Internal Standards Provide Direct Quality Control for NGS Testing

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AccuGenomics
A higher standard of accuracy

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NIIMBL® The National Institute for Innovation in Manufacturing Biopharmaceuticals

Abstract

Improve standardization of adventitious agent testing using Internal Standards

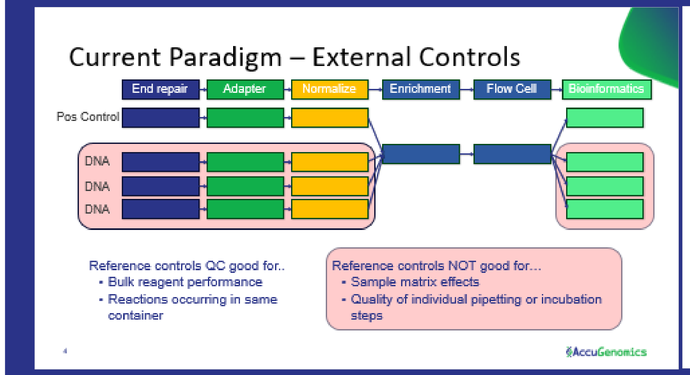
- Standardized viral abundance
- Capture yield quality control
- Lyophilized for shelf and shipping stability

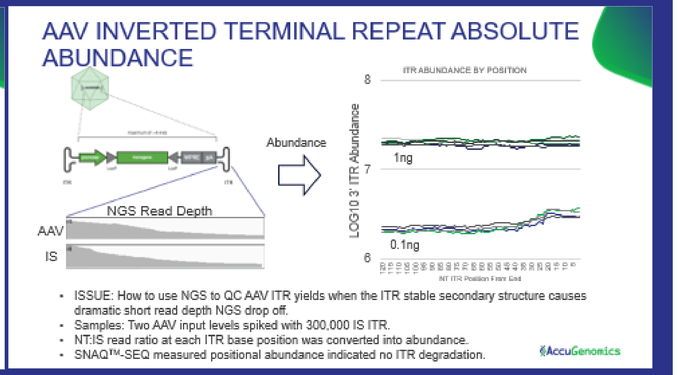
Introduction

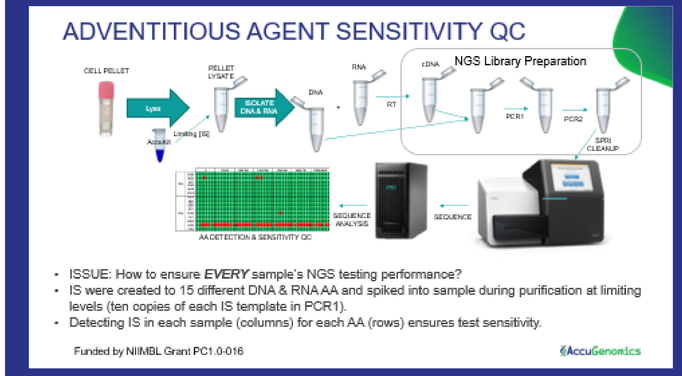
NGS has great potential as an adventitious agent surveillance tool

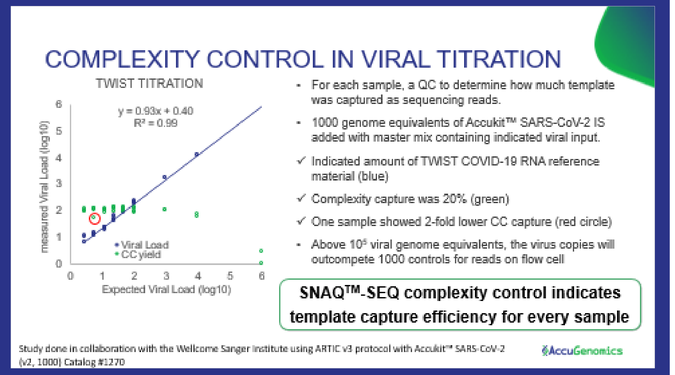
Lack of NGS standardization creates silos of inconsistent testing performance

Use of internal standards, which biochemically mimic NGS targets, will support the existing need for per sample quality controls to ensure standardization of NGS surveillance









Results

Positive Control samples do not provide direct QC for each sample

Existing NGS metrics are convoluted indirect surrogates of testing performance

Internal standards provide direct overarching QC for each sample

Internal standards will standardize results across laboratories

Internal standards provide a direct assessment of abundance measurements, complexity capture and sensitivity QC

Public Impact

NGS monitoring of product yields, vector & host contamination and adventitious agent detection shows potential, but NGS complexity requires robust relevant quality controls

SNAQ™ internal standards provide a direct and comprehensive quality control to standardized testing between every sample and testing site

Acknowledgements

A portion of the study data were funded by NIIMBL Grants PC1.0-016 & ARP-22

Abstract

AccuGenomics manufactures and supports the integration of internal standards to improve quality control and standardization of routine NGS manufacturing tests. Standardized Nucleic Acid Quantification for sequencing (SNAQ™-SEQ) internal standards are mixtures of synthetic nucleic acid controls that when added to each sample biochemically covary in yield and sequence detection in targeted or non-targeted NGS assays. Additionally, AccuGenomics supports the integration of internal standards into the manufacturing bioinformatic pipeline. The poster will provide examples of using internal standards to measure test capture efficiency and duplication rate; limit controls as a sensitivity QC for adventitious agents; and ddPCR like accuracy for abundance measurements of manufacturing host/vector and therapeutic vector.

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Introduction

Next Generation Sequencing (NGS) has demonstrated great potential as a single method to detect many biomarkers with high sensitivity and specificity. Unfortunately, NGS is highly complex and relies on analytical surrogates that indirectly demonstrate testing proficiency. AccuGenomics makes internal standards (IS) for NGS testing in a similar approach as analytic mass spectrophotometry uses to ensure testing accuracy. The IS are designed to biochemically and bioinformatically covary with biomarkers of interest to directly ensure testing sensitivity and analytic accuracy for every sample tested. The IS are manufactured to have < 10^{-8} base error rate and IS mixtures are prepared using 2 to 3 different abundance methods under quality control systems to create reference quality materials. AccuGenomics tunes the IS mixture for the testing platform and supports integration into bioinformatic pipeline. This poster will discuss uses of the IS mixtures relevant to biomanufacturing environment.

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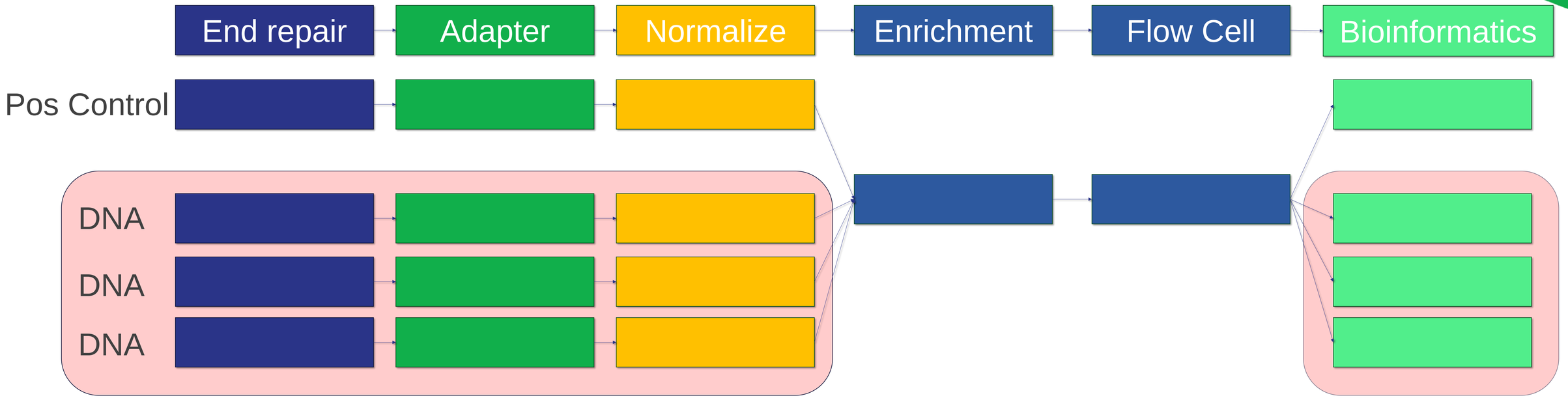
Current Paradigm – External Controls



Reference controls QC good for..

- Bulk reagent performance
- Reactions occurring in same container

Reference controls NOT good for…

- Sample matrix effects
- Quality of individual pipetting or incubation steps

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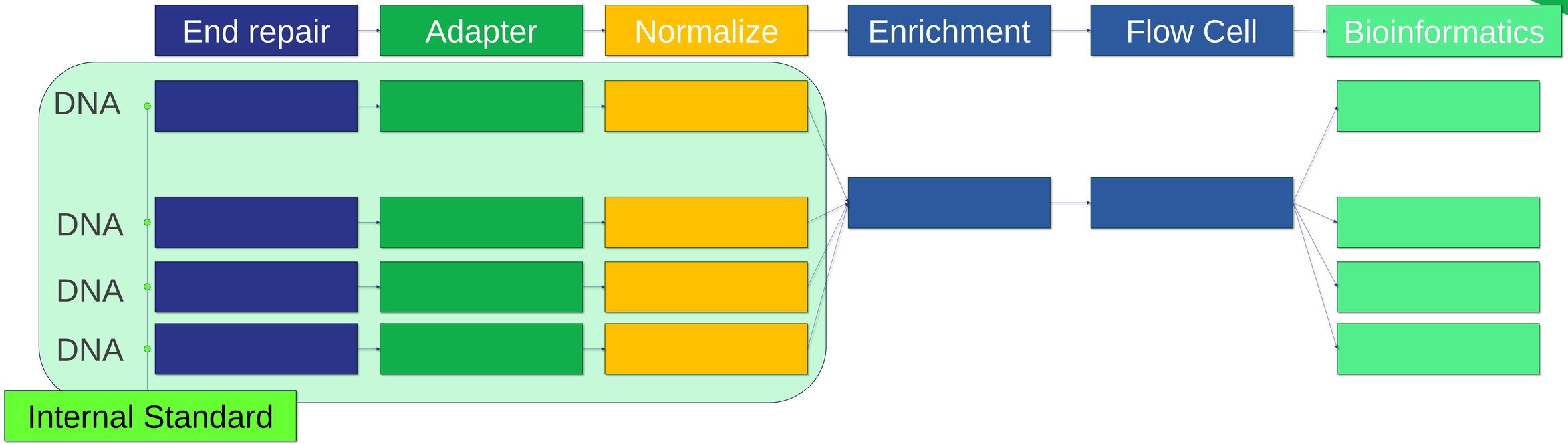
Standardized Nucleic Acid Quantification For Sequencing (SNAQ™-SEQ)



SNAQ™-SEQ Internal Standards (IS)

- Engineered to biochemically mimic target regions
- Spiked-in at limiting levels
- IS variants are unique
- Compatible with existing library preps and analysis pipelines

Internal reference controls good for..

- **Sensitivity QC in EVERY sample**
- Increases testing throughput
- Standardized abundance

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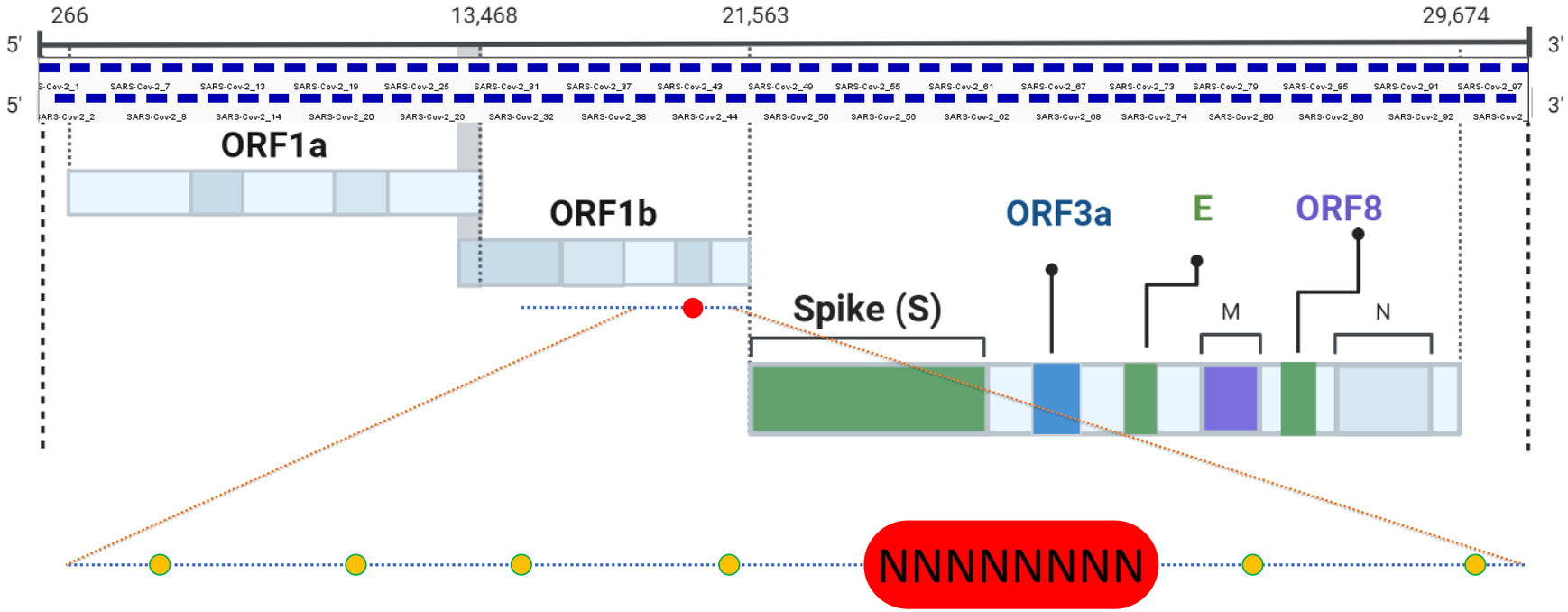
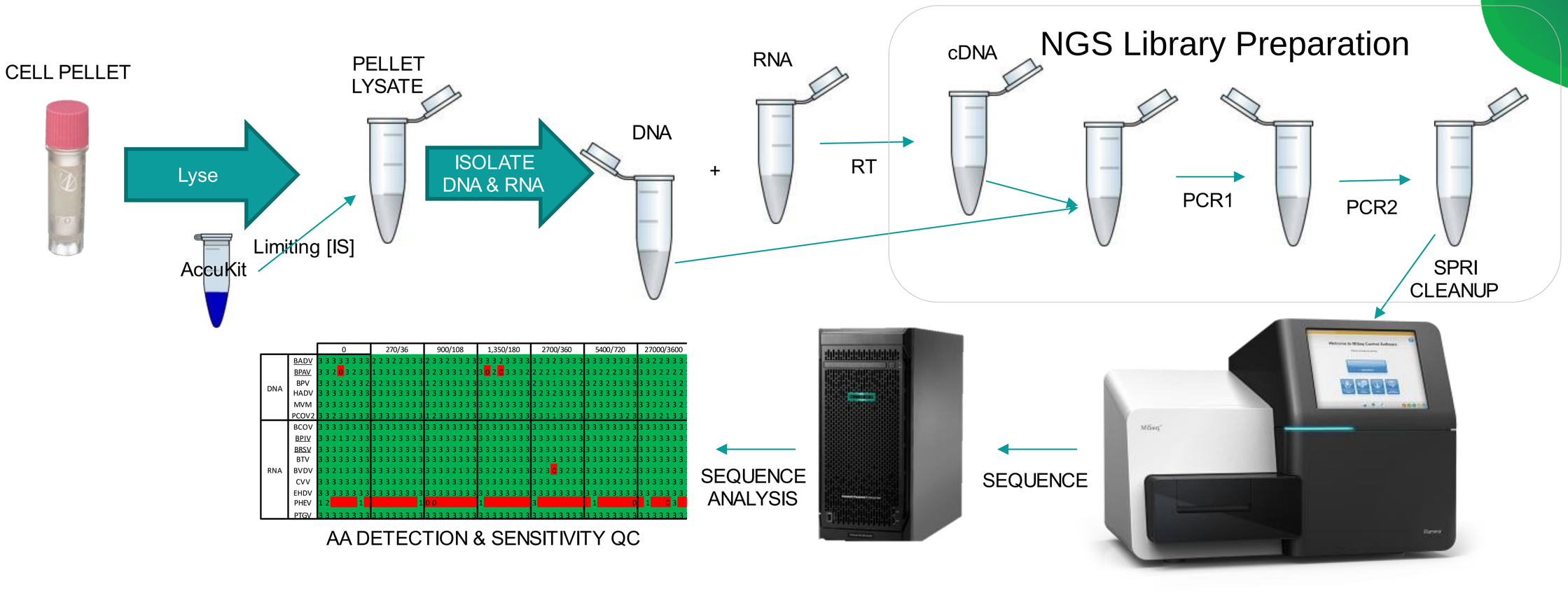
SNAQ™-SEQ Internal standards



- AccuGenomics internal standards are manufactured to end users NGS testing needs.
- IS can correspond to small target regions (e.g., AAV ITR) for limit control or abundance, or tiled across entire organism to also measure sensitivity.
- Unique base changes allow bioinformatic identification (yellow circles)
- May include degenerate bases for estimation of library complexity capture (red Ns).

ADVENTITIOUS AGENT SENSITIVITY QC



- ISSUE: How to ensure ***EVERY*** sample's NGS testing performance?
- IS were created to 15 different DNA & RNA AA and spiked into sample during purification at limiting levels (ten copies of each IS template in PCR1).
- Detecting IS in each sample (columns) for each AA (rows) ensures test sensitivity.

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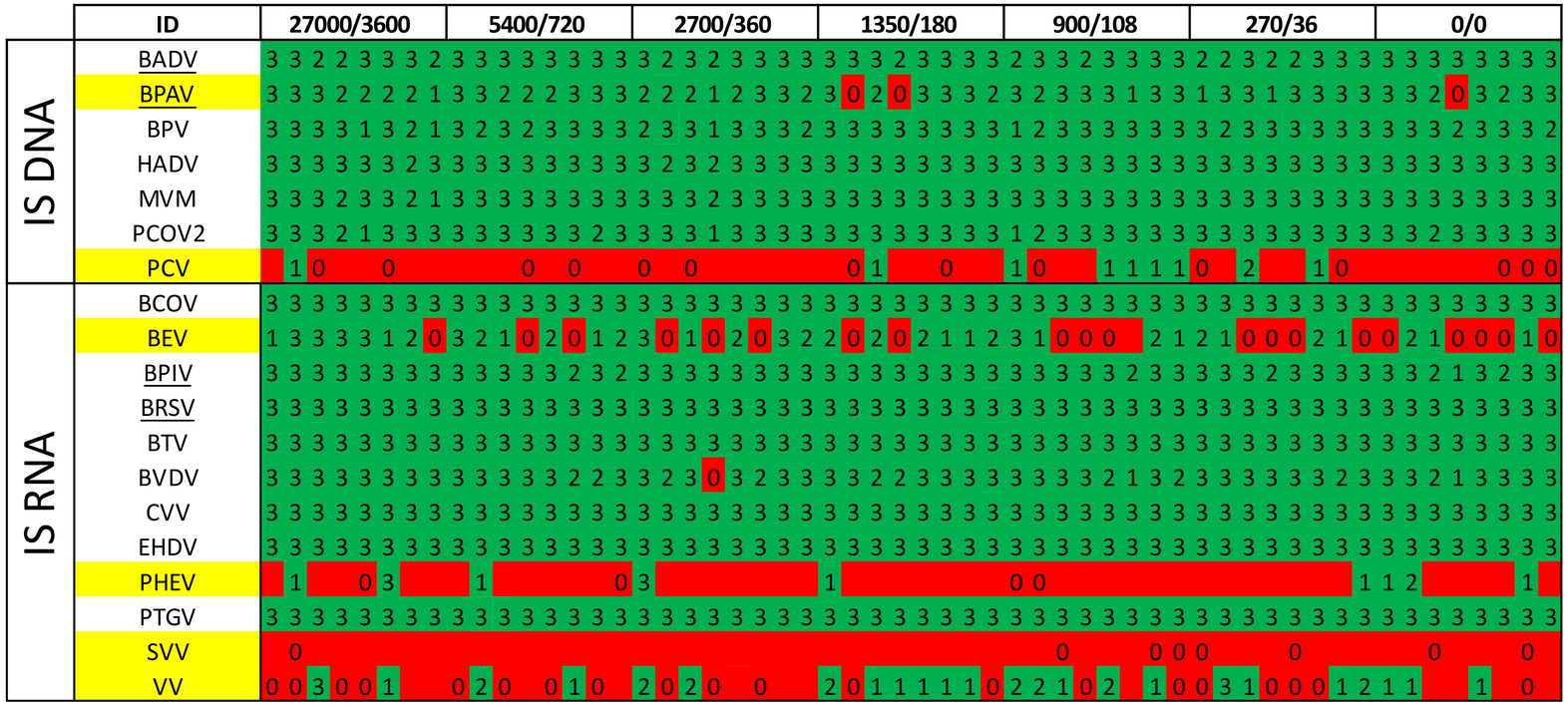
SENSITIVITY QC ANALYSIS



	ID	27000/3600	5400/720	2700/360	1350/180	900/108	270/36	0/0
IS DNA	BADV	3 3 2 2 3 3 3 2	3 3 3 3 3 3 3 3	3 2 3 2 3 3 3 3	3 3 3 2 3 3 3 3	2 3 3 2 3 3 3 3	2 2 3 2 2 3 3 3	3 3 3 3 3 3 3 3
	BPAV	3 3 3 2 2 2 2 1	3 3 2 2 2 3 3 3	2 2 2 1 2 3 3 2	3 0 2 0 3 3 3 2	3 2 3 3 3 1 3 3	1 3 3 1 3 3 3 3	3 3 2 0 3 2 3 3
	BPV	3 3 3 3 1 3 2 1	3 2 3 2 3 3 3 3	2 3 3 1 3 3 3 2	3 3 3 3 3 3 3 3	1 2 3 3 3 3 3 3	2 3 3 3 3 3 3 3	3 3 3 2 3 3 3 2
	HADV	3 3 3 3 3 3 2 3	3 3 3 3 3 3 3 3	3 2 3 2 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3
	MVM	3 3 3 2 3 3 2 1	3 3 3 3 3 3 3 3	3 3 3 2 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3
	PCOV2	3 3 3 2 1 3 3 3	3 3 3 3 3 3 3 2	3 3 3 3 1 3 3 3	3 3 3 3 3 3 3 3	1 2 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 2 3 3 3 3 3 3
	PCV	1 0 0	0 0	0 0	0 1 0	1 0 1 1 1 1	0 2 1 0	0 0 0
IS RNA	BCOV	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3
	BEV	1 3 3 3 3 1 2 0	3 2 1 0 2 0 1 2	3 0 1 0 2 0 3 2	2 0 2 0 2 1 1 2	3 1 0 0 0 2 1 2	1 0 0 0 2 1 0 0	2 1 0 0 0 1 0
	BPIV	3 3 3 3 3 3 3 3	3 3 2 3 2 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	2 3 3 3 3 2 3 3	3 3 3 2 1 3 2 3
	BRSV	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3
	BTV	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3
	BVDV	3 3 3 3 3 3 3 3	3 3 3 2 2 3 3 2	3 0 3 2 3 3 3 3	2 2 3 3 3 3 3 3	3 2 1 3 2 3 3 3	3 3 2 3 3 3 2 1	3 3 3 3
	CVV	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3
	EHDV	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3
	PHEV	1 0 3	1 0 3	1	0 0	1 1 2	1	
	PTGV	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3
	SVV	0	0	0	0 0 0	0	0	0 0
	VV	0 0 3 0 0 1	0 2 0 0 1 0	2 0 2 0 0	2 0 1 1 1 1 1 0	2 2 1 0 2 1 0 0	3 1 0 0 0 1 2 1 1	1 0

- QUESTION: Did the new nucleic purification kit was used to process samples, would this affect AA testing sensitivity?
- Each column is a different sample, each row a different AA, cell color indicates IS sensitivity PASS QC (green) or FAIL QC (red). Cell numbers indicate technical replicates with IS target read counts above NGS method's limit of blank, yellow target names indicate AA targets failing QC.
- AccuKit QC indicated six AA templates failed ten copy sensitivity requirement.
- *IS controls detected library prep inhibitors present in the new purified nucleic acid method used.*

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AA TITRATION NGS DETECTION RESPONSE

[DNA]/[RNA]		0/0	270/36	900/108	1,350/180	2700/360	5400/720	27000/3600
DNA	BADV	0 0	1 1 0 2	1 0 2 2 3 1	1 1 1 2 1 2 1	2 1 3 3 3 3 3	1 3 2 2 3 3 3 1	3 3 3 3 3 3 3 3
	BPAV	0 0	1 2 1 2 0 0 1 3	2 2 3 3 3 2 3 2	2 3 3 0 3 3 3 3	3 3 3 0 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3
	BPV	1 0 0 1 0	1 1 2 3 1 3 2 3	2 1 3 3 3 2 3 3	3 3 3 3 3 2 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3
	HADV	0 0 0 0	1 2 2 2 2	1 1 0 3 2 1	2 1 2 1 2 3 2 3	1 2 3 2 2 1 1	2 2 2 3 1 2 2 2	3 3 3 3 3 3 3 3
	MVM	2 0 0 1	0 1 2 1 0 2 2 3	1 3 3 3 3 3 3 3	3 3 3 2 3 2 3 3	3 3 3 2 3 3 3 3	3 3 3 3 3 3 3 3	3 3 3 3 3 3 3 3
	PCOV	0	1 0 1 1 1 1 3 1	3 3 1 1 3 1 3 3	1 1 3 0 2 3 3 3	1 2 3 2 3 3 3 3	3 3 3 3 3 3 2 3	3 3 3 3 3 3 3 3
RNA	BPIV		0 1 0 0 0	3 1 0 0 1 0 2 0	2 0 0 0 3 1 2 0	3 1 3 0 3 3 2 1	3 3 3 1 3 3 3 2	3 3 3 3 3 3 3 3

- QUESTIONS: Do the IS affect detection of AA?
- Different levels of AA nucleic acid spiked into CHO lysate, from zero to the same levels as IS (27000/3600 DNA/RNA).
- IS indicated all samples exhibited 10 copy sensitivity
- AA detection levels demonstrated expected response to input level.
- The MVM5 positive in zero sample (yellow circle) was an environmental contaminate detected in only this sample.
- The AccuKit internal standards ensured the integrity of each measurement.

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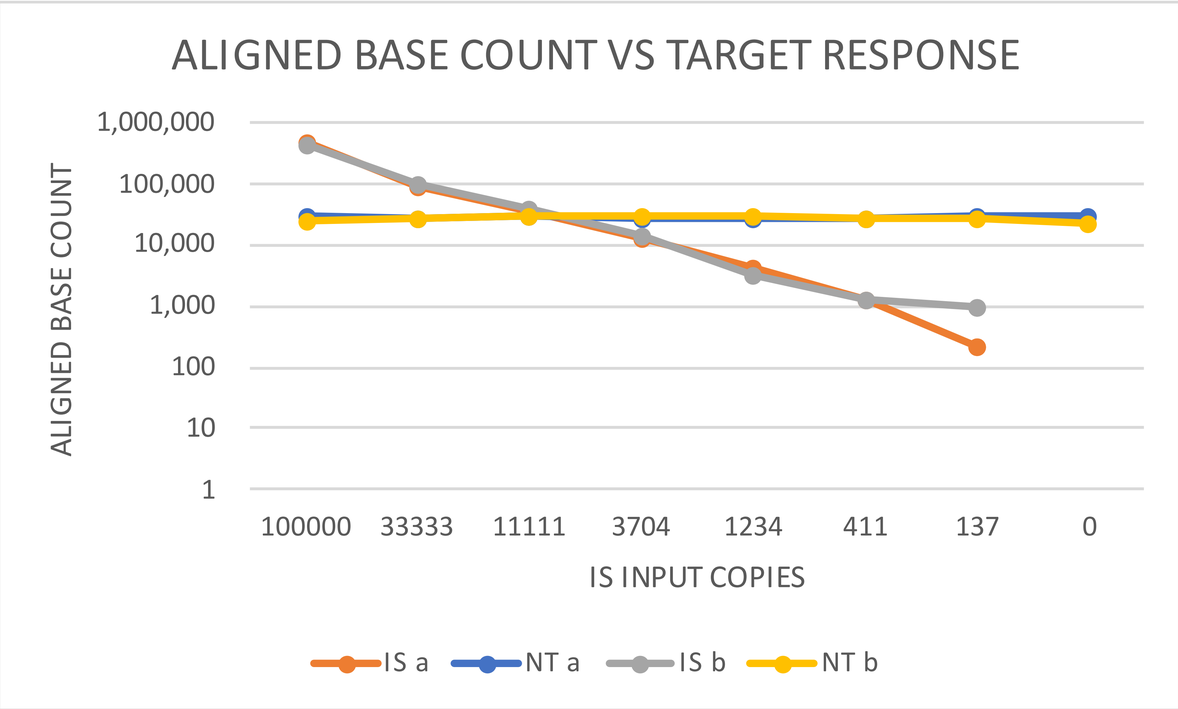
POSITIVE CONTROL IN AGNOSTIC AGENT DETECTION ASSAY



Samples:

- 37 kb COVID-19-like IS sequence added to sample at indicated levels
- 3000 AA genome
- 8 million reads per sample

Results

- No IS false positives in zero IS sample
- IS did not alter NT detection
- IS detection indicating stochastic behavior at 137 copy input

- ISSUE: How do gauge the per sample AA sample testing performance when using a non-targeted method that balances sensitivity with broad AA detection?
- AA samples can be spiked with limiting levels of IS that are sequence similar to a variety of AA.
- Above example demonstrates that IS spike could be detected down to methods 137 IS genomes while not interfering with detection of limiting levels of a 3kb AA.

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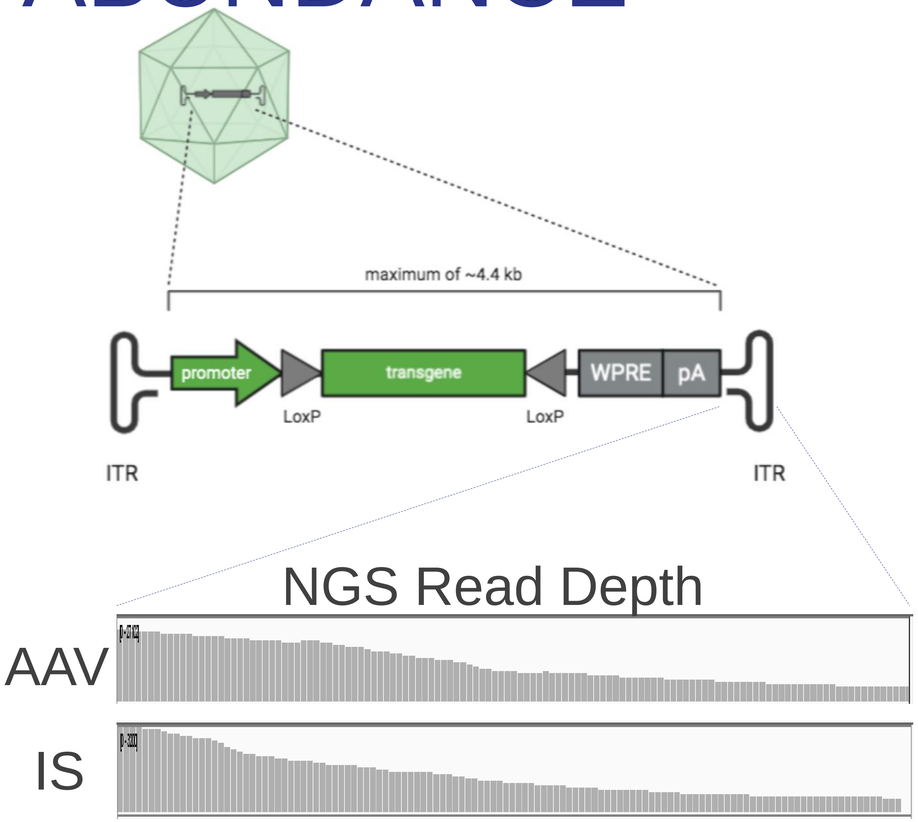
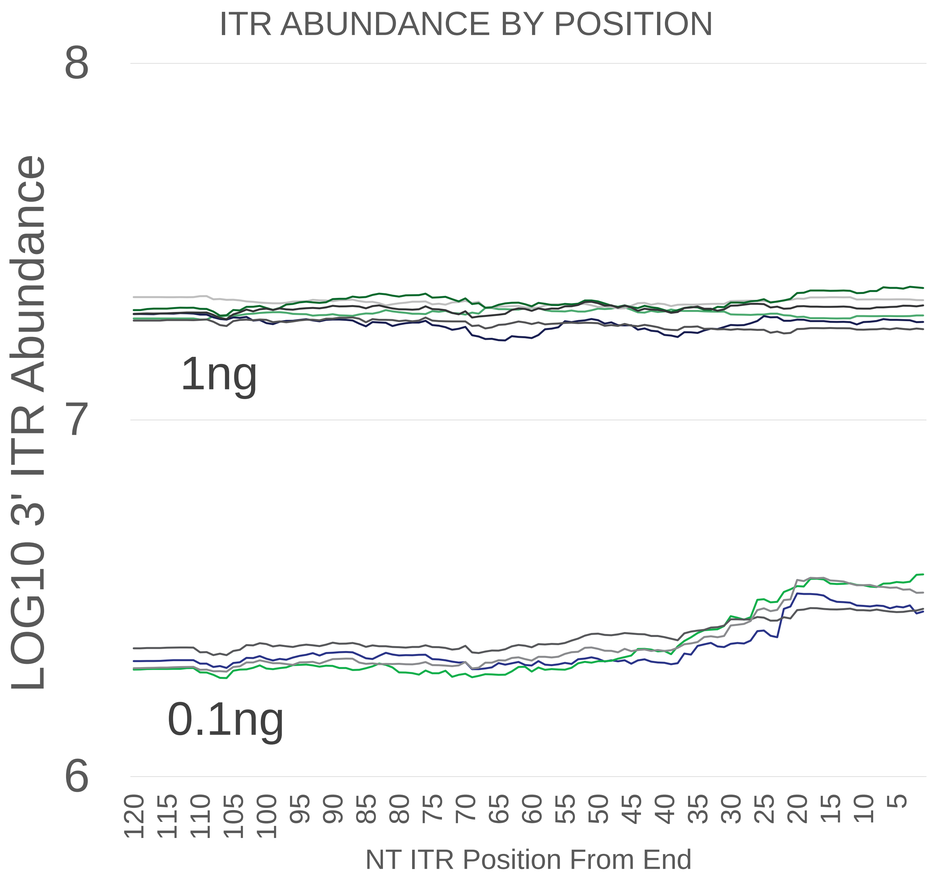
AAV INVERTED TERMINAL REPEAT ABSOLUTE ABUNDANCE



Abundance



- ISSUE: How to use NGS to QC AAV ITR yields when the ITR stable secondary structure causes dramatic short read depth NGS drop off.
- Samples: Two AAV input levels spiked with 300,000 IS ITR.
- NT:IS read ratio at each ITR base position was converted into abundance.
- SNAQ™-SEQ measured positional abundance indicated no ITR degradation.

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Standardized Target Abundance

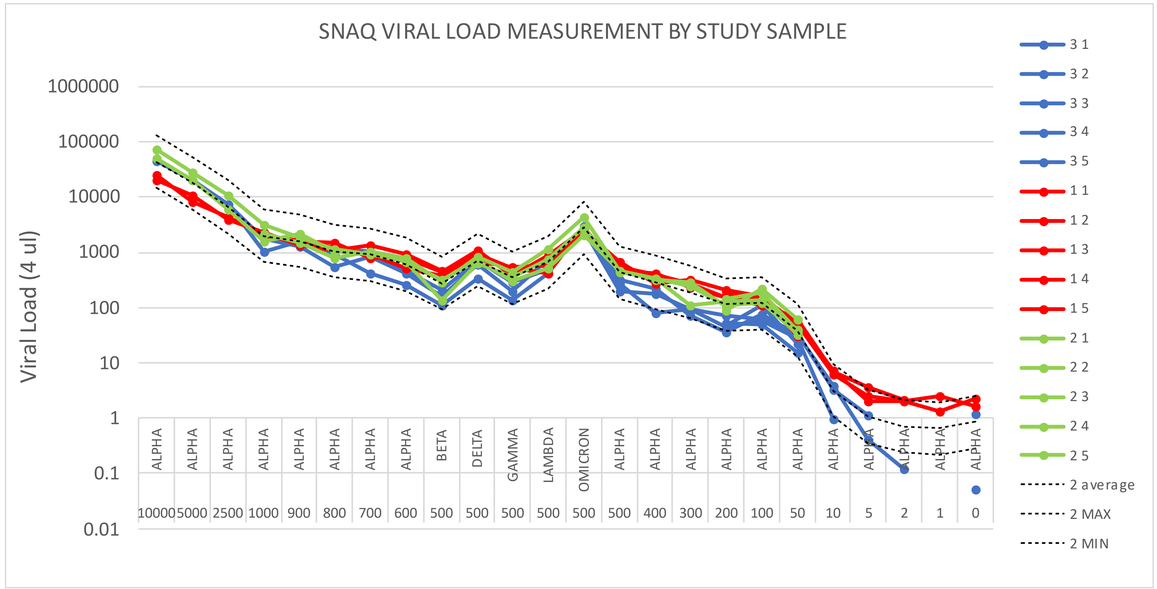

Figure 2. SNAQ™ Based Viral Load Measurement of Study Samples. Study FASTQ were analyzed using SNAQ™-VSOFT v1.2beta to estimate viral load (y-axis) of each study sample (x-axis). X-axis indicates COVID-19 genome and approximate genomic input; legend indicates lab and replicate number. The mean (central dashed line) and three-fold difference from mean (outer dashed lines) indicate that 163 of 165 samples with greater than 100 genomic abundance were within 3-fold of mean.

- Poor test results can arise from lower-than-expected genomic input or errors in NGS testing procedure
- SNAQ™ abundance directly measures capturable genomic input
- Results demonstrate SNAQ™ inter laboratory abundance measurements varied less than three-fold for samples ranging from 10 to >10^4 viral genomes with NGS tests using different sequencing instruments and altered ARTIC COVID-19 NGS protocols

❖ SNAQ™ standardizes viral abundance measurements

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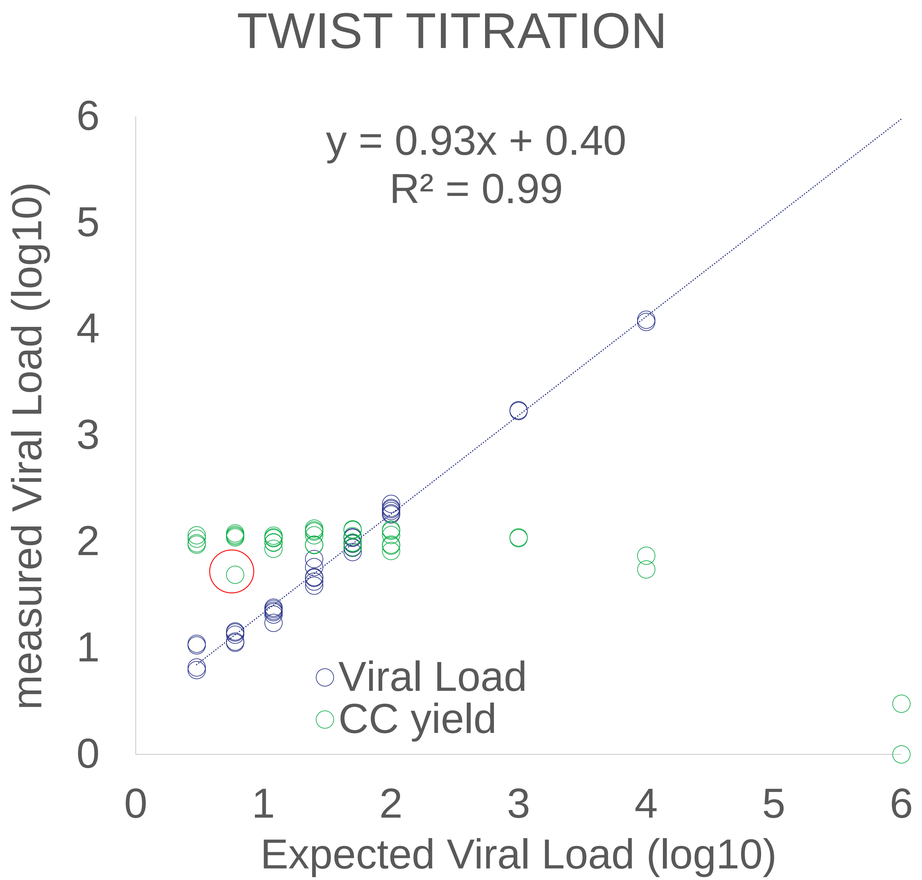
COMPLEXITY CONTROL IN VIRAL TITRATION



- For each sample, a QC to determine how much template was captured as sequencing reads.
- 1000 genome equivalents of Accukit™ SARS-CoV-2 IS added with master mix containing indicated viral input.
- ✓ Indicated amount of TWIST COVID-19 RNA reference material (blue)
- ✓ Complexity capture was 20% (green)
- ✓ One sample showed 2-fold lower CC capture (red circle)
- Above 10^5 viral genome equivalents, the virus copies will outcompete 1000 controls for reads on flow cell

SNAQ™-SEQ complexity control indicates template capture efficiency for every sample

Study done in collaboration with the Wellcome Sanger Institute using ARTIC v3 protocol with Accukit™ SARS-CoV-2 (v2, 1000) Catalog #1270

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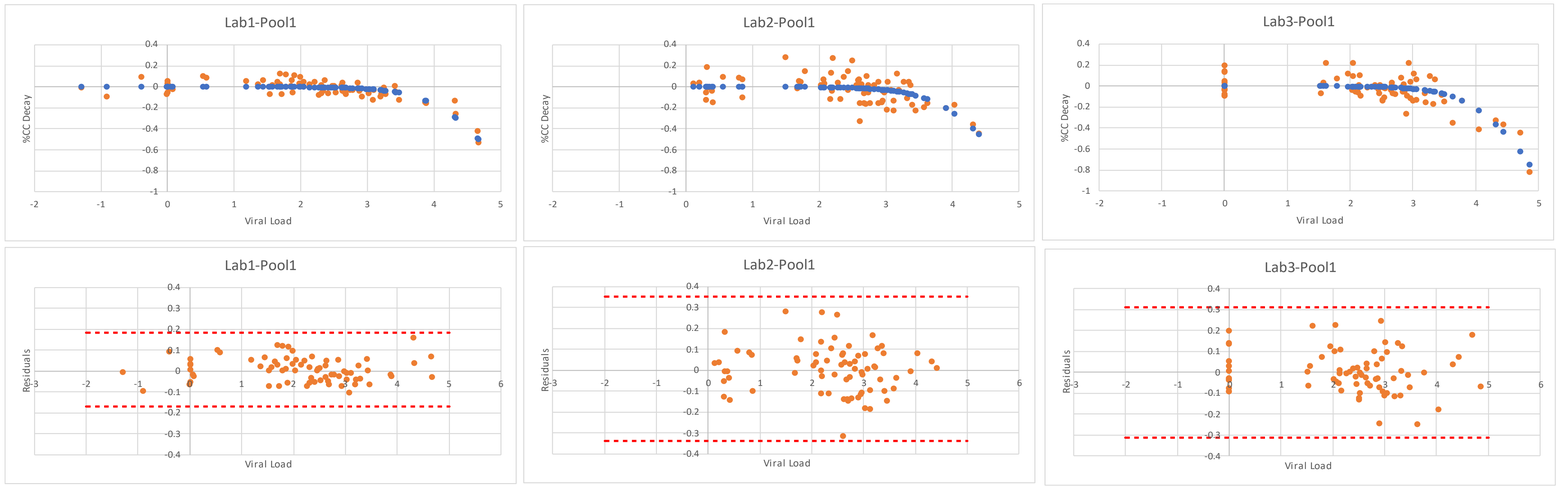
COMPLEXITY CAPTURE QUALITY CONTROL

	Lab1	Lab2	Lab3
POOL1	11.4 ± 1.6%	3.2 ± 0.9%	3.6 ± 0.9%
POOL2	17.1 ± 1.7%	7.2 ± 1.9%	6.1 ± 1.2%



- How to more accurately detect NGS library preparation testing errors?
- SNAQ™ complexity capture (CC) acts as a full process control to measure how well each sample's viral genome was captured as sequencing reads
- Table indicates each lab's nominal CC rate
- A CC model was created from each lab's CC vs viral Load response; the residuals to this model indicate if a sample CC is nominal
- SNAQ™ could detect a >two-fold drop in complexity capture with high specificity.

❖ SNAQ™ CC indicated each sample met nominal testing performance

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