KAPA HyperCap SARS-CoV-2 panel

End to End viral surveillance workflow – from RNA to report

May 2021

Markos Mihalatos, PhD – Sr. International Product Manager

Intended Use:

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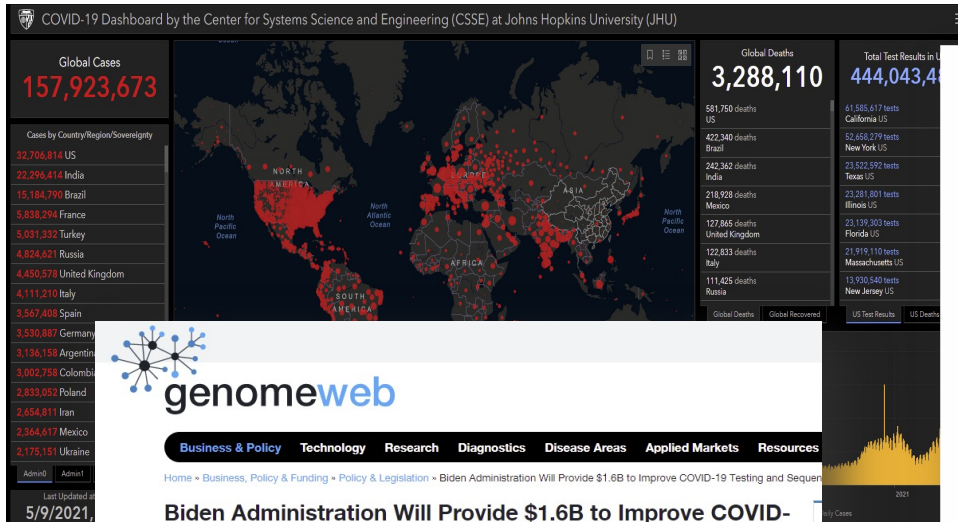Not for use in diagnostic procedures.

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SARS-CoV-2 spreads unprecedented challenges
First pandemic where the importance of Next Generation Sequencing (NGS) is broadly recognized

- Ongoing **pandemic** has huge **health and economic cost**

- **Emerging variants** spread fast posing **risks** for vaccine efficiency

- The **need for fast and confident sequencing** is broadly acknowledged

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Sequencing is important in following viral spread and evolution
Hybrid capture based target enrichment offers advantages for COVID-19 sequencing

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Detect **phylogenetic relationships**
(follow viral spread – surveillance and evolution)

Detect **nucleotide changes** in patient isolates
(e.g. D614G, B.1.1.7, B.1.351)

Identify **potential new strains**

Detect **emerging resistance mutations** to antiviral drugs and vaccines
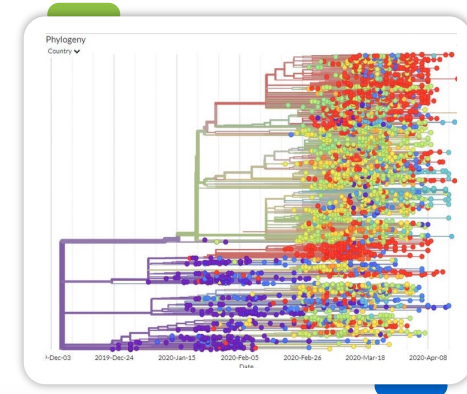
Genomic sequencing of SARS-CoV-2

A guide to implementation for maximum impact on public health
8 January 2021

World Health Organization

*"**One advantage** of using a capture-based approach over a PCR amplicon-based approach (section 6.5.4) is that **capture-based** approaches can **tolerate sequence differences** from the probe sequences **of 10–20%.** This is **higher than** the mismatch tolerated by **PCR,** where such a divergence from the primer sequences would result in a **high risk of amplicon failure.**"*

https://apps.who.int/iris/rest/bitstreams/1326052/retrieve: 6.5.3 Targeted capture-based approaches

Sources:
James Hadfield, Colin Megill, Sidney M Bell, John Huddleston, Barney Potter, Charlton Callender, Pavel Sagulenko, Trevor Bedford, Richard A Neher, Nextstrain: real-time tracking of pathogen evolution, Bioinformatics, Volume 34, Issue 23, 01 December 2018, Pages 4121–4123, https://doi.org/10.1093/bioinformatics/bty407

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Viral surveillance with KAPA Sample Prep Tools and Exatype platform
End-to-end workflow from RNA to analysis and meaningful reporting

Viral RNA in host RNA background → Library Prep with KAPA RNA HyperPrep Kit → Target Enrichment with KAPA HyperCap SARS-CoV-2 panel → Final library enriched with viral targets → Next Gen Sequencing → Analysis and reporting using the Exatype platform
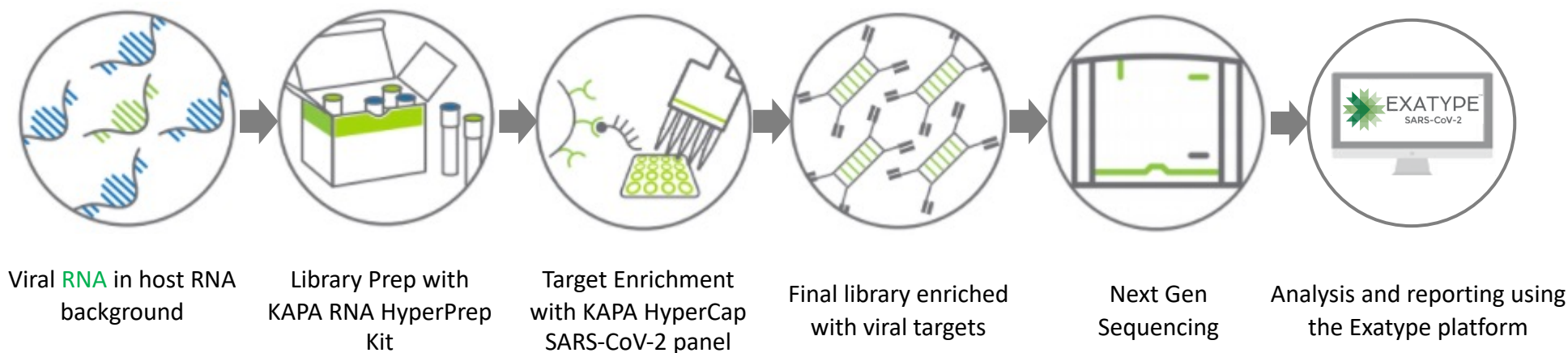
KAPA RNA HyperCap Workflow for Sample Preparation with Exatype Platform analysis and reporting:

- **Single day & automatable** from RNA to Sequencer (with **1h hybridization**)

- **Single vendor offering** across the entire sample prep workflow

- **Long 120 bp probes** "resistant" to underlying variants

- **Secure on-cloud Exatype platform parallel analysis and reporting** by Hyrax Biosciences

- Purpose-built **user friendly analysis** with **5 simple steps** to **variant and lineage/clade reporting**

Exatype Platform by Hyrax Biosciences
Purpose built from viral sequence analysis experts for reliable SARS-CoV-2 surveillance

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High quality results

Confidently detect and report variants with the Exatype platform
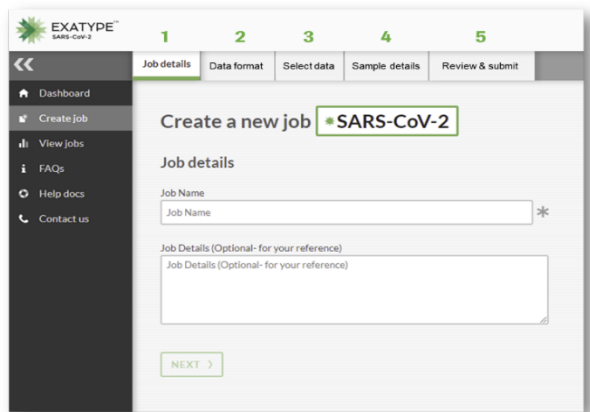
Minimize time

Report your findings **faster by on-cloud parallel processing** using the intuitive interface of the Exatype platform

Scalability

Scale up with ease using Hyrax Biosciences' **streamlined and secure, cloud-based IT infrastructure**

Proven Expertise

Hyrax Biosciences' **proven expertise in viral sequence and reporting** offers **reliable** SARS-CoV-2 **surveillance**

HYRAX
BIOSCIENCES

Lineage/Clade Typing

Sample	Pangolin (2.3.9 - 2021-04-23)[1]	Nextstrain clade (0.14.2)[2]	Nextstrain AA substitutions (0.14.2)[2]	Nextstrain AA deletions (0.14.2)[2]
Sample 1	B.1.1.7	✓ 20I/501Y.V1	ORF1a: T1001I, A1708D, I2230T ORF1b: P314L, T797I, K1383R S: N501Y, A570D, D614G, P681H, T716I, S982A, H1058N, D1118H ORF8: Q27*, R52I, Y73C N: M1X, D3L, R203K, G204R, S235F ORF7b: *44Q	S: Y144-

DOWNLOAD AS CSV

[1] https://github.com/cov-lineages/pangolin
[2] https://clades.nextstrain.org/

EXATYPE
SARS-CoV-2

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Exatype Platform by Hyrax Biosciences
Purpose built from viral sequence analysis experts with intuitive user interface

Exatype Platform by Hyrax Biosciences

Purpose built from viral sequence analysis experts with intuitive user interface

My jobs

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Page 1 of 1 · 30 items per page

Job name	Date	Info	Pathogen	Status	Platform	Analysis
SARS-CoV-2 surveillance	2021-05-09 06:14 pm	Show	SARS-CoV-2	Running 0%	Illumina	Pending

Analysis **starts automatically** after uploading

EXATYPE™ SARS-CoV-2

Intuitive **reports generated automatically**

Lineage/Clade Typing

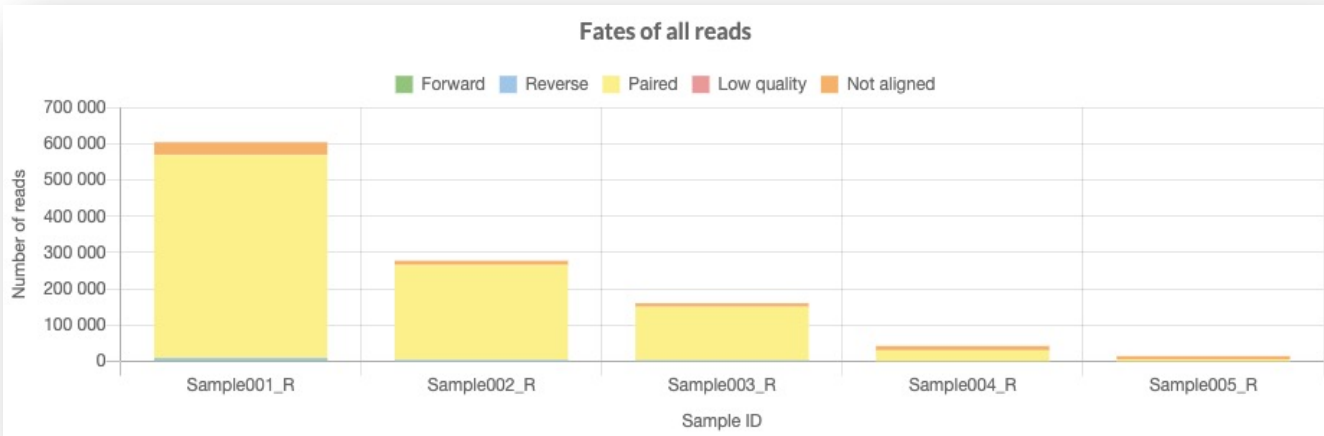| Sample	Pangolin (2.3.9 - 2021-04-23)[1]	Nextstrain clade (0.14.2)[2]	Nextstrain AA substitutions (0.14.2)[2]	Nextstrain AA deletions (0.14.2)[2]
UHR_100ng_MS2_0pg_SCV2_14_10000copy_3_DesignV3_singleplex_S21	B.1.1.7	✓ 20I/501Y.V1	ORF1a: T1001I, A1708D, I2230T ORF1b: P314L, T797I, K1383R S: N501Y, A570D, D614G, P681H, T716I, S982A, H1058N, D1118H ORF8: Q27*, R52I, Y73C N: M1X, D3L, R203K, G204R, S235F ORF7b: *44Q	S: Y144-

DOWNLOAD AS CSV

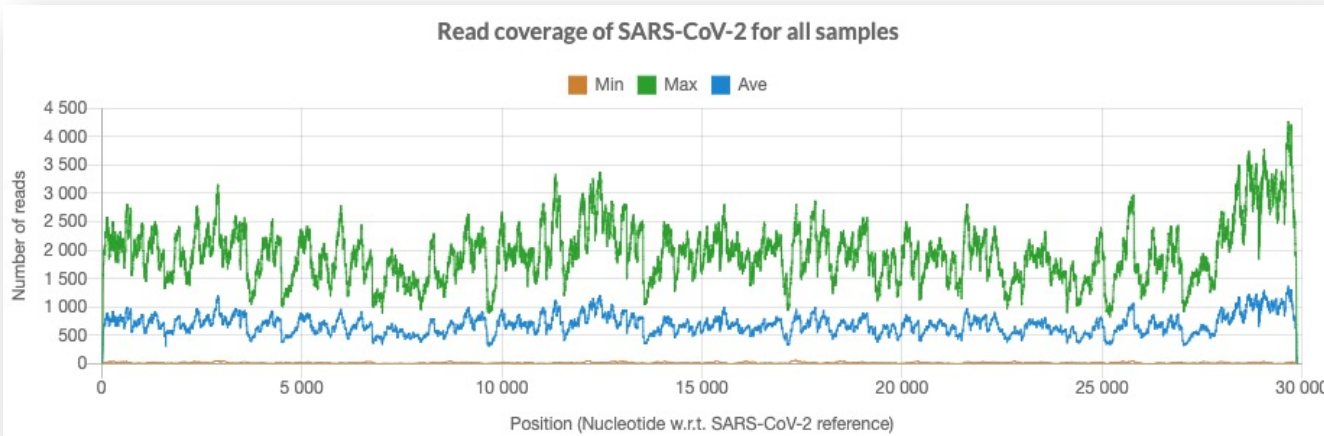[1] https://github.com/cov-lineages/pangolin
[2] https://clades.nextstrain.org/

Exatype Platform by Hyrax Biosciences
Purpose built from viral sequence analysis experts with intuitive user interface

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Fates of all reads

Legend: Forward · Reverse · Paired · Low quality · Not aligned

Number of reads (700 000 – 0)

Sample IDs: Sample001_R, Sample002_R, Sample003_R, Sample004_R, Sample005_R

Plethora of **downloadable reports,** from **sequencing metrics** through **variant frequency** reports and output files for **GISAID and Nextstrain**

Read coverage of SARS-CoV-2 for all samples

Legend: Min · Max · Ave

Number of reads (4 500 – 0)
Position (Nucleotide w.r.t. SARS-CoV-2 reference): 0 – 30 000

High-prevalence amino acid mutations

Observed at >=80% prevalence relative to the SARS-CoV-2 reference (MN908947.3)

Locus	Wild type	Locus position	Variant	Prevalence
ORF1a	T	1001	I	100%
ORF1a	A	1708	D	100%
ORF1a	I	2230	T	100%
ORF1b	P	314	L	100%
ORF1b	K	491	*	100%
ORF1b	T	797	I	100%
ORF1b	K	1383	R	100%
S	VY	143	V	100%
S	N	501	Y	100%
S	A	570	D	100%
S	D	614	G	100%
S	P	681	H	100%
S	T	716	I	
S	S	982	A	
S	H	1058	N	
S	D	1118	H	
ORF8	Q	27	*	
ORF8	R	52	I	
ORF8	Y	73	C	
N	D	3	L	
N	R	203	K	
N	G	204	R	
N	S	235	F	

EXATYPE™
SARS-CoV-2

Low-prevalence amino acid mutations

Observed at <80% prevalence relative to the SARS-CoV-2 reference (MN908947.3)

⚠ Low-prevalence mutations may be due to co-infection or within-host evolution, but also due to contamination. Proceed with caution.

Locus	Wild type	Locus position	Variant	Prevalence
ORF1a	L	3606	frameshift(TTtG)	22%
ORF1a	L	3606	frameshift(TTttG)	12%
ORF1a	Q	4392	K	11%
ORF1b	D	490	V	70%
ORF1b	I	2465	T	10%

Downloads

All consensus sequences from SARS-CoV-2 samples

Combined
⬇ Version3_10k_copies_3_consensus_sequences.sars-cov-2.fasta

One-per-file
⬇ Version3_10k_copies_3_aligned_consensus_sequences.zip

All mutations in SARS-CoV-2 samples present at >= 10% prevalence

⬇ Version3_10k_copies_3_coding_prevalences.zip
⬇ Version3_10k_copies_3_noncoding_prevalences.zip

Lineage/Clade files

⬇ Version3_10k_copies_3_nextclade.csv
⬇ Version3_10k_copies_3_nextclade.json
⬇ Version3_10k_copies_3_nextclade.tree
⬇ Version3_10k_copies_3_pangolin.csv

Benefits of KAPA RNA HyperPrep, KAPA HiFi DNA Polymerase and KAPA HyperCap Probes

KAPA RNA HyperPrep Kit including KAPA HiFi DNA Polymerase

Single-day automation-friendly library construction	inclusive of RNA enrichment
KAPA HiFi DNA Polymerase	offers uniform and robust library amplification
Robust and reliable performance	across different sample types and input amounts
Higher success rates	with lower input and degraded samples

KAPA HyperCap Target Enrichment probes

Renowned probe design & content expertise	uncovers difficult genomic regions
Manufactured with KAPA HiFi DNA Polymerase	enrich with higher fidelity and higher specificity
High uniformity and low duplication	deliver high sequencing efficiency
NGS probe pool QC	provides consistent quality

KAPA HyperCap SARS-CoV-2 Panel

- Covers **100% of the reference genome** (RefSeq) – target size ~30 Kb

- Covers >99.7% of another 183 genomic sequences from GenBank

- **Dense probe tiling**

- Probes targeting MS2 RNA for optional spike-in internal control

- **Based on hybrid capture** which **may advance** characterization, surveillance and viral evolution **research of divergent isolates**[1]

Ordering details:

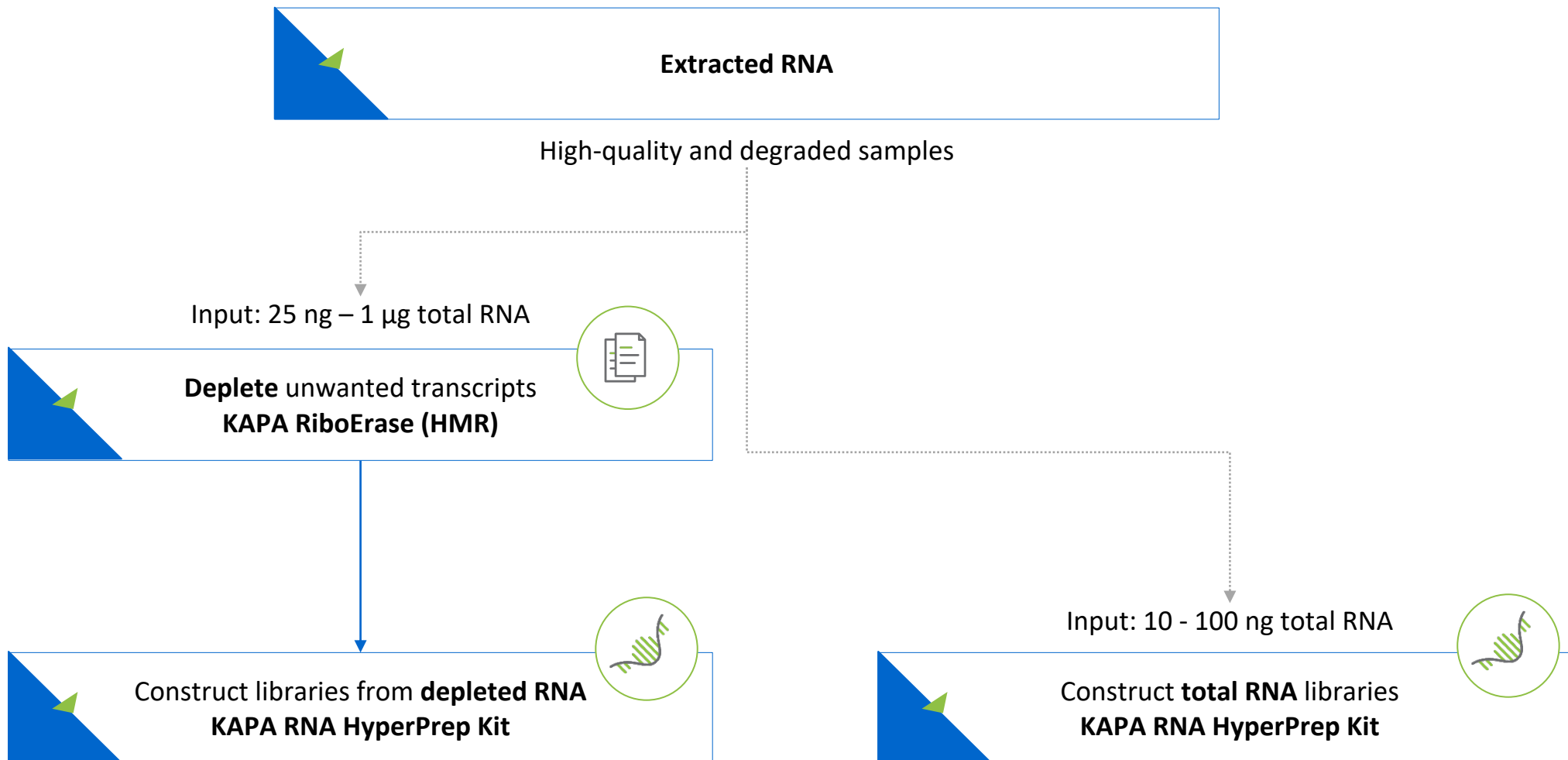|	
09436499001	KAPA HyperCap SARS-CoV-2 panel 12 rxn
09436502001	KAPA HyperCap SARS-CoV-2 panel 24 rxn
09436529001	KAPA HyperCap SARS-CoV-2 panel 48 rxn
09436537001	KAPA HyperCap SARS-CoV-2 panel 96 rxn

1. Genomic sequencing of SARS-CoV-2: a guide to implementation for maximum impact on public health. 8 Jan 2021 | *COVID-19: Laboratory and diagnosis.* World Health Organization.

KAPA HyperCap SARS-CoV-2 Target Enrichment Panel
Research protocol combines KAPA RNA HyperPrep and HyperCap Probes benefits

Extracted RNA

High-quality and degraded samples

Input: 25 ng – 1 µg total RNA

Deplete unwanted transcripts
KAPA RiboErase (HMR)

Construct libraries from **depleted RNA**
KAPA RNA HyperPrep Kit

Input: 10 - 100 ng total RNA
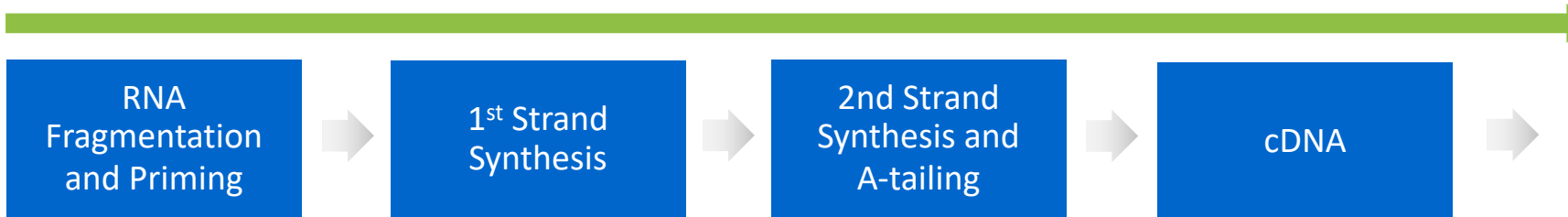
Construct **total RNA** libraries
KAPA RNA HyperPrep Kit

KAPA HyperCap SARS-CoV-2 Target Enrichment Panel
Research protocol combines KAPA RNA HyperPrep and HyperCap Probes benefits

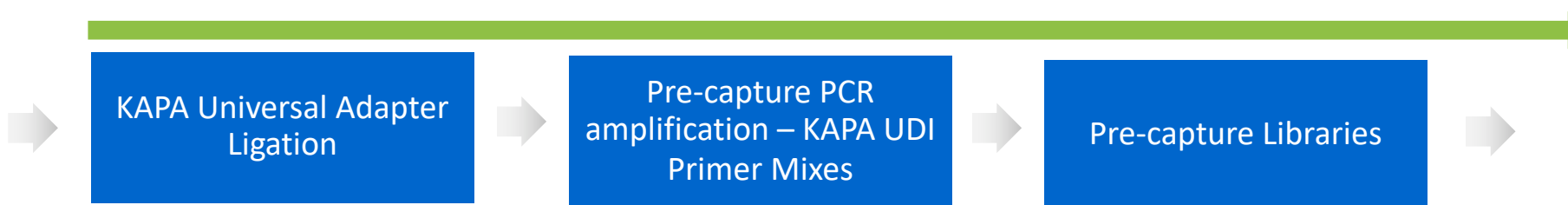KAPA RNA HyperPrep Kit

rRNA depleted Or total RNA →

| RNA Fragmentation and Priming | → | 1st Strand Synthesis | → | 2nd Strand Synthesis and A-tailing | → | cDNA | → |

KAPA RNA HyperPrep Kit

cDNA →

| KAPA Universal Adapter Ligation | → | Pre-capture PCR amplification – KAPA UDI Primer Mixes | → | Pre-capture Libraries | → |

KAPA HyperCap v3.0 Workflow   Sequencing   Analysis

Pre-capture libraries →

| Singleplex (1000 ng) or pre-capture multiplex (1500 ng) | → | KAPA HyperCap SARS-CoV-2 Design (30kb) | → | Hybridization and capture | → | Illumina Sequencing | → | **Exatype platform** |

EXATYPE™ SARS-CoV-2
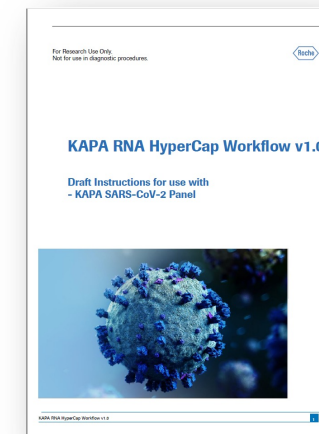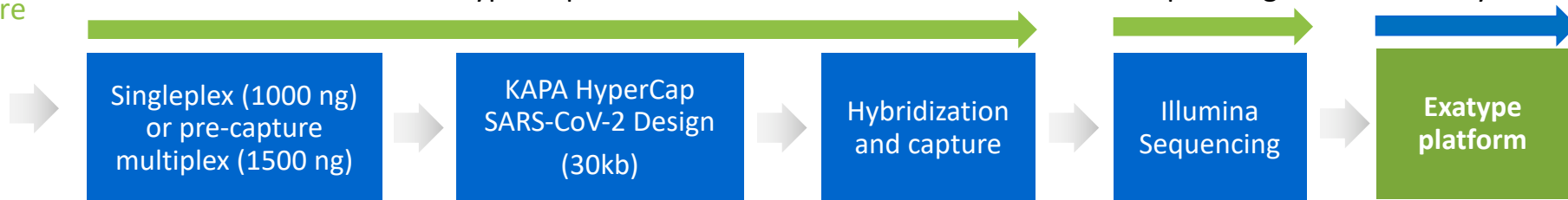
Minor protocol modifications
- Use 5 µL of KAPA Universal Adapter in ligation (instead of 10 µL)
- Omit first post ligation clean-up, perform only the 0.7X 2nd post ligation clean-up

KAPA HyperCap SARS-CoV-2 Target Enrichment Panel
Strong performance from low to high starting viral copies in the library preparation

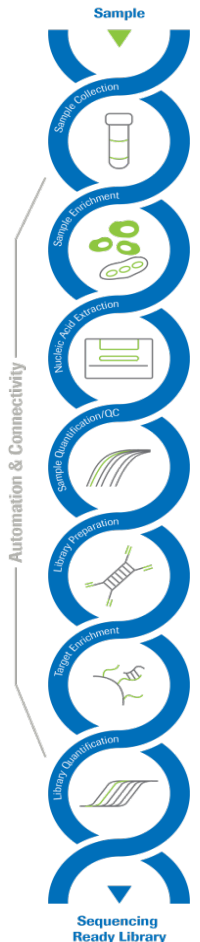**The KAPA HyperCap SARS-CoV-2 panel and target-enriched RNA-Seq workflow enables high-throughput sequencing of the SARS-CoV-2 genome**

It was determined through testing the panel and workflow that:

- **About 97% 1x SARS-CoV-2 genome coverage** can be achieved **down to 1,000 viral copies** and 0.5 million NextSeq™ clusters (2 x 75 bp)

- **Genomic sequence (6% - 6.8%)** can be obtained **from as few as 10 viral input copies**

- SARS-CoV-2 **coverage can further be increased by adding KAPA RiboErase** for rRNA depletion prior to library preparation

- **Variants from six different SARS-CoV-2 isolates** were **detected** in the same sample **near their expected frequency**

- A **complex 10nt deletion was detected down to 1,000 total viral copies** (166 copies of the isolate with the deletion)

Data on file.

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Demonstrate performance of the SARS-CoV-2 panel under the following conditions:

- **From very low (10) to very high (1M)** starting viral copies

- **Pre-capture multiplexing** of **8** or **up to 16** sample libraries per hybridization

- **One-hour** compared to overnight **hybridization**

Data on file.

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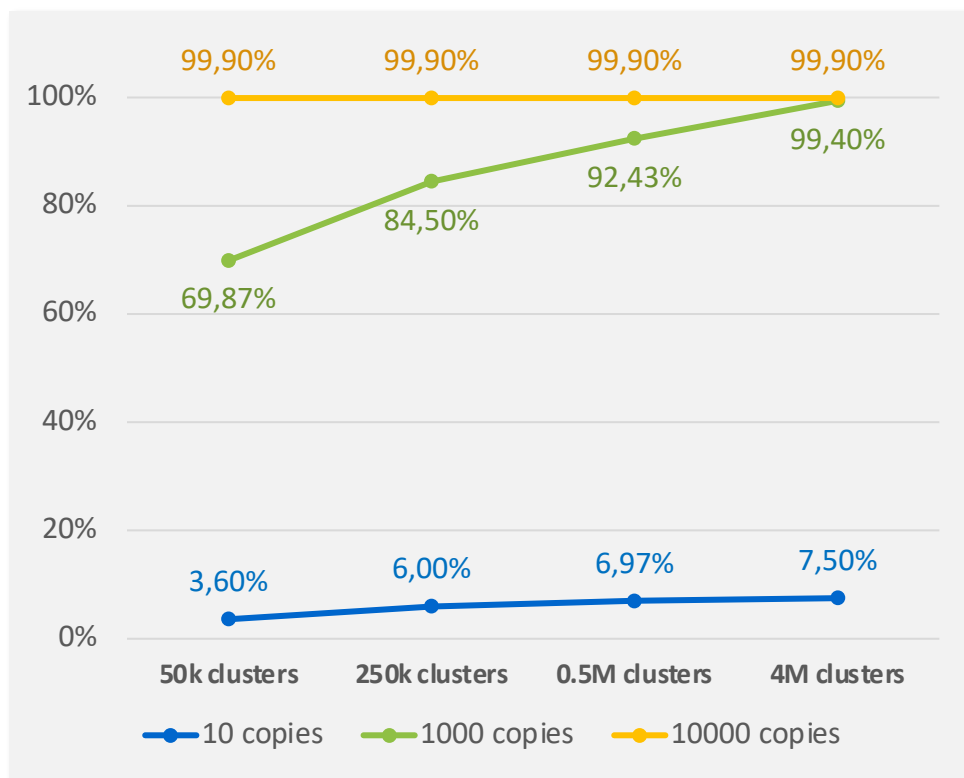
KAPA HyperCap SARS-CoV-2 Target Enrichment Panel
Recovers viral reads even from just 10 starting viral copies
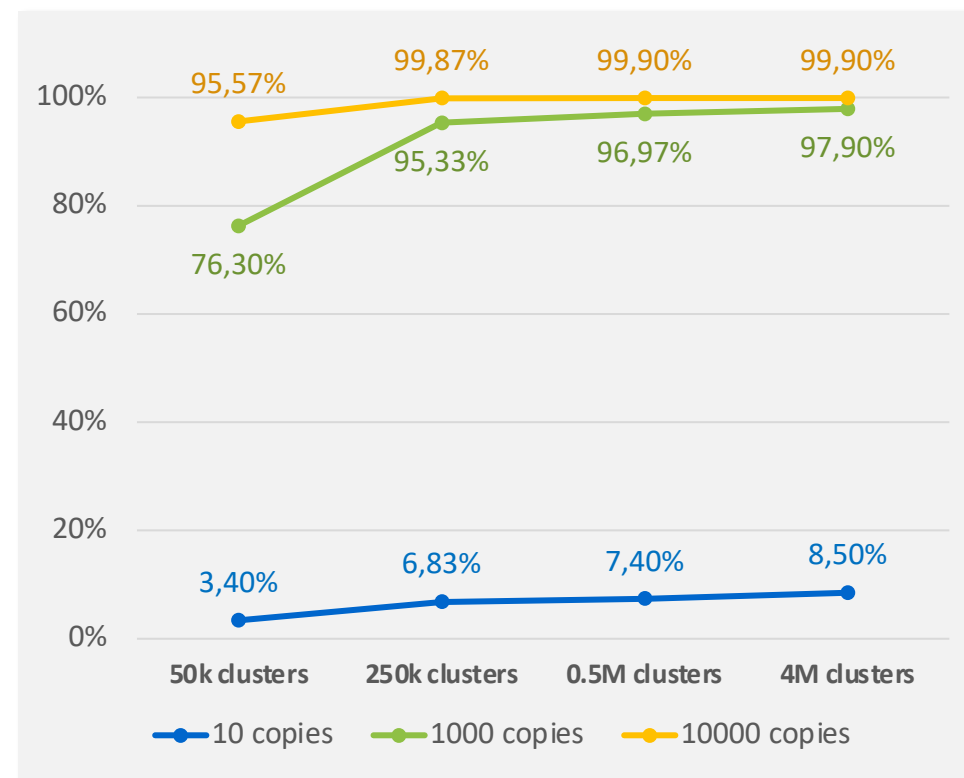
- As few as **250k clusters** can cover the genome of just **10 viral copies** by **6% - 6.8%**

- Starting from **1000 viral copies, 0.5M clusters cover 92.4% or 96.9%** of the genome in 20 ng or 100 ng UHR background

% bases ≥ 1x

20 ng   **UHRR background**   **100 ng**

Data on file.
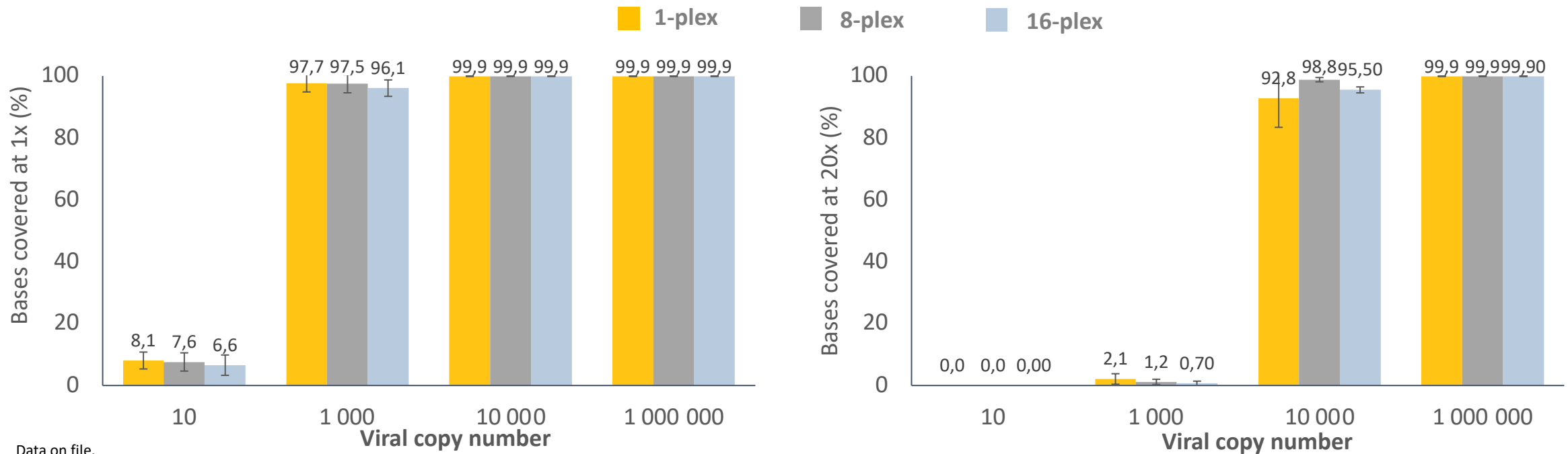
UHRR: Universal Human Reference RNA

KAPA HyperCap SARS-CoV-2 panel with pre-capture multiplexing
Pre-capture multiplexing performance is concordant to single library capture

Pre-capture multiplexing of 8 or up to 16 libraries (8-plex or 16-plex) compared to single captures (1-plex):

- **Coverage metrics are similar across input viral copies for either 1-plex, 8-plex or up to 16-plex captures**

- **Robust capture performance offers convenience of pooling in the same hybridization**

■ **1-plex**   ■ **8-plex**   ■ **16-plex**

Data on file.

100 ng UHRR background, 1 million NextSeq™ clusters (2 x 75 bp)

KAPA HyperCap SARS-CoV-2 panel with pre-capture multiplexing
Pre-capture multiplexing considerations for successful libraries pooling

- When pre-capture multiplexing avoid mixing libraries with vastly different Ct values or viral loads

- **In the same capture high viral load libraries may compete for reads** with the low viral load libraries
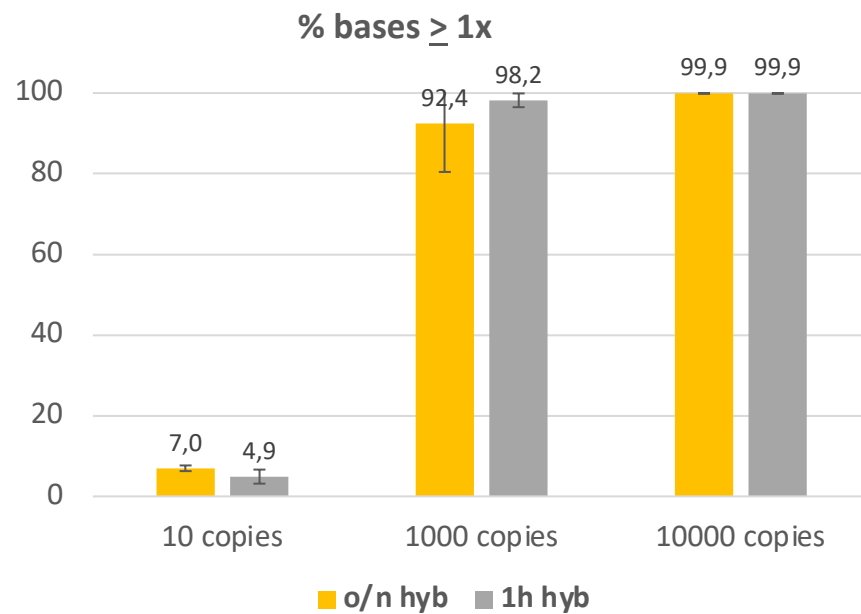
8-plex Capture
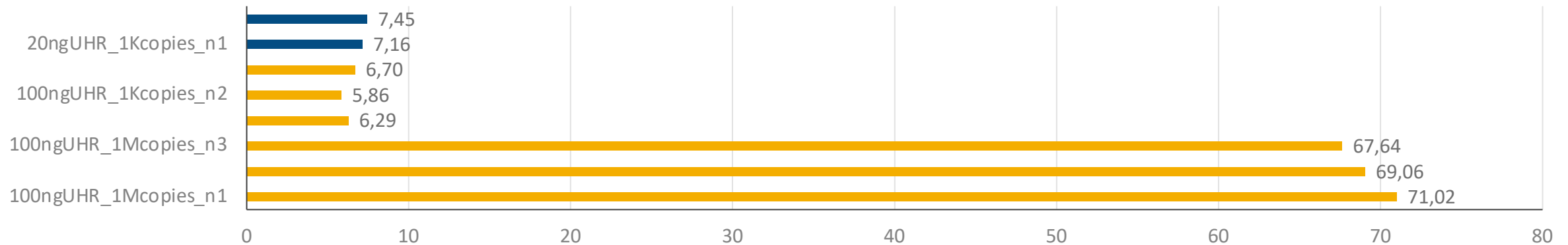
Data on file.

20 ng UHRR, 100 ng UHRR

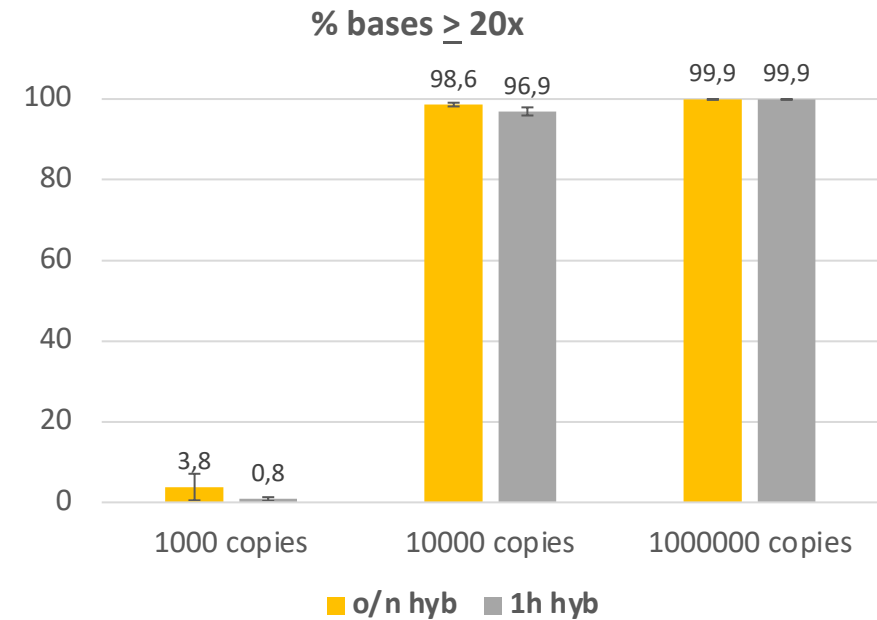# KAPA HyperCap SARS-CoV-2 panel with 1-hour hybridization
Quick 1-hour hybridization offers similar performance to overnight in a single day workflow

Roche

One hour hybridization offers good balance between workflow speed and performance at low viral concentrations

% bases > 1x

% bases > 20x

o/n hyb   1h hyb

o/n hyb   1h hyb

1 hour hyb samples subjected to 3 additional post-capture PCR cycles than overnight

20 ng UHRR background, 0.5 million NextSeq™ clusters (2 x 75 bp)

Data on file.

n=3 for each data point

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KAPA HyperCap SARS-CoV-2 Target Enrichment Panel
One hour hybridization provides broad coverage with low sequencing requirements

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% bases uniquely covered at different levels vs 2 x 100 bp clusters from 10,000 viral copies
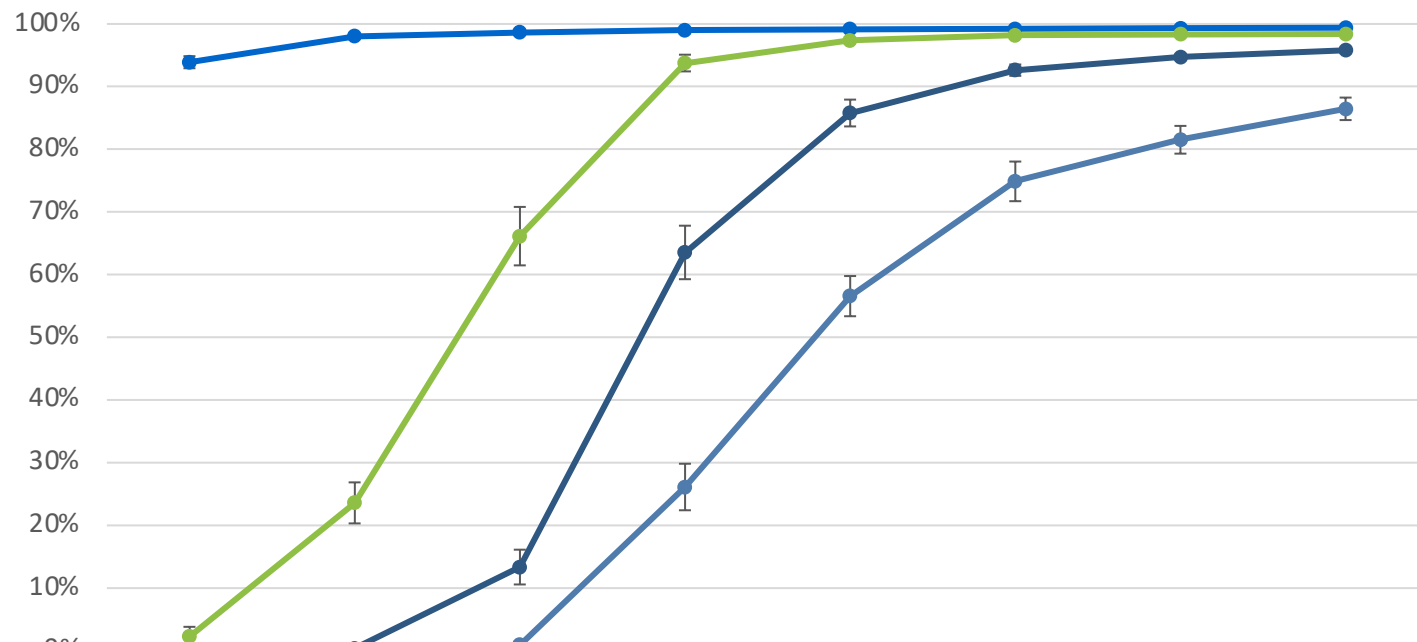
	12500	25000	50000	125000	250000	500000	1000000	4000000
1x	93,90%	98,03%	98,67%	99,00%	99,17%	99,23%	99,30%	99,40%
10x	2,30%	23,63%	66,17%	93,77%	97,37%	98,17%	98,33%	98,40%
20x	0,00%	0,43%	13,40%	63,57%	85,80%	92,63%	94,73%	95,80%
30x	0,00%	0,00%	1,00%	26,17%	56,60%	74,90%	81,53%	86,47%

Data on file. KAPA RNA HyperCap Workflow with 1 hour hybridization. Viral copies in 50 ng UHRR. Sequenced on a NovaSeq6000 System.
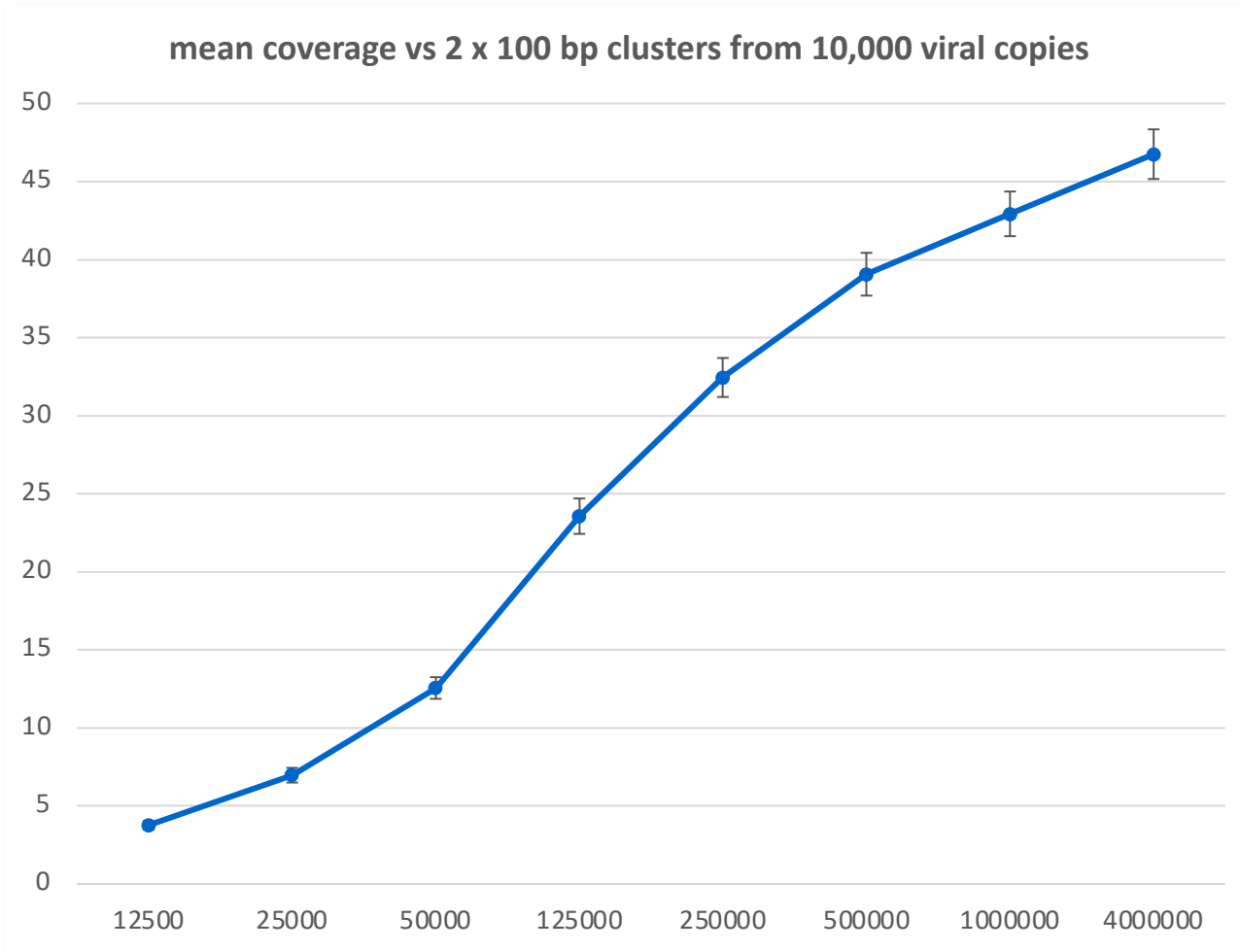
Robust performance with **1-hour hybridization:**

- **> 98%** of the viral genome **covered** by **1x unique molecule depth** with only **50,000 clusters** of 2 x 100 bp.

- **> 98%** of the viral genome **covered** by **10x unique molecule depth** with only **500,000 clusters** of 2 x 100 bp.

KAPA HyperCap SARS-CoV-2 Target Enrichment Panel
One hour hybridization provides broad coverage with low sequencing requirements

mean coverage vs 2 x 100 bp clusters from 10,000 viral copies

Robust performance with **1-hour hybridization:**

- **23x** mean **unique coverage** with **only 125,000 clusters** of 2 x 100 bp.

- **39x** mean **unique coverage** with **only 500,000 clusters** of 2 x 100 bp.

Data on file. KAPA RNA HyperCap Workflow with 1 hour hybridization. Viral copies in 50 ng UHRR. Sequenced on a NovaSeq6000 System
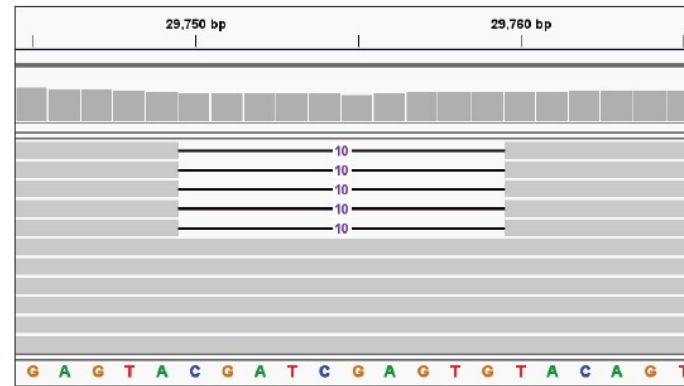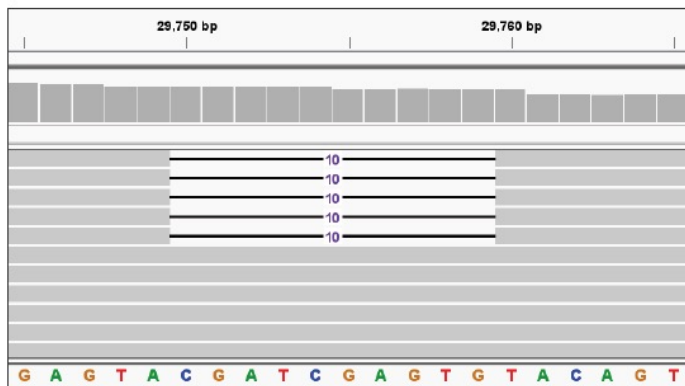
KAPA HyperCap SARS-CoV-2 minority variant detection
Concordant performance between expected and measured variant frequencies
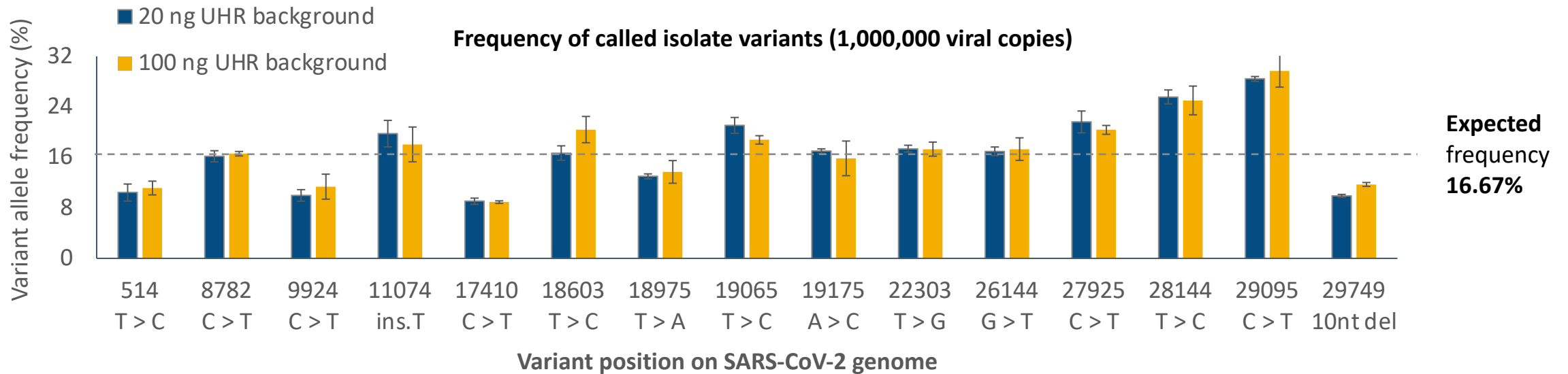
Most complex variant – a 10nt deletion detected down to 1,000 and 10,000 copies in 100 ng human RNA

1 million NextSeq™ clusters (2 x 75 bp) – duplicates retained
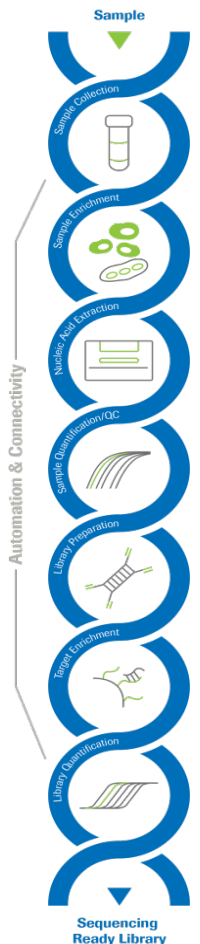
KAPA HyperCap SARS-CoV-2 Target Enrichment Panel
Strong performance from low to high starting viral copies in the library preparation

The KAPA HyperCap SARS-CoV-2 panel and target-enriched RNA-Seq workflow enables high-throughput sequencing of the SARS-CoV-2 genome
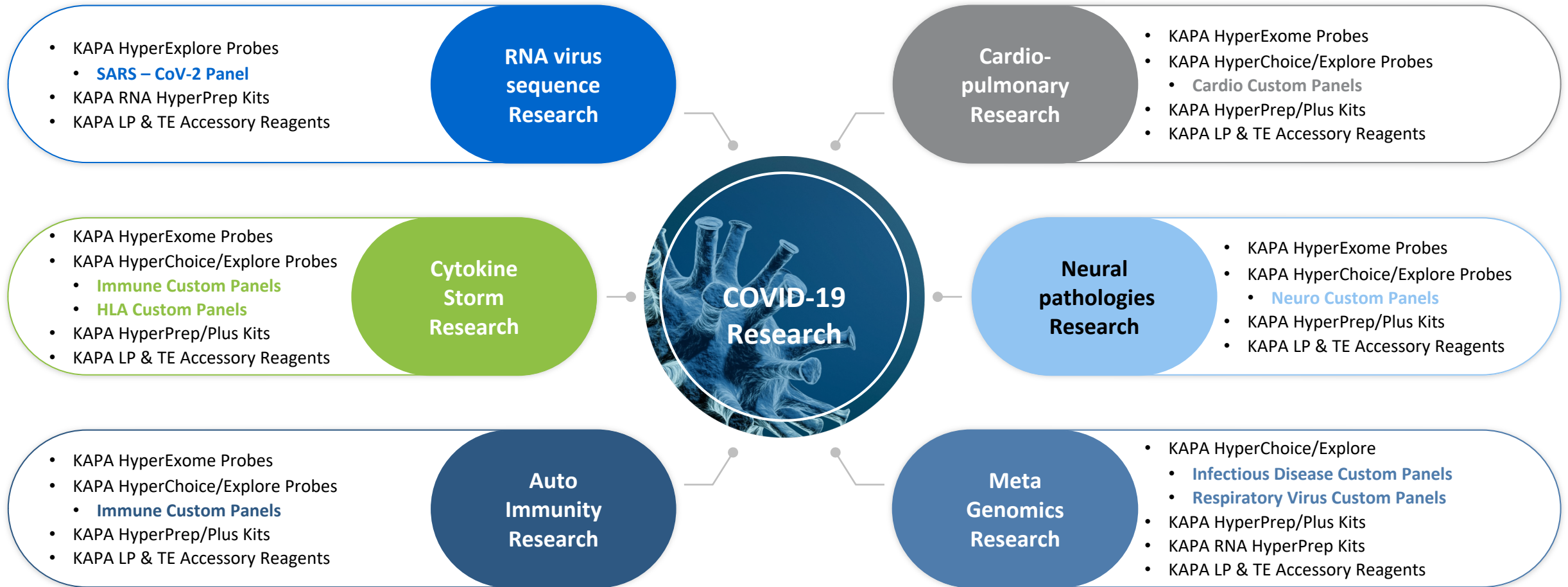
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- **Genomic sequence (6% - 6.8%)** can be obtained **from as few as 10 viral input copies**

- SARS-CoV-2 **coverage can further be increased by adding KAPA RiboErase** for rRNA depletion prior to library preparation

- **Variants from six different SARS-CoV-2 isolates** were **detected** in the same sample **near their expected frequency**

- A **complex 10nt deletion was detected down to 1,000 total viral copies** (166 copies of the isolate with the deletion)

KAPA Sample Prep Tools advance NGS COVID-19 research

Breadth of additional offerings in the fight against the pandemic

Roche

RNA virus sequence Research

- KAPA HyperExplore Probes
  - **SARS – CoV-2 Panel**
- KAPA RNA HyperPrep Kits
- KAPA LP & TE Accessory Reagents

Cardio-pulmonary Research

- KAPA HyperExome Probes
- KAPA HyperChoice/Explore Probes
  - Cardio Custom Panels
- KAPA HyperPrep/Plus Kits
- KAPA LP & TE Accessory Reagents

Cytokine Storm Research

- KAPA HyperExome Probes
- KAPA HyperChoice/Explore Probes
  - **Immune Custom Panels**
  - **HLA Custom Panels**
- KAPA HyperPrep/Plus Kits
- KAPA LP & TE Accessory Reagents

COVID-19 Research

Neural pathologies Research

- KAPA HyperExome Probes
- KAPA HyperChoice/Explore Probes
  - **Neuro Custom Panels**
- KAPA HyperPrep/Plus Kits
- KAPA LP & TE Accessory Reagents

Auto Immunity Research

- KAPA HyperExome Probes
- KAPA HyperChoice/Explore Probes
  - **Immune Custom Panels**
- KAPA HyperPrep/Plus Kits
- KAPA LP & TE Accessory Reagents

Meta Genomics Research

- KAPA HyperChoice/Explore
  - **Infectious Disease Custom Panels**
  - **Respiratory Virus Custom Panels**
- KAPA HyperPrep/Plus Kits
- KAPA RNA HyperPrep Kits
- KAPA LP & TE Accessory Reagents

Exatype Platform by Hyrax Biosciences
Purpose built from viral sequence analysis experts for reliable SARS-CoV-2 surveillance

High quality results

Achieve **uniform and broad genome coverage** with the **KAPA RNA HyperCap workflow Confidently detect** and **report variants** with the **Exatype platform**

Minimize time

Save valuable **time** and **maintain** data **quality by 1-hour hybridization** with the KAPA HyperCap SARS-CoV-2 panel Report your findings **faster by on-cloud parallel processing** using the intuitive interface of the Exatype platform

Scalability

Scale up with **ease** using the **streamlined, automation friendly** KAPA HyperCap workflow and Hyrax Biosciences' **secure, cloud-based IT infrastructure**

Proven Technology

Hybrid capture based methods may better detect relatively divergent SARS-CoV-2 sequences of new emerging variants[1]

Proven Expertise

Roche and Hyrax Biosciences combine their **proven expertise in sample prep and reporting** to offer a **complete, end-to-end solution for SARS-CoV-2 surveillance**

Viral RNA in host RNA background | Library Prep with KAPA RNA HyperPrep Kit | Target Enrichment with KAPA HyperCap SARS-CoV-2 panel | Final library enriched with viral targets | Next Gen Sequencing | Analysis and reporting using the Exatype platform

1. Genomic sequencing of SARS-CoV-2: a guide to implementation for maximum impact on public health. 8 Jan 2021 | *COVID-19: Laboratory and diagnosis.* World Health Organization.

Doing now what patients need next

BACKUP SLIDES

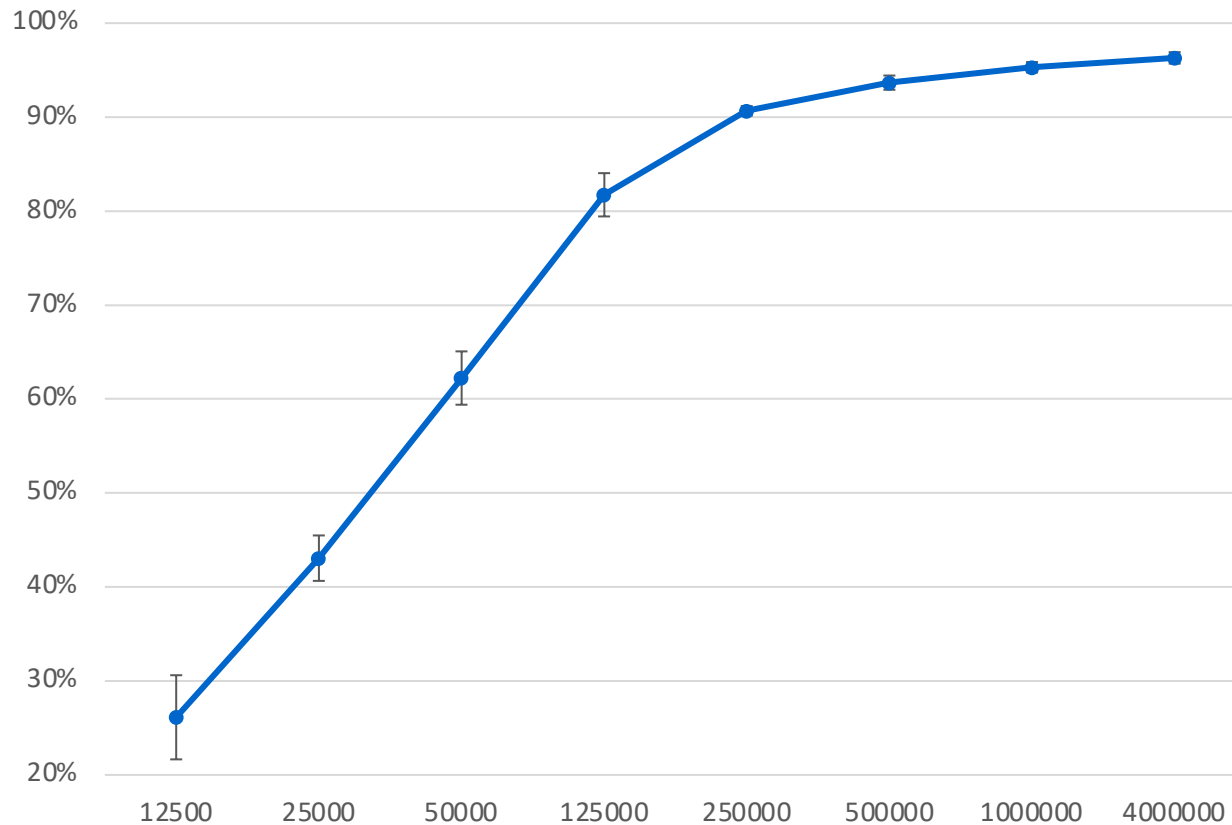For Research Use Only. Not for use in diagnostic procedures.

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KAPA HyperCap SARS-CoV-2 Target Enrichment Panel
One hour hybridization provides broad coverage with low sequencing requirements

% viral genome uniquely covered ≥ 1x vs 2 x 100 bp clusters from 1,000 viral copies

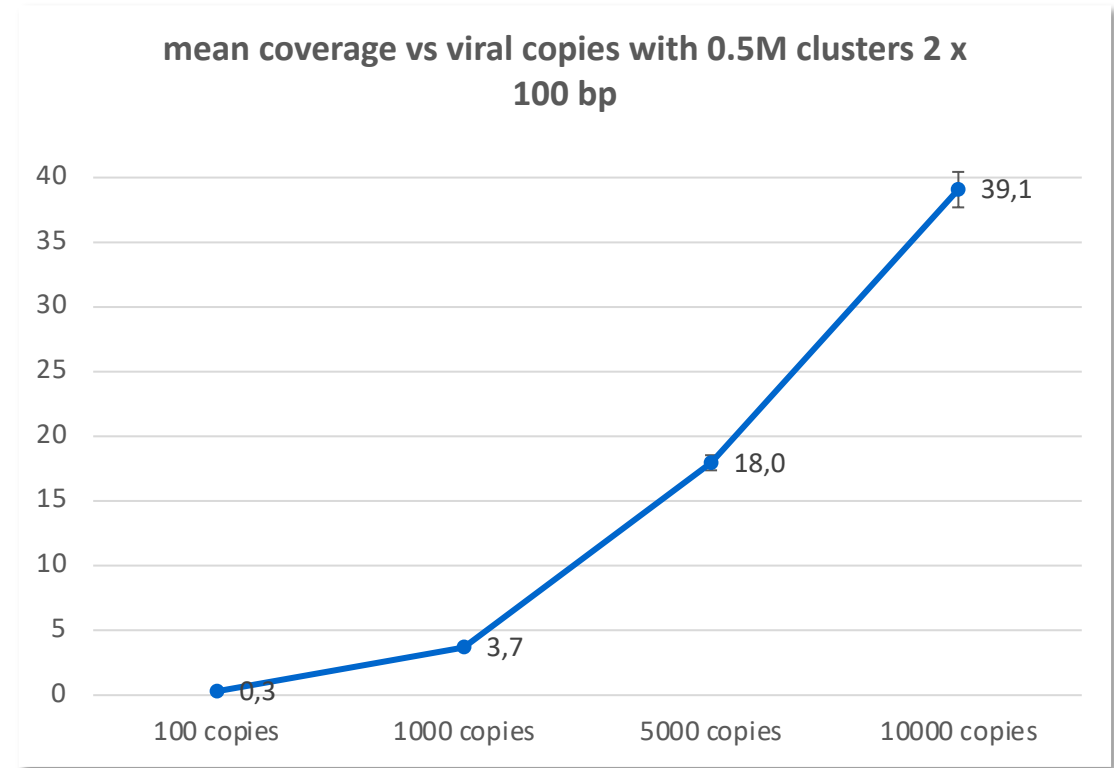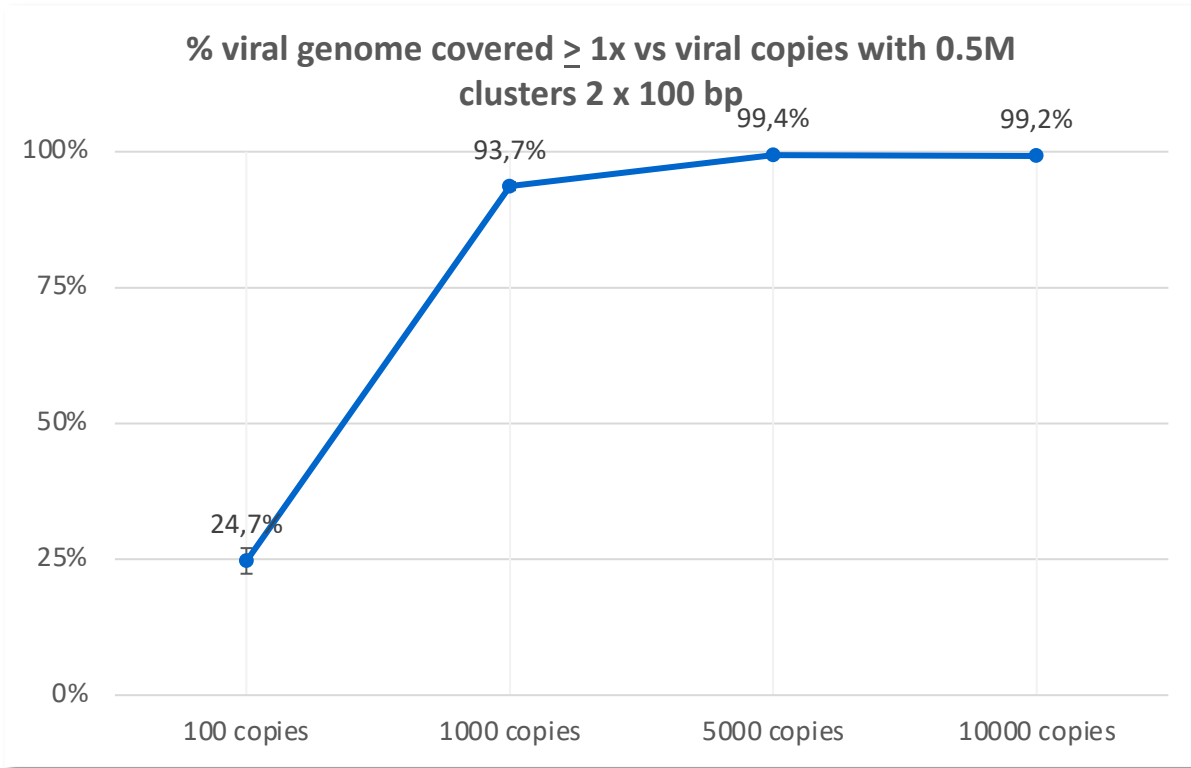**Robust performance** with **1-hour hybridization from just 1,000 copies:**

- **More than 90% of the viral genome is uniquely covered by only 250,000 clusters of 2 x 100 bp.**

Data on file. KAPA RNA HyperCap Workflow with 1 hour hybridization. Viral copies in 50 ng UHRR. Sequenced on a NovaSeq6000 System

UHRR: Universal Human Reference RNA

KAPA HyperCap SARS-CoV-2 Target Enrichment Panel
One hour hybridization provides broad coverage with low sequencing requirements

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% viral genome covered ≥ 1x vs viral copies with 0.5M clusters 2 x 100 bp

mean coverage vs viral copies with 0.5M clusters 2 x 100 bp

Robust performance with **1-hour hybridization with only 0.5M clusters of 2 x 100 bp:**

- **> 99%** of the viral genome **uniquely covered from 5,000 copies**

- **18x mean unique coverage** of the viral genome **from 5,000 copies**

Data on file. KAPA RNA HyperCap Workflow with 1 hour hybridization. Viral copies in 50 ng UHRR. Sequenced on a NovaSeq6000 System

KAPA HyperCap SARS-CoV-2 Target Enrichment Panel
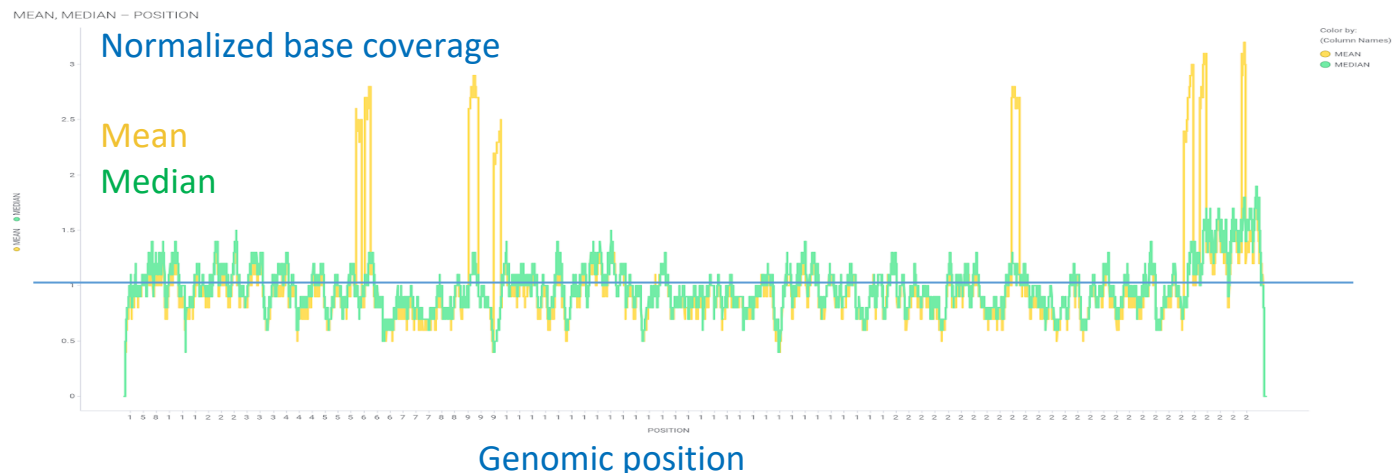*Great customer feedback for uniformity, specificity and genome coverage even at low virus loads*

Early Access Customer positive feedback:

- **Highly specific** (high on-target rates)

- **Highly sensitive** (reads from samples with Ct>32)

- **Broad Genome coverage**

- **Streamlined and user friendly** protocol

Data on file.

Early Access Customer positive feedback:

- **Sequencing Uniformity**

- **Minority species analysis**

- **Cleaner data -** No oligo-based amplification artifacts

- **Streamlined protocol**

Data on file.

Normalized base coverage

Mean

Median

Normalized coverage of 1 (optimum)

Genomic position

Workflow length comparisons

HOURS

32

24

16

8

0

11:50   10:50

KAPA HyperCap o/n Hyb   KAPA HyperCap 1h Hyb   ARTIC

With RiboErase   Without RiboErase

Data on file.

KAPA HyperCap SARS-CoV-2 with KAPA RNA HyperPrep:

- **Single tube** enrichment & automatable
- **Single day** from RNA to Sequencer
- **Long 120 bp probes** "resistant" to underlying variants
- **Single vendor offering** across the workflow
- **Superior uniformity** compared to amplicon based methods

ARTIC based workflows:*

- Legacy standard method
- Cannot call variants on either edge of the genome
- Split tubes (2) protocol
- New variants pose dropout risks, may need frequent updates
- Real-life TAT is 1.5 days to get to a sequencing library

* https://www.protocols.io/view/ncov-2019-sequencing-protocol-v3-locost-bh42j8ye

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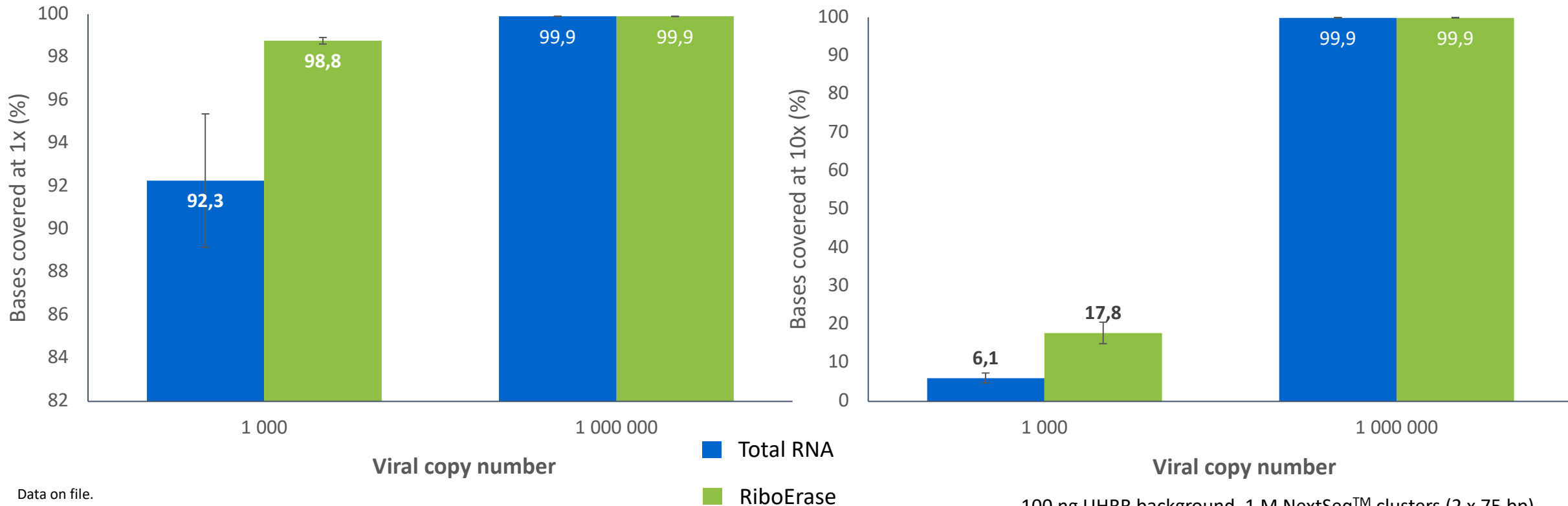
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KAPA HyperCap SARS-CoV-2 panel with ribosomal RNA depletion
RiboErase depletion improves coverage at low viral loads

Depletion of rRNA with KAPA **RiboErase provides better coverage at low viral concentrations**

- **Broadening coverage** of the viral genome **from 92.3% to 98.8%**

- **Increasing ~3x** the **percent** of bases **covered by ≥ 10x**

Data on file.

100 ng UHRR background, 1 M NextSeq™ clusters (2 x 75 bp)