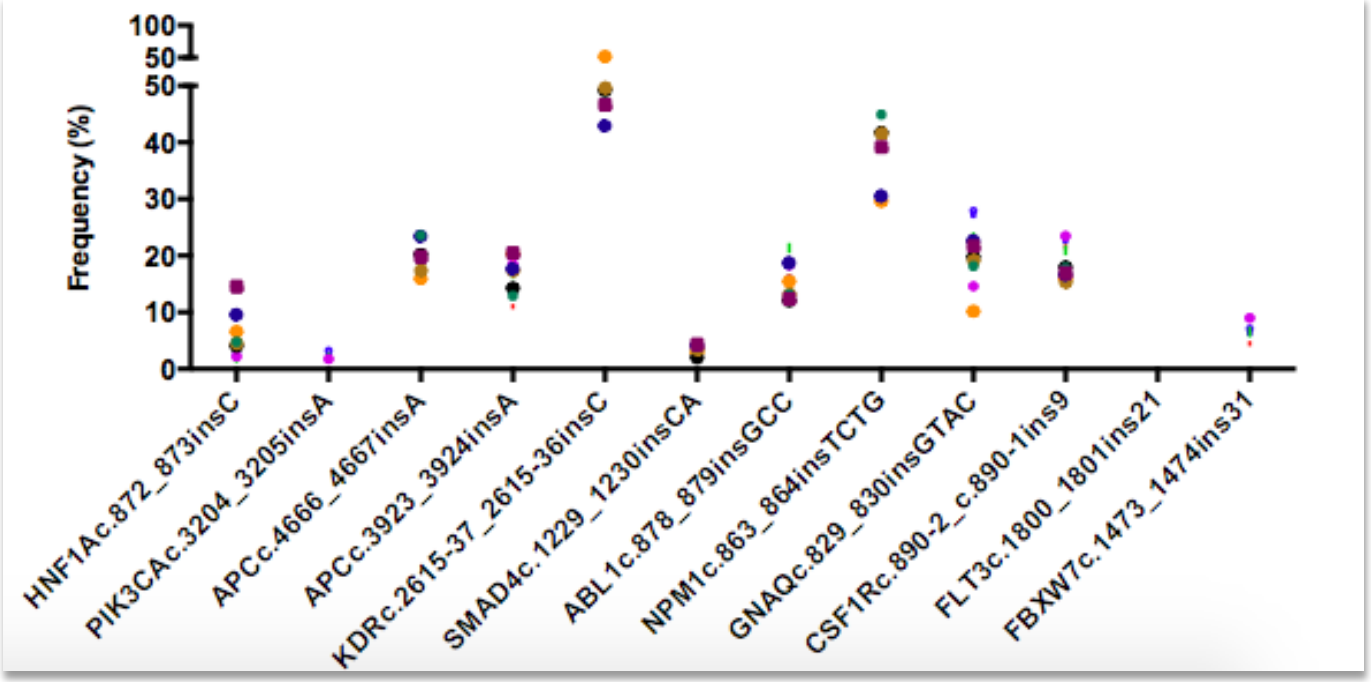
Global Allelic Frequency Study

Insertions



- AcroMetrix Oncology Hotspot Control was tested globally in 30+ sites
- Data shown are from one NGS panel
- Most insertions and deletions were detected
- Reported frequency varied from lab to lab

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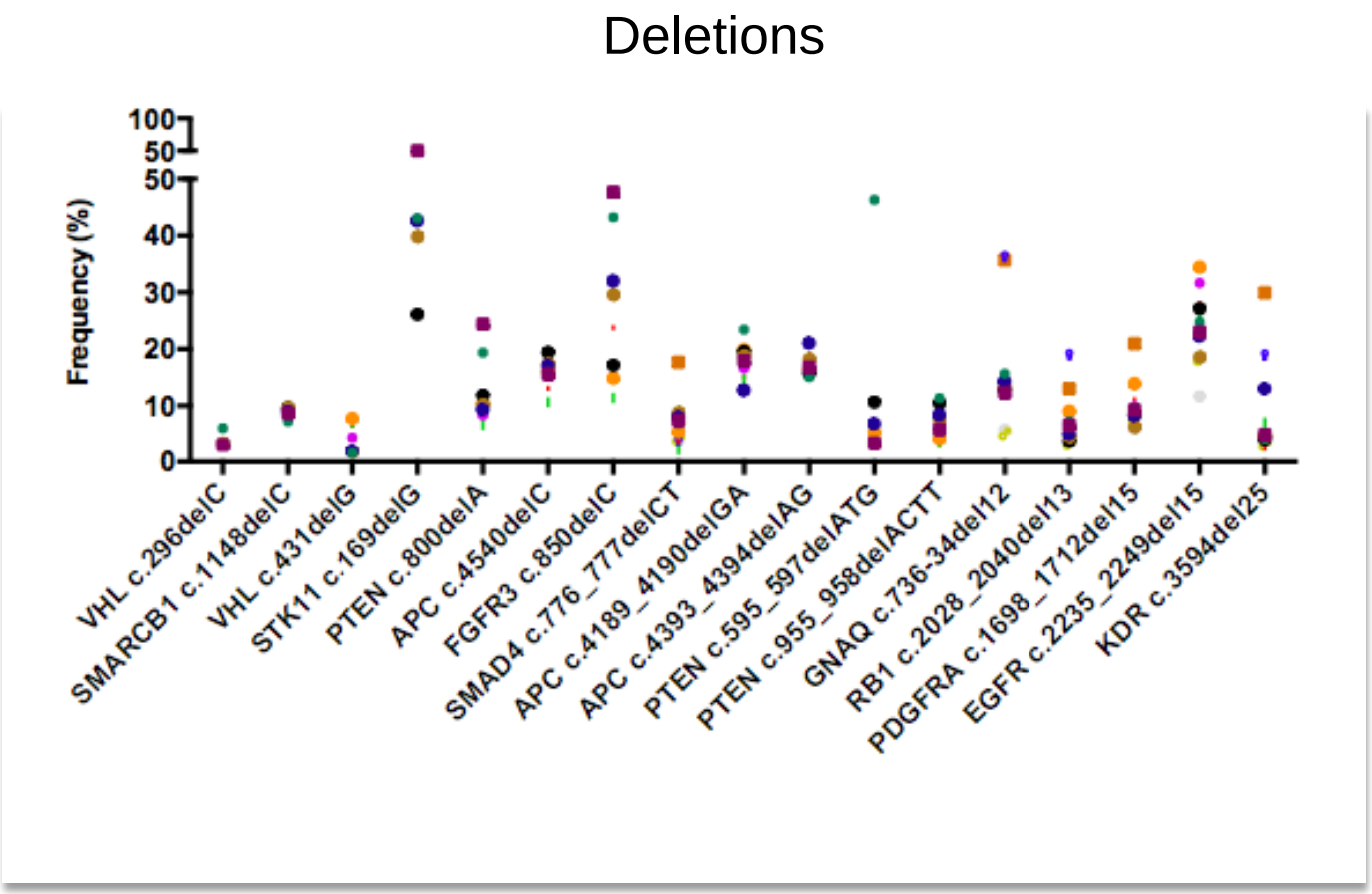
Frequency: It's All Relative



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- Most insertions and deletions were detected
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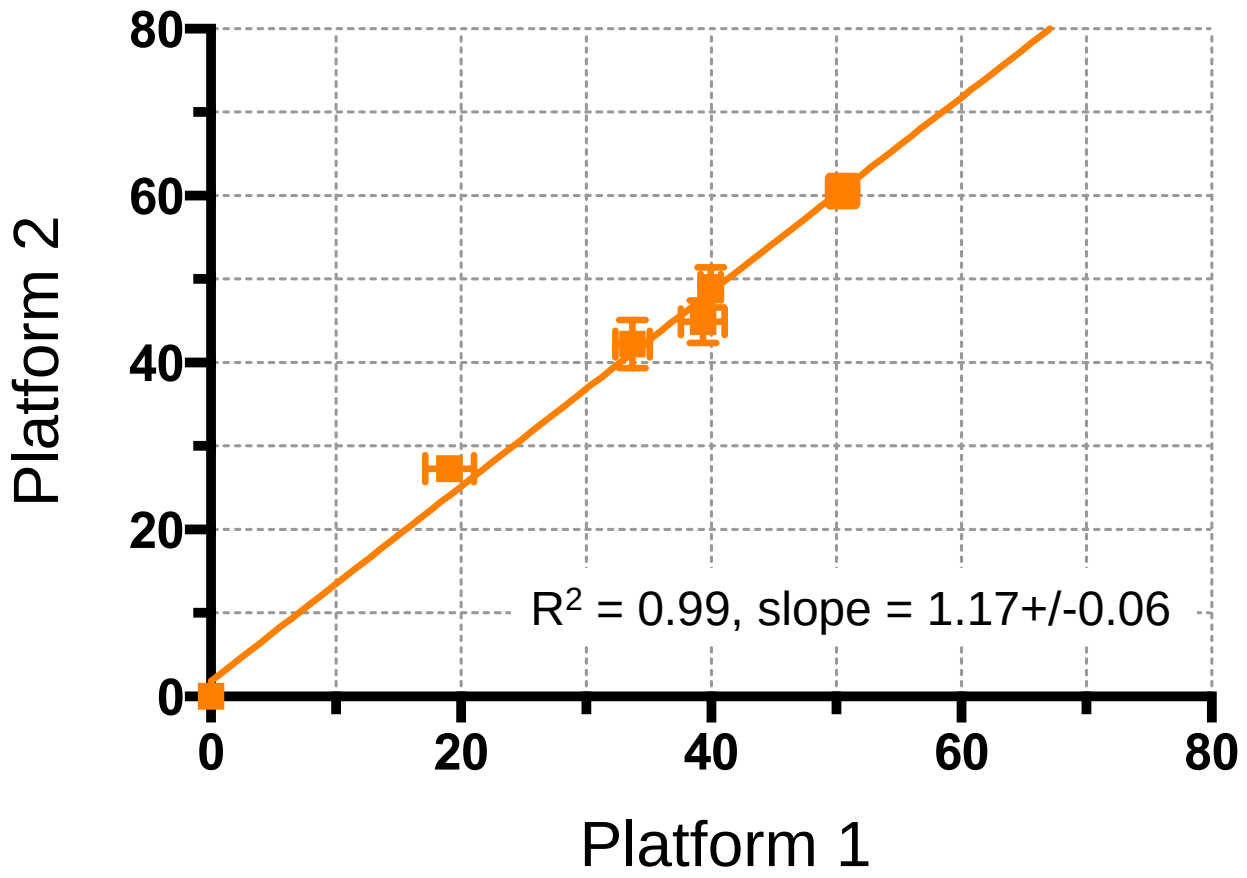
Human FFPE Samples Also Demonstrate Frequency Variation Across Platforms

Human tumor FFPE sample tested on 2 NGS platforms

10% difference in measured frequency



APC chr 5:112175770:G>A

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Traceable Reference Materials Promote Measurement Standardization

- Gold Standard Truth
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- There is currently no standardization across platforms for allelic frequency
- Variation seen in both control materials and in human samples
- Expect cross-platform differences

> Traceability & standardization will become more important as monitoring is implemented

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How do you choose a *good* quality control for NGS?

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- How well does a contrived sample mimic a human sample?
- Two examples: FFPE and circulating tumor DNA

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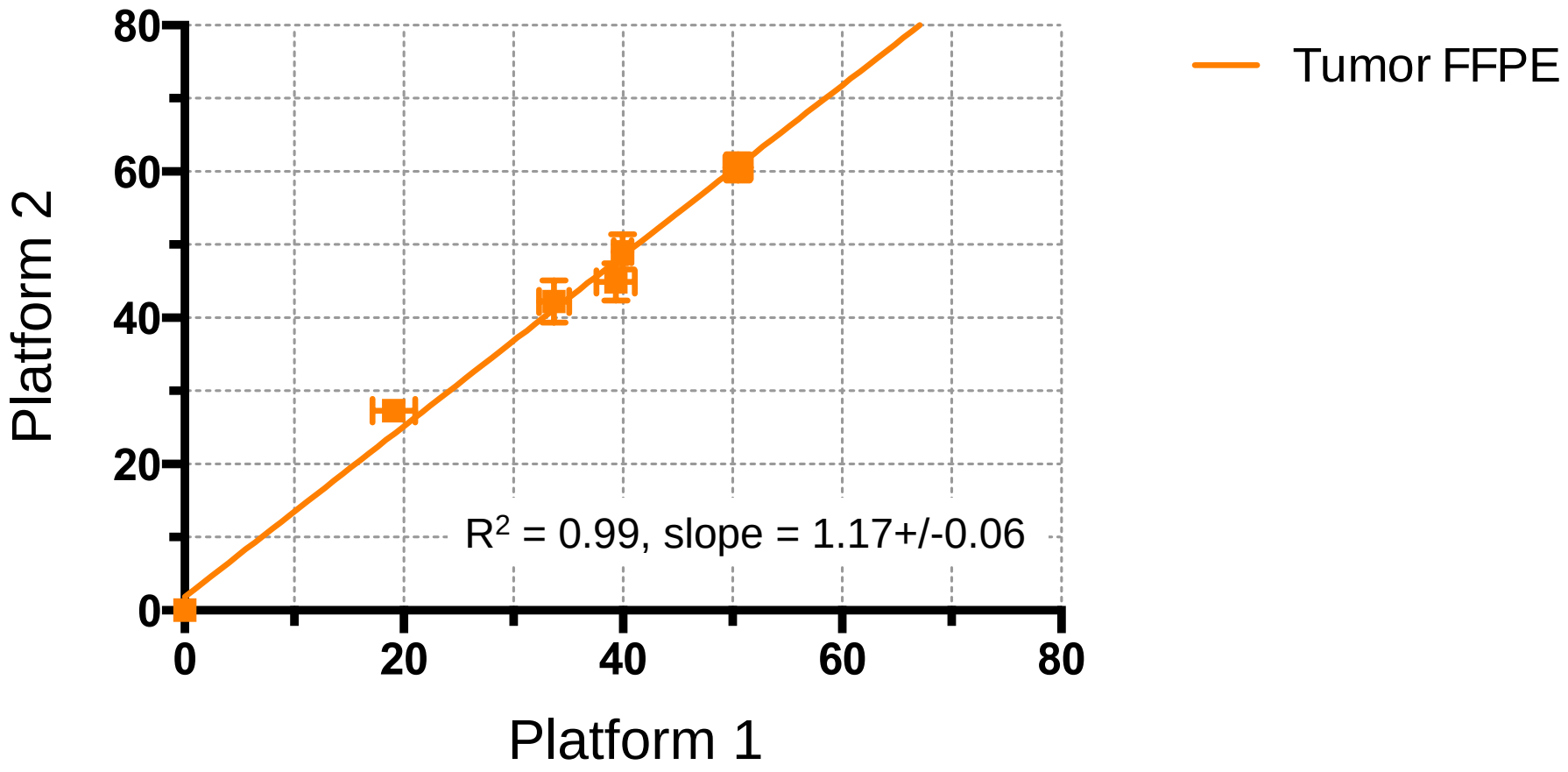
Commutability Between FFPE And Synthetic DNA/Cell Lines

Human tumor FFPE sample tested on 2 NGS platforms
Relative performance is linear



APC chr 5:112175770:G>A

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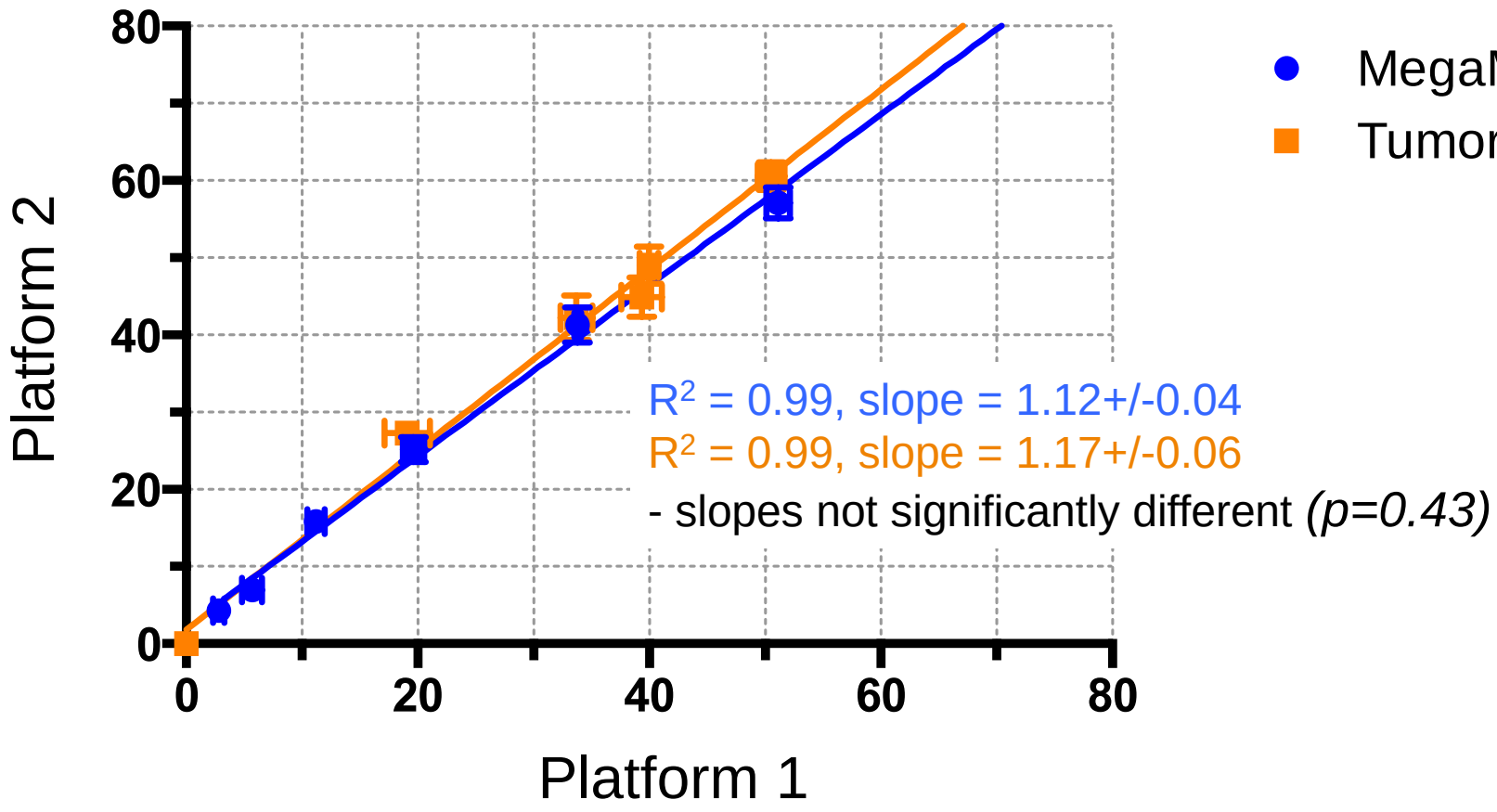
Commutability Between FFPE And Synthetic DNA/Cell Lines

AcroMetrix Oncology Hotspot Control derivative material compared to a human tumor FFPE sample
SNV frequency behaves like a tumor FFPE sample



APC chr 5:112175852:G>T
APC chr 5:112175770:G>A

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Can ctDNA Be Made To Mimic A Human Sample?

1. Start with **real human plasma**

Human Plasma Screening	
Target	**Result**
Antibodies to HIV-1 and HIV-2	Not reactive
Antibody to HCV	Not reactive
Hepatitis B surface antigen (HBsAg)	Not reactive
Antibodies to HTLV I-II	Not reactive
HBV DNA	Not reactive
HCV RNA	Not reactive
HIV-1 RNA	Not reactive
CMV DNA	Not reactive
WNV RNA	Not reactive
Bioburden (bacterial count)	≤ 10 CFU/mL

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Can ctDNA Be Made To Mimic A Human Sample?

2. Alleles of interest may need to be negative: confirm to appropriate level of sensitivity depending on platform

Target	Result
EGFR p.L858R mutation	Negative
KRAS p.G12D mutation	Negative
NRAS p.G12D mutation	Negative
EGFR exon 19 deletion	Negative
EGFR p.T790M mutation	Negative

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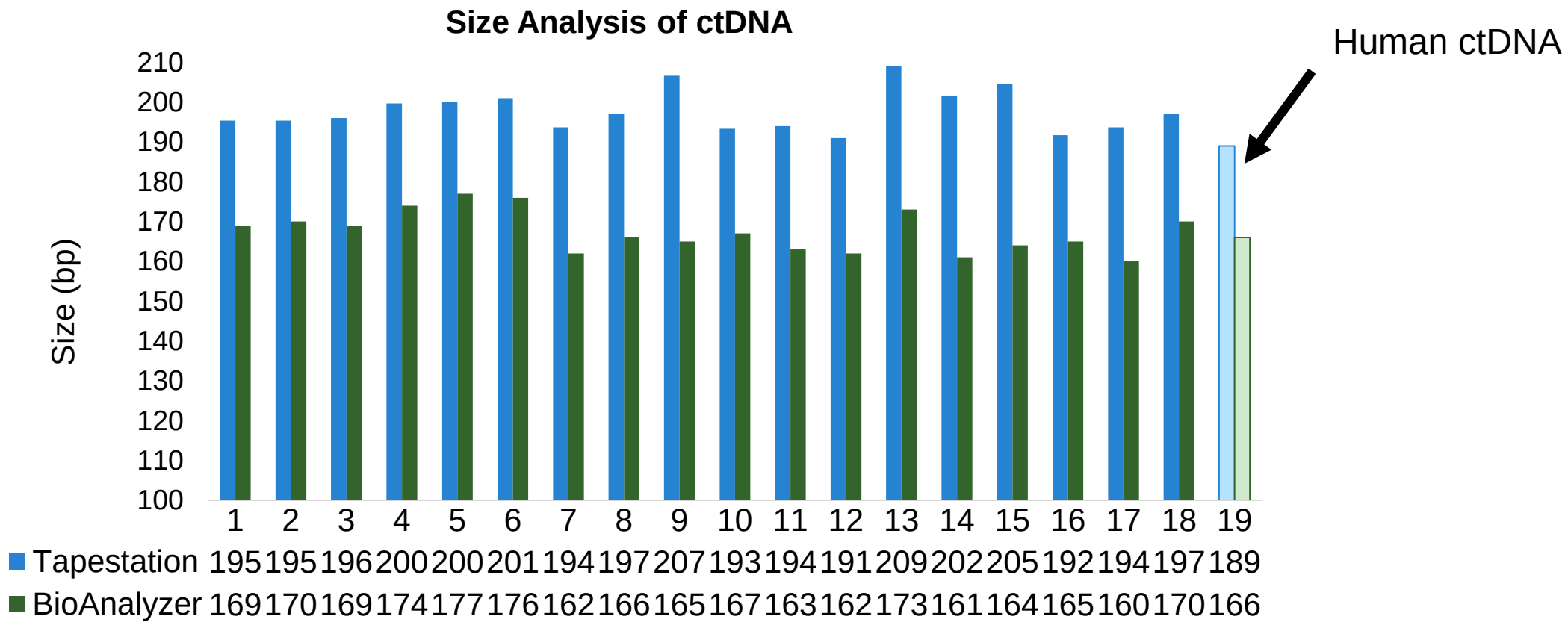
Can ctDNA Be Made To Mimic A Human Sample?

3. Fragmentation to mimic human sample

Be careful about your measurement platform!



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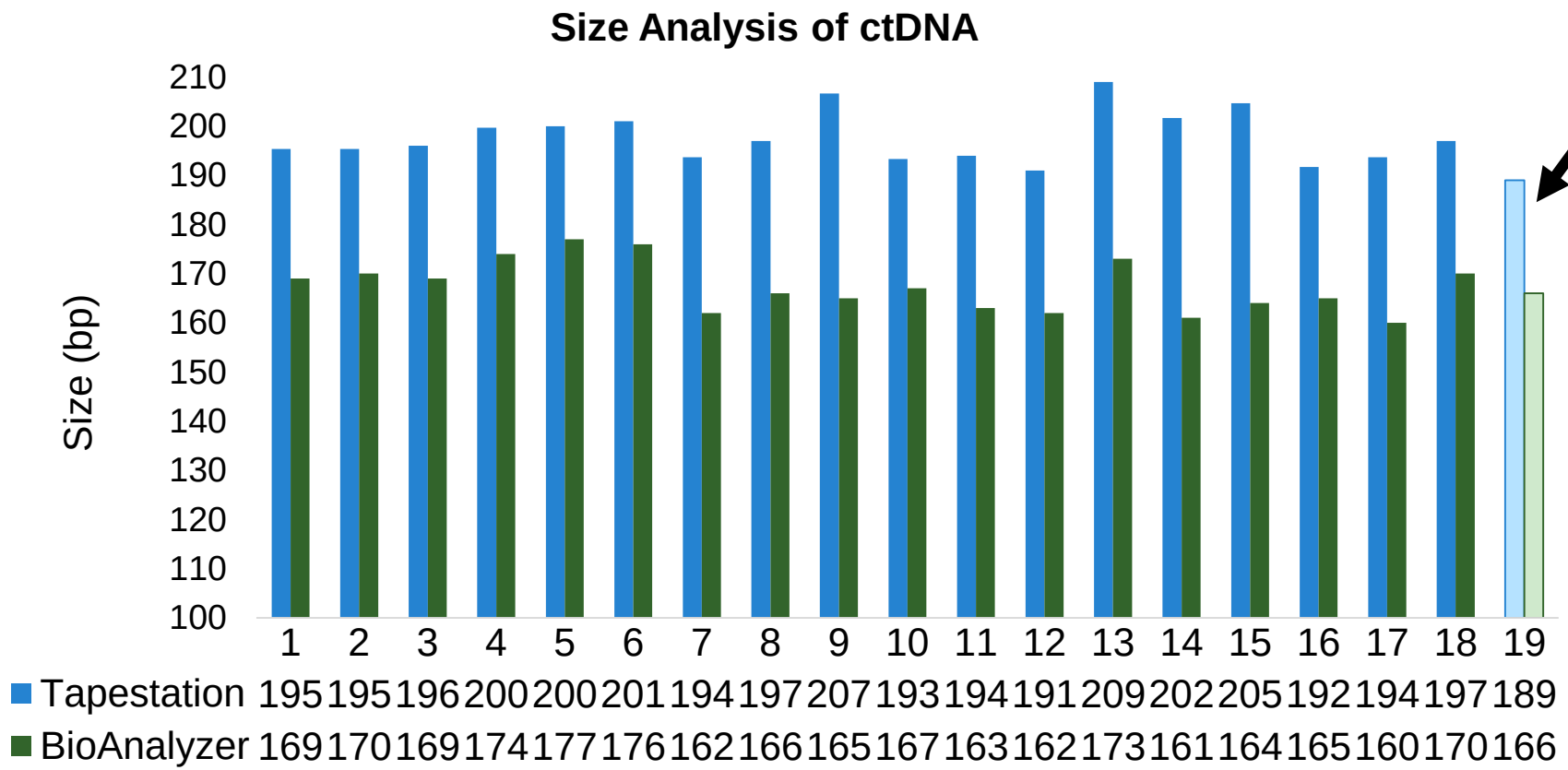

Human ctDNA

...stay tuned!

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How Do You Choose A *Good* Quality Control For NGS?

- Gold Standard Truth
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- Commutability is when:
  - the relative performance of a contrived sample mimics the relative performance of a human sample across multiple platforms
- Controls can be generated that closely mimic human samples

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How Do You Choose A *Good* Quality Control For NGS?

- Gold Standard Truth
- Traceable
- Commutable
- Streamlined
- Consistent

- Making reference materials takes time and money
  - Finding Materials
  - Variant Confirmation on Multiple Platforms
  - Value Assignment
  - Dilutions
  - Testing
  - Stability Confirmation

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Reference Materials Need To Be Prepared For Many Experiments

What strategy is used today for NGS assay analytical validation?

- **Accuracy**: The degree of agreement between the nucleic acid sequences derived from the assay and a reference sequence.
- **Precision**: The degree to which repeated sequence analyses give the same result- repeatability and reproducibility.
- **Analytical sensitivity**: Likelihood that the assay will detect the targeted sequence variations, if present.
- **Analytical specificity**: Probability that the assay will not detect a sequence variation when none are present (the false positive rate).
- **Reportable range**: Region of the genome in which sequence of an acceptable quality can be derived by the laboratory test.
- **Reference range**: Reportable sequence variations the assay can detect that are expected to occur in an unaffected population.

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Well-designed Preconfigured Materials Make QC Simpler

Reduce # of runs, cost and time with well-designed reference materials

- **Accuracy**: The degree of agreement between the nucleic acid sequences derived from the assay and a reference sequence.
- **Precision**: The degree to which repeated sequence analyses give the same result- repeatability and reproducibility.
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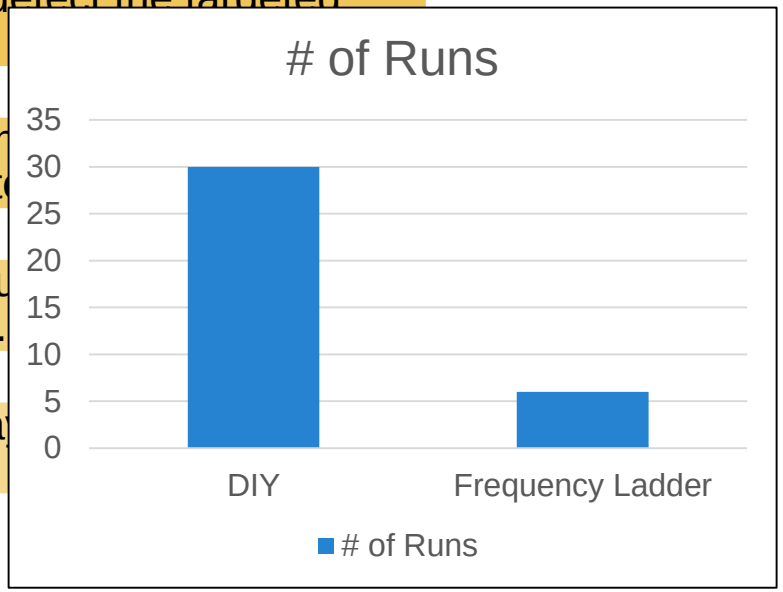

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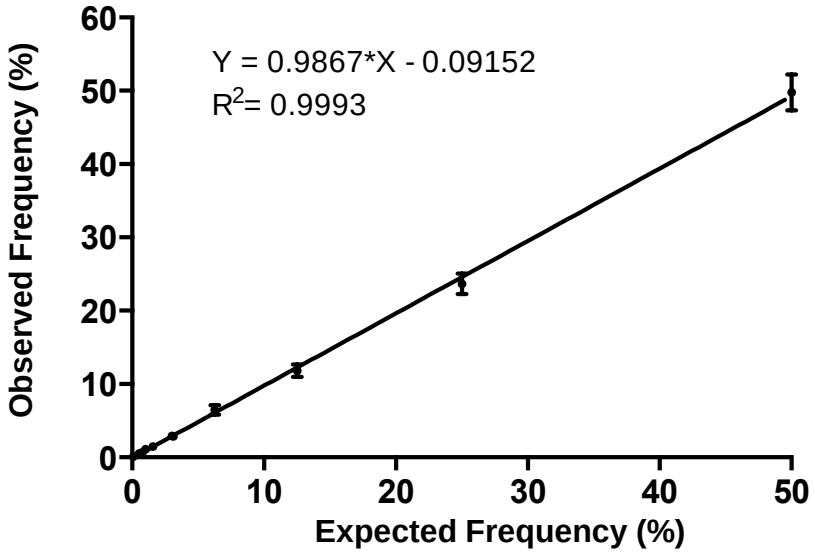
What Is A Frequency Ladder?

- Multiplexed variants all in one tube within a defined frequency range
- Linear dilution series to below LOD
- Run Frequency Ladder multiple times to capture assay reproducibility

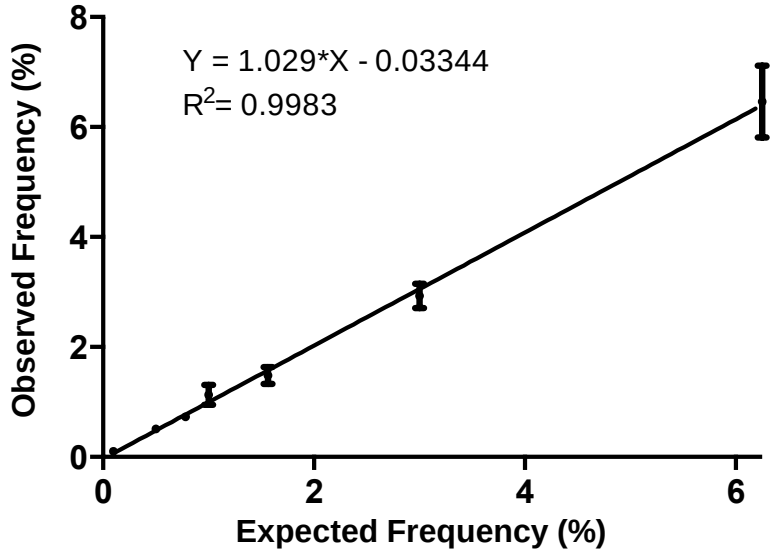
Solid Tumor

0.1% to 50% Frequency



Liquid Biopsy

0.1% to 6.25% Frequency



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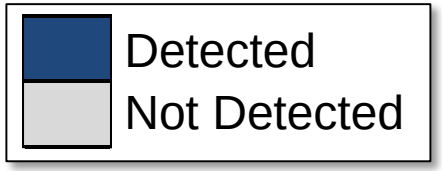
Streamlined Verification Study With Six Highly Multiplexed Samples

Tube # :	1	2	3	4	5	6
Frequency (%):	3	5	11	18	30	48

Gene	Mutation CDS	Mutation AA	1	2	1	2	1	2	1	2	1	2	1	2
ALK	c.3824G>A	p.R1275Q	3.8	4.4	7.7	8.7	15.5	15.9	25.8	27.2	41.2	47.2	63.1	58.5
ATM	c.1009C>T	p.R337C	4	4	7.5	7.3	13.7	11.8	21.9	21	36.8	33.6	56.1	57.9
BRAF	c.1799T>A	p.V600E	4	4.7	8.6	8.3	12.2		26.4	28.6	39	43.9	58	57.4
CDKN2A	c.172C>T	p.R58*	2.9	4.8	8.7	11.7	16.7	14.3	31.4	28.8	48	45.1	64.6	62
FGFR3	c.746C>G	p.S249C	4.2	3.6	5.5	5.4	10.7	10.5	23.1	20.6	36.3	37.8	54.2	51.1
FGFR3	c.1948A>G	p.K650E									25.3	24.8	42.1	44.8
GNAS	c.601C>T	p.R201C	4.6	4.2	8.3	7.5	15.5	12.4	24.4	26.9	39.1	42.4	55.5	58
HRAS	c.182A>G	p.Q61R	3.9	5.2	8	7.1	14.5	14.6	25.4	24.4	44.3	42.9	59.5	60.8
IDH1	c.395G>A	p.R132H	4.3	3.9	8.1	9	13.4	17.4	26.4	25.5	42.2	43.8	61.6	59.6
IDH2	c.419G>A	p.R140Q	2.8	3.2	5.6	6.8	10.1	10.9	19.9	20.9	30.9	31	51.7	50.1
JAK2	c.1849G>T	p.V617F		2.8	6.5	7.6	11.4	11.1	23	20.7	38	35	57.9	53.3
KRAS	c.35G>A	p.G12D	4.6	3.4	8.1	6.8	15.2	12.8	24.5	24.5	36	36.7	57.4	60
NRAS	c.182A>G	p.Q61R	3.2	2.9	6	7.4	10	10.7	22.8	24.6	35.1	35.7	54.3	55.5
NOTCH1	c.4799T>C	p.L1600P			3.1	3	3.8	6.5	11.9	9.2	23.4	19.5	38.7	36.8
PDGFRA	c.2525A>T	p.D842V	3.3	3.3	5.9	6.9	13.4	9.3	21.2	19.8	35.4	34.4	54.4	53
PIK3CA	c.1624G>A	p.E542K	3.4	2.7	8.5	7.4	13.2	12.8	26.5	22.2	40.5	33.3	57.7	54.7
RET	c.2753T>C	p.M918T		3.2	5.3	6.3	10	11.3	21.2	20.8	37.2	29.5	56.3	53.4
TP53	c.1015G>T	p.E339*	3		6.1	5.4	12.1	11.1	20.3	20.4	33.6	34.2	55.7	49.8
TP53	c.743G>A	p.R248Q	3	3.6	7.1	7.1	11.2	12.2	21.1	23.7	32.8	35.8	54.1	55.6
VHL	c.277G>C	p.G93R	4.8	4.7	6.9	9.1	14.3	14.3	23.5	25.5	39.8	41.2	59	57

- Variant stratification / bucketing



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ThermoFisher SCIENTIFIC

Streamlined Verification Study With Six Highly Multiplexed Samples

Tube #:			1		2		3		4		5		6	
Frequency (%):			3		5		11		18		30		48	
Gene	**Mutation CDS**	**Type**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**	**1**	**2**
RET	c.1894_1906>AGCT	Cmplx						10.3	14.3		27.3	27.3	46.5	46.6
APC	c.3923_3924insA	INS-1					16.3	16	27.8	24.5	37.5	39.9	59.6	56.9
APC	c.4666_4667insA	INS-1												36.1
ERBB2	c.2324_2325ins12	INS-12	3.1	3.1	5.5	5.7	10.6	10.9	19.4	18.3	31.7	29.8	48.4	47.6
KIT	c.1509_1510ins6	INS-6		3	5.3	7.2	7.8	11.9	20	31.3	30.8	45.3	46.7	65.2
FLT3	c.1800_1801ins21	INS-21							11.6	7.8	15.5	14.1	36.1	39.4
NPM1	c.863_864insTCTG	INS-4	3.7	5.2	8.2	8.1	15	17.7	27.3	33.5	40.8	49.6	59.7	63.4
PIK3CA	c.3204_3205insA	INS-1	2.8	3.1	5.8	6.7	11.7	11.8	22.9	20.7	37.4	34.9	54.8	54.8
SMAD4	c.1229_1230insCA	INS-2	4	4.3	6.9	6.3	13.6	13.5	25.1	27.1	35.9	43.3	58.7	59.1
GNAQ	ACA>TGC	MNP			6.2	7.6	12.7	13.8	23.2	21.4	40.2	33.5	58.6	56.2
EGFR	c.2235_2249del15	DEL-15					18.3	16.8	26.2	27.7	41.4	43.6	61.2	59.5
PTEN	c.800delA	DEL-1												
RB1	c.2028_2040del13	DEL-13			4.3	6		8.4	14.9				45.6	45.6
SMAD4	c.776_777delCT	DEL-2	2.9	3.8	7.9	7.8	15	14.6	24.5	27.4	35.7	36.5	59.6	56.7
VHL	c.431delG	DEL-1	3.9	3.2	7.6	7.6	14.3	12	26.7	23.6	38.8	36.1	58.9	56.8
VHL	c.296delC	DEL-1								20.7	42.2	43.1	69.8	62

- Variant stratification / bucketing

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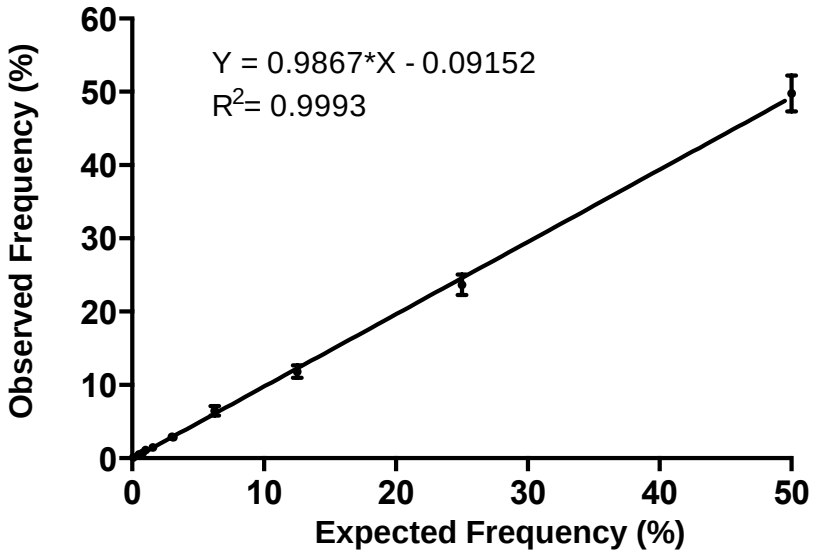
How Do You Choose A *Good* Quality Control For NGS?

- Gold Standard Truth
- Traceable
- Commutable
- Streamlined
- Consistent

- What is a Frequency Ladder?
- Choose variants of interest
- Lowest frequency in a level defines the limit of detection

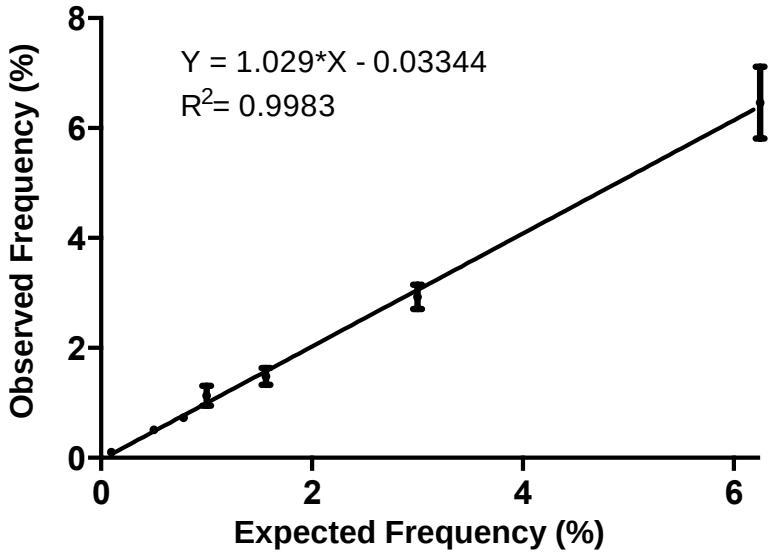
Solid Tumor

0.1% to 50% Frequency



Liquid Biopsy

0.1% to 6.25% Frequency



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How Do You Choose A *Good* Quality Control For NGS?

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Quality Controls need to be high quality

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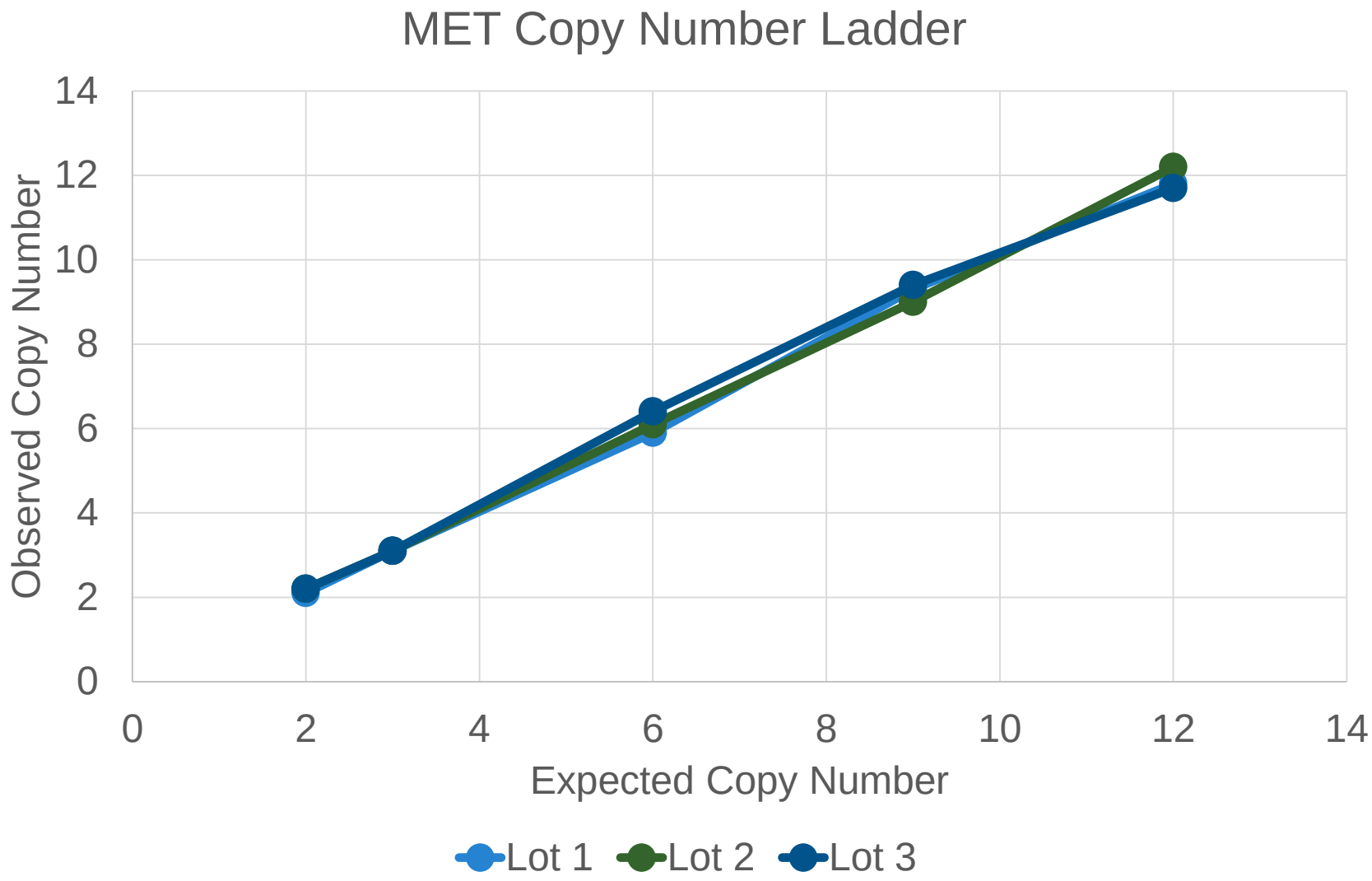
Lot To Lot Control Consistency Enables Detection Of Changes In The Assay System

- Is it the Control or the Assay?
- Reliable lot to lot performance removes the guesswork



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Agenda

1. How do you choose a quality control for NGS?
2. How do you choose a *good* quality control for NGS?
3. Why should you perform regular QC for NGS?

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Why Are Reference Materials Needed For Regular QC?

Important indicator of run quality

Bioinformatics control

1. Global study: Running controls prevents surprises
2. Study: Read coverage is *not* a good proxy for regular QC

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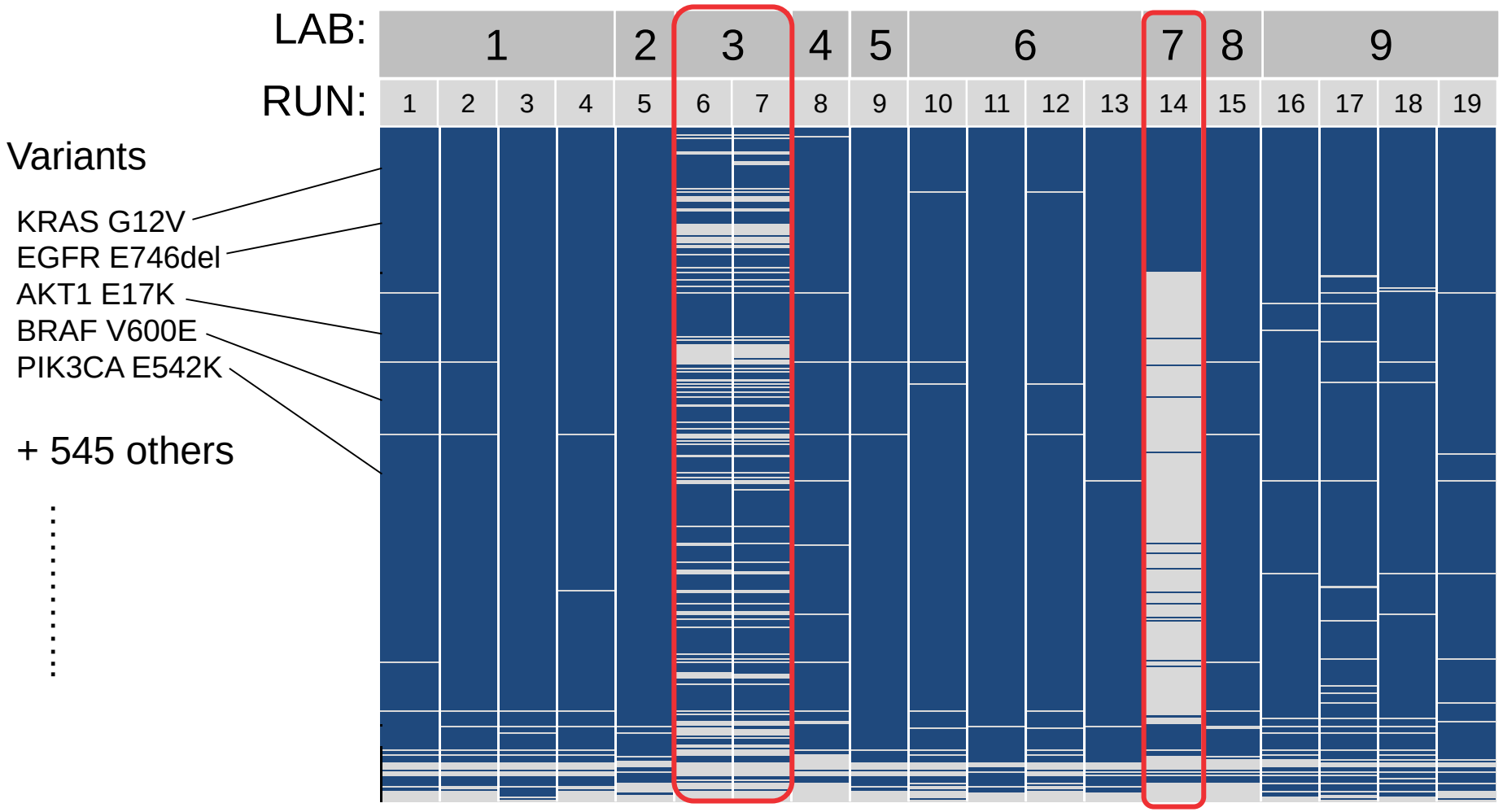
Regular QC Prevents Surprises

- 9 labs, same assay, same instrument:



Labs did not have a regular run control that revealed this issue

Detected
Not Detected

Missed most variants

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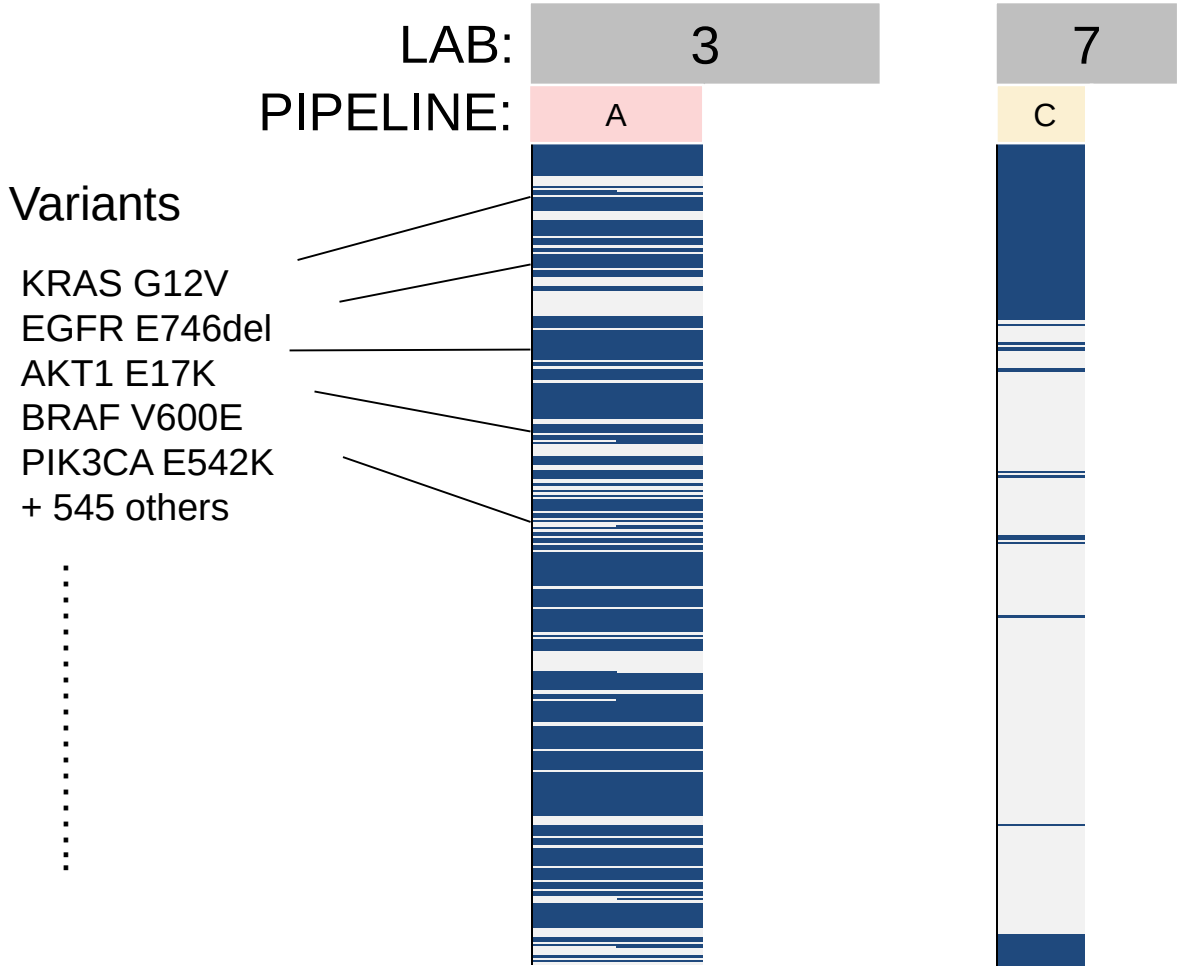
Regular QC Prevents Surprises



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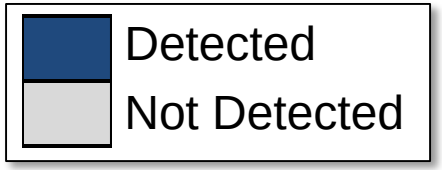

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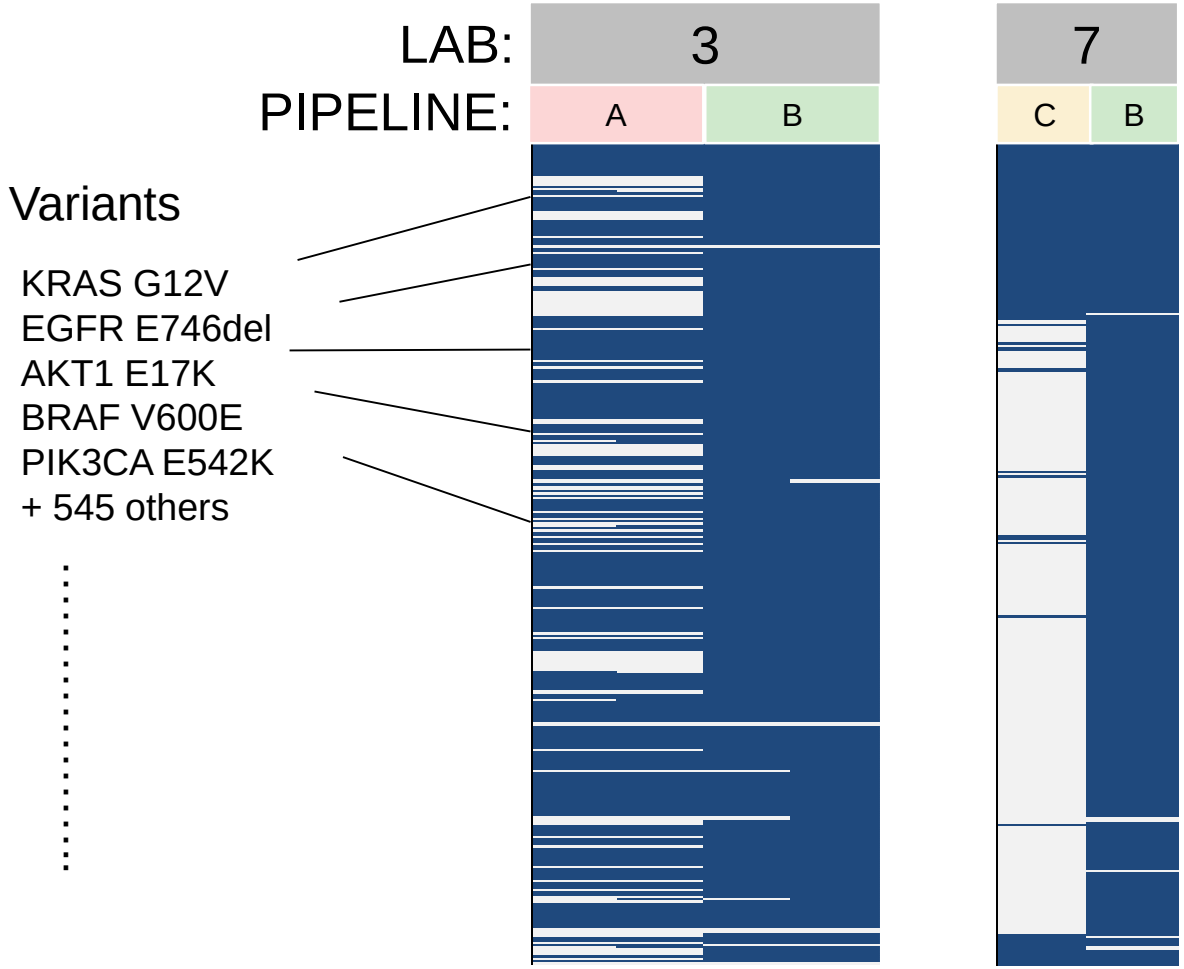
Regular QC Prevents Surprises



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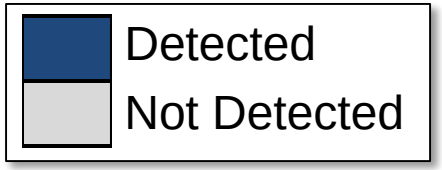

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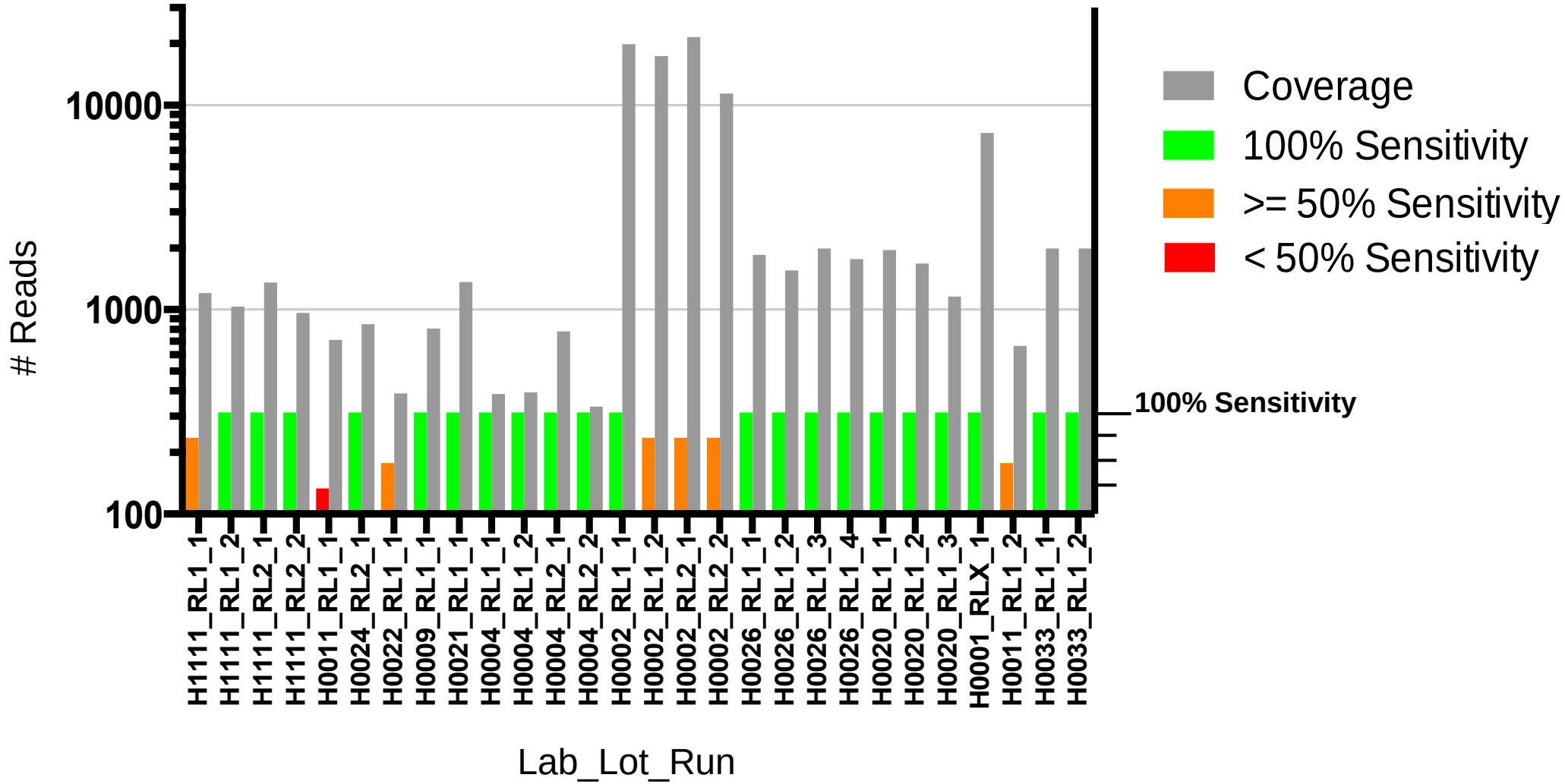
Study: Read coverage is *not* a good proxy for regular QC

Average coverage was calculated over 28 runs in 12 labs for one amplicon (in TP53), which contains 4 variants in the AcroMetrix Oncology Hotspot Control.



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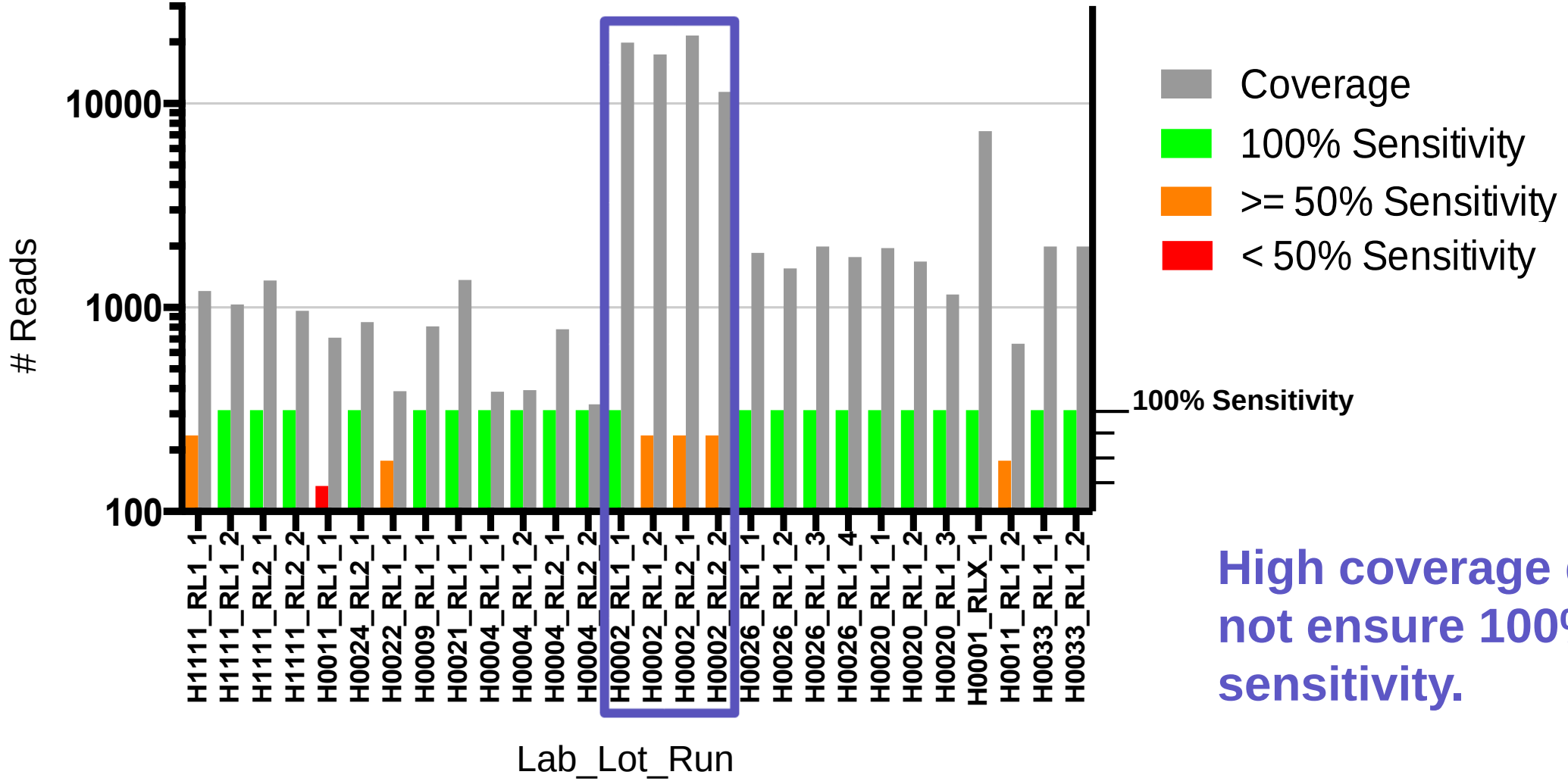

High coverage did not ensure 100% sensitivity.

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Important indicator of run quality

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Thank You!

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