



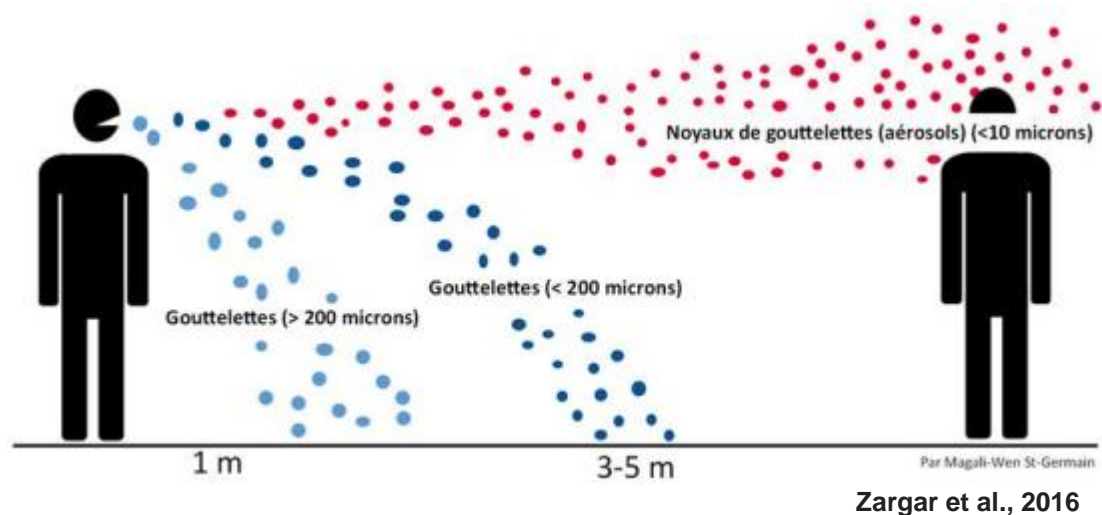
« Applications du NGS, du microbiome au metabarcoding »

*Le pouvoir de notre solution AmpliSeq au service
d'Ion Torrent*

Bouchera DOUAH & Emeric ROUX
Product Specialist NGS France

 The world leader in serving science

Precept of the composition of the air



Highlighting also **the importance of daily mask** use during this outbreak

Covid crisis has further highlighted the presence of **airborne microorganisms**

Le niveau de filtration des différents masques en France

Le port de certains masques en tissu est déconseillé et la distance de sécurité passe de un mètre à **deux mètres** entre chaque personne**

MASQUES ARTISANAUX	MASQUES DE CATÉGORIE 2	MASQUES DE CATÉGORIE 1	MASQUES CHIRURGICAUX	MASQUES FFP 2
À éviter après l'apparition de nouveaux variants	Recommandés	Recommandés	Recommandés	Les plus filtrants, en théorie réservés aux soignants
Aucun contrôle sur la performance	Selon les normes Afnor. En tissu réutilisable filtrent 70 % des particules de 3 microns*	Selon les normes Afnor. En tissu réutilisable 90 % des particules de 3 µm	95% à 98% des particules de 3 µm	Au moins 94 % des particules de 0,6 µm

Source : gouvernement français

*les aérosols (fines gouttelettes) du coronavirus mesurent 1 µm

**recommandations du Haut conseil de la santé publique

AFP

Bioaerosol and Surface Analysis in Subway Station



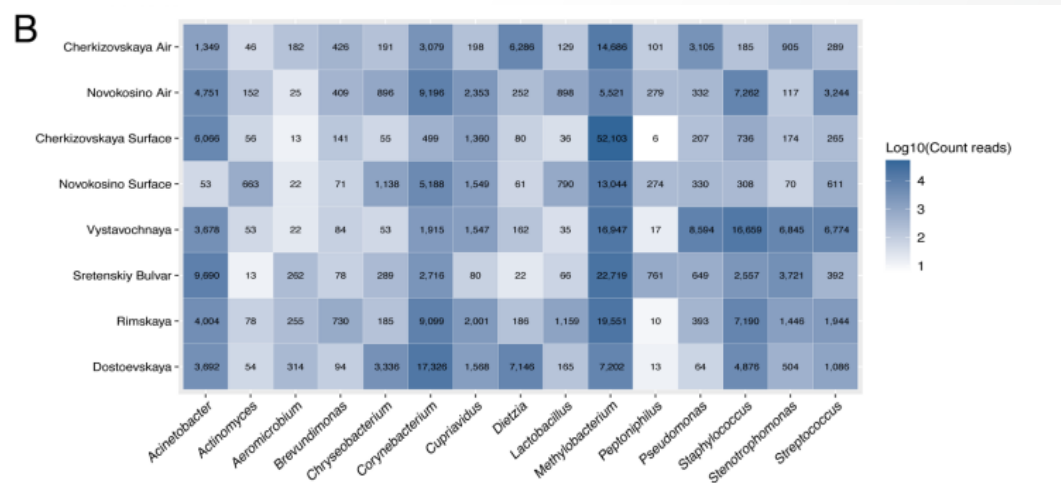
Article

Microbiological Characteristics of Some Stations of Moscow Subway

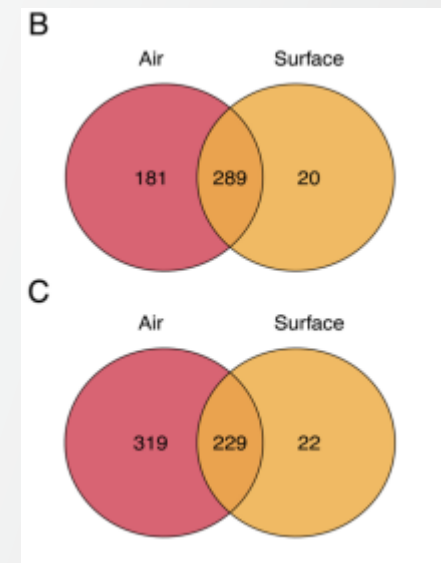
Andrei A. Pochtovyi ^{1,2,*}, Daria V. Vasina ¹, Bakhtiyar I. Verdiev ¹, Alexey M. Shchetinin ¹, Anton G. Yuzhakov ^{1,3}, Roman S. Ovchinnikov ^{1,4}, Artem P. Tkachuk ¹, Vladimir A. Gushchin ^{1,2,*} and Alexander L. Gintsburg ^{1,5}

¹ Federal State Budget Institution "National Research Centre for Epidemiology and Microbiology Named after Honorary Academician N.F. Gamaleya" of the Ministry of Health of the Russian Federation, 123098 Moscow, Russia; d.v.vasina@gmail.com (D.V.V.); yuryevpolskei@yandex.ru (B.I.V.); shchetinin.am@gmail.com (A.M.S.); anton_oskol@mail.ru (A.G.Y.); rsovchinnikov@mail.ru (R.S.O.); artem.p.tkachuk@gmail.com (A.P.T.); gintsburg@gamaleya.org (A.L.G.)

performed using the
Ion 16S™
Metagenomics Kit



In this study, they compared the composition of the **microbiome in the air compared** to the **microbiome on the surfaces** of the metro.



They focused on **two different stations** of the Moscow. The obtained data indicated the **formation of a core microbiome** at **each station**, depending on the characteristics of the station and the level of passenger traffic. It also identified the distribution of microorganisms associated with human microflora, **which are not susceptible to seasonal fluctuations**.



AerosolSense by ThermoFisher Scientific

The Foundation of Ion Torrent NGS Solutions



Gene **panels** or
exomes

As low as **1 ng**
DNA and RNA

Transcriptome
and gene
expression

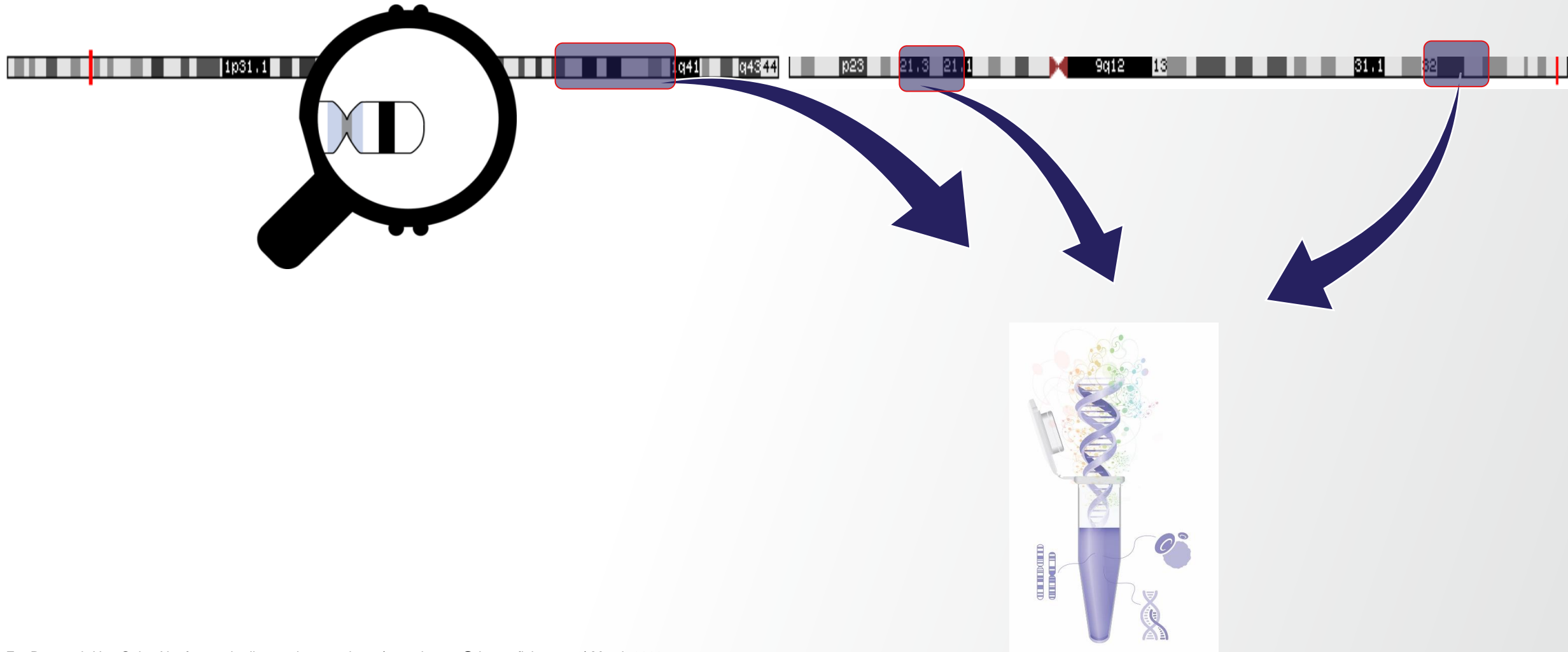
Multiple
applications

SNPs,
indels,
CNVs, fusions

Single-tube
workflow

What is Targeted Sequencing?

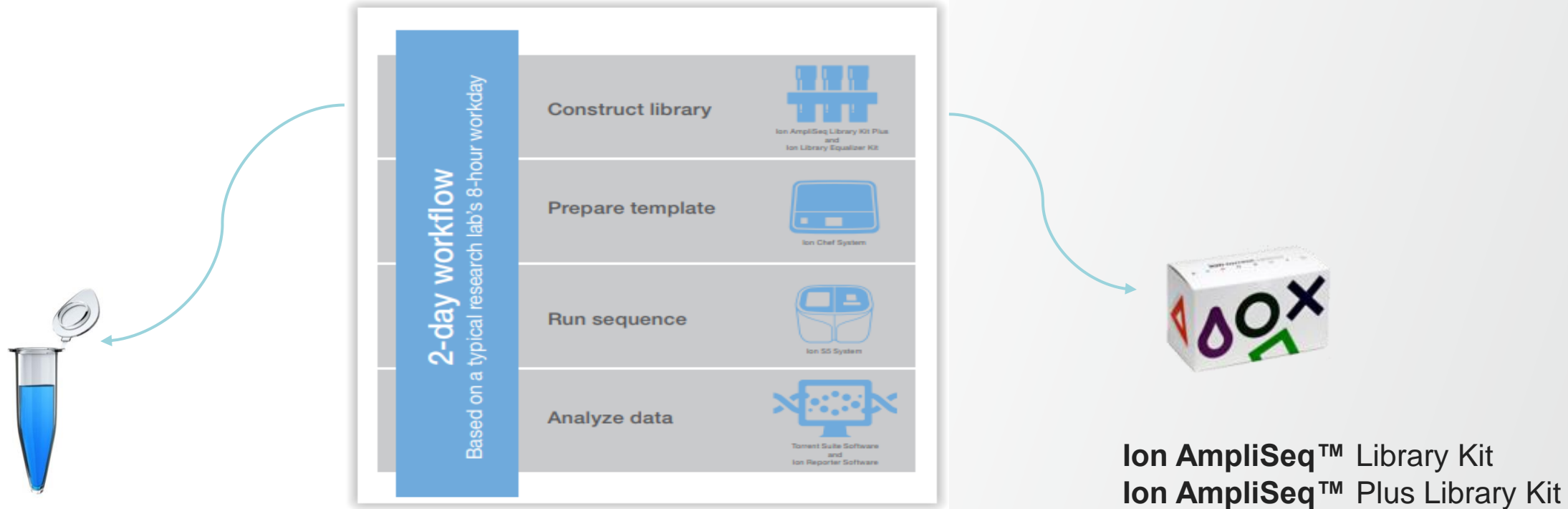
Targeted Sequencing isolates and **focuses** your sequencing on **specific genes or genomic regions** of interest rather than surveying the whole genome.



Ion AmpliSeq™ Technology: As Simple As PCR

ThermoFisher
SCIENTIFIC

Single-tube, ultra-high multiplex for targeted resequencing



12-24 576 primers pairs per tube

Ion AmpliSeq™ Custom Panels
OR
Ion AmpliSeq™ Ready-to-Use Panels

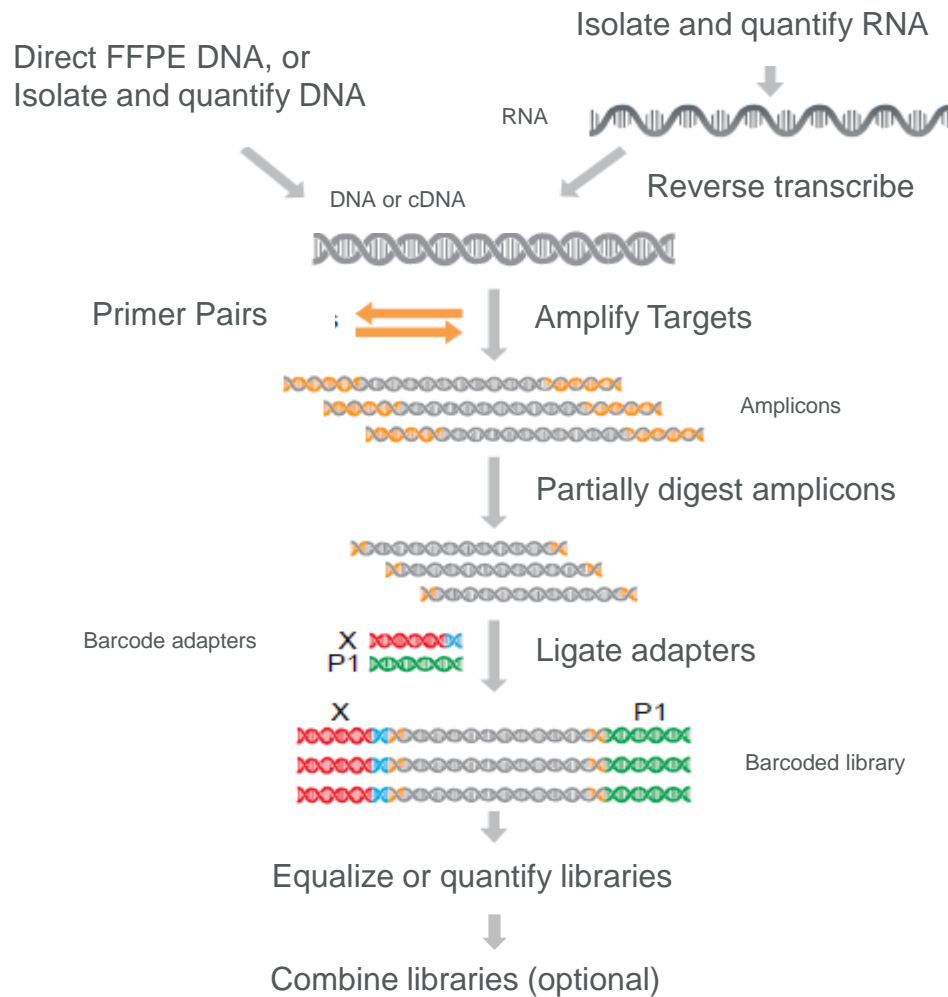
+



+

1-100 ng DNA per tube

Targeted Sequencing Ciblé- Ion AmpliSeq Technology



- Custom Designs with **any Genome**
- As little as **1-100 ng** of input DNA, enabling FFPE analysis
- Rapid results with 3-5-hour manual library construction
- Library construction **automation** on the Ion Chef instrument

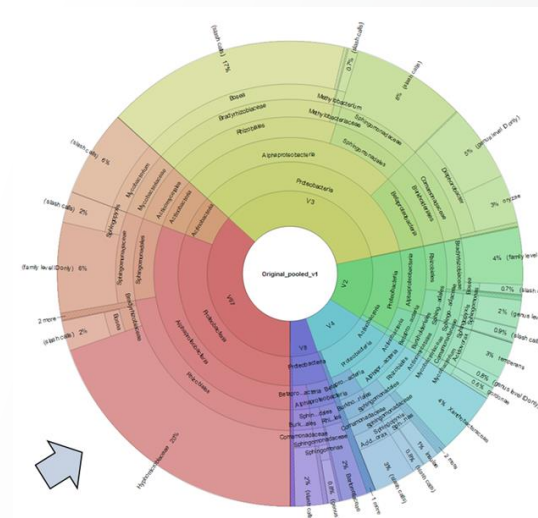


Ion 16S Metagenomics Kit

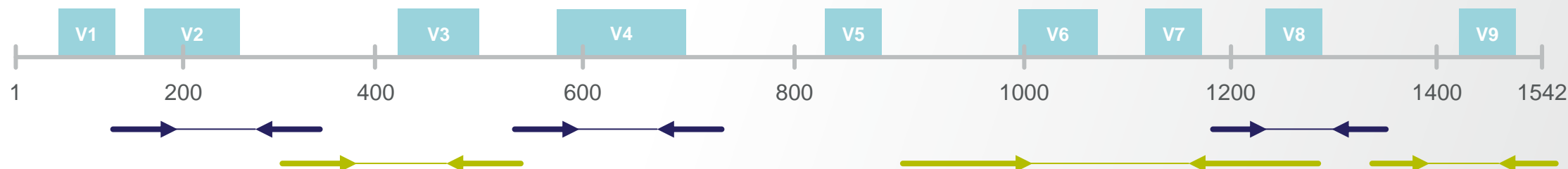
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Primer sets

- Set V2-4-8
- Set V3-6,7-9



16S gene



- **Stringent Bioinformatic Assay Design:** Analyses against the entire GreenGenes database as well as the 26K species of primary metagenomic interest.
- **Broad Coverage Primer Sets:** Addresses multiple groups of organisms using multi-amplicon approach (amplicon sizes 200–300bp range)
- **Maximum Read Length:** Uses 400bp sequencing chemistry

Study of SarsCov2 mutation in WasteWater



OMICS DATA SETS



Genome Sequencing of Wastewater Confirms the Arrival of the SARS-CoV-2 Omicron Variant at Frankfurt Airport but Limited Spread in the City of Frankfurt, Germany, in November 2021

Shelesh Agrawal,^a Laura Orschler,^a Simona Tavazzi,^b Robert Greither,^c Bernd Manfred Gawlik,^b Susanne Lackner^a

^aTechnical University of Darmstadt, Department of Civil and Environmental Engineering Sciences, Institute IWAR, Chair of Water and Environmental Biotechnology, Darmstadt, Germany

^bEuropean Commission, Joint Research Centre, Ispra, Varese, Italy

^cLife Technologies, Darmstadt, Germany

In November 2021, they found all characteristic mutations of Omicron in wastewater originating from Frankfurt Airport **before the first** confirmed clinical report from an arriving passenger on 26 November 2021.

the library was prepared using the **Ion AmpliSeq SARS-CoV-2 research panel** on Ion S5 Sequencer

They show that **the surveillance of wastewater** from transportation hubs, such as airports, using sequencing can support **tracking of the import of SARS-CoV-2 variants**.

TABLE 1 Summary of the sequencing data for the samples

Sample location and collection date (day-mo-yr)	Total no. of reads	No. of mapped reads	Avg target base coverage depth (X)	Avg read identity vs target (%) ^a	GC content (%)	BioSample accession no.	SRA accession no.
Airport							
4-11-2021	1,169,001	345,149	319.5	95.61	62.9	SAMN24156791	SRR17258655
23-11-2021	8,299,055	3,245,669	9,693	98.36	55.8	SAMN24156792	SRR17258654
City							
4-11-2021	1,972,935	620,663	753.4	98.72	62.2	SAMN24156793	SRR17258653
23-11-2021	9,908,030	3,825,450	11,834	98.02	56.8	SAMN24156794	SRR17258652

^a The target sequence was the SARS-CoV-2 reference genome (Wuhan-Hu-1 [GenBank accession numbers [NC_045512](#) and [MN908947.3](#)]).

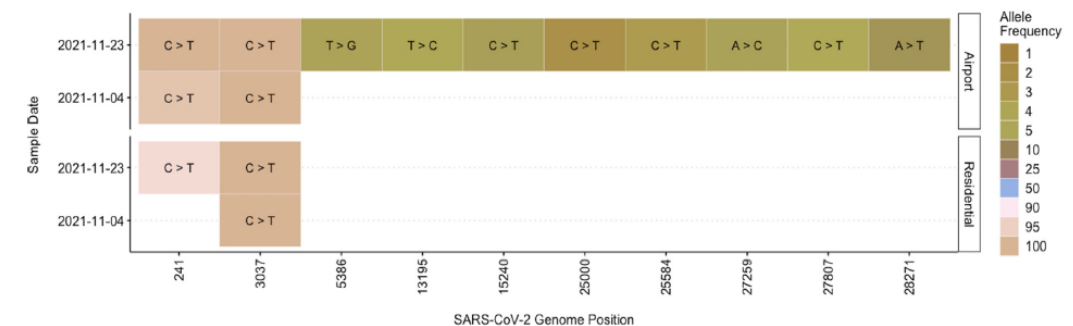


FIG 1 (Top) Occurrence (i.e., the numbers of reads corresponding to each mutation) of characteristic S, E, M, and N protein mutations and ORF1ab mutations of the Omicron variant. (Bottom) Heatmap showing the allele frequency and the alternate nucleotide of the characteristic synonymous mutations of the Omicron variant. Airport, wastewater sample with a significant fraction from Frankfurt Airport; Residential, influent wastewater sample collected from the WWTP of Frankfurt City.

Ion AmpliSeq SARS-CoV-2 Insight Research Panel

Higher sensitivity for earlier identification of variants in **low titer samples (Ct>28)** to inform public health guidance and intervention strategies

- **237 amplicons specific** to SARS-CoV-2 with **addition of variant-tolerant primers for emerging variants**
- **5 newly-designed human expression controls** for sample **confirmation** and **quality** assessment
- Amplicon length range: **125-275 bp**
- **>99% coverage** of SARS-CoV-2 genome (~30kb)
- **All** potential serotypes **covered**



- **Increase productivity** by running **more samples** per chip with the insight panel

Chip type	Samples*/chip
510	2-3
520	4-6
530	16-32
540	80-160
550	130-200

* We recommend that you allow 500,000–1,000,000 reads per sample for the Ion AmpliSeq SARS-CoV-2 Insight Research Panel dependent on sample quality

Ampliseq Designer tool: custom panel design



Home

2

My Designs



Notifications



Chip Calculator

13

Genomes

70

Fixed Panels




Help

 Community Ion AmpliSeq SARS-CoV-2 Insight Research Assay now available to support COVID-19 research


[▶ Getting Started with AmpliSeq](#)

In stock

Ships in 4 – 10 working days; fewer reactions 

[Check gene availability](#)

Made-to-order

Ships in 2 – 3 weeks; more reactions 

Product and description

Oncomine™ tumor specific panels

Select from catalog of solid tumor panels from > 301 pretested genes and fusion drivers

[Browse tumor types](#)

[Or Add gene list](#)

Ion AmpliSeq™ On-Demand panels

Select from catalog of > 5,000 pre-tested genes

[Create On-Demand panel](#)

[Or browse disease research areas](#)

Ion AmpliSeq™ Custom panels

Customizable for any genome

[Create Custom panel](#)

Ion AmpliSeq™ HD Custom panels

Customizable with ultrahigh allelic sensitivity for applications including liquid biopsy

[Create Custom HD panel](#)

Genome

Human

Human

Any

Human

Nucleic acid

DNA / RNA

DNA only

DNA / RNA

DNA / RNA

Sample type

FFPE

Genomic DNA

Any

cfDNA / FFPE

Target type

Genes

Genes

Genes, regions, hotspots, amplicons, expression, fusions

Genes, regions, hotspots, amplicons, fusions


Limit of detection 

5%


5 – 20%

5%

0.1%

Pre-curated 



Pre-tested 



AmpliSeq™ technology: Chose target on any Genome

2 Application type *

DNA designs may include a mix of gene and region targets, including small hotspot regions.



DNA
Gene designs
(multi-pool)



DNA
Hotspot designs
(single-pool)



RNA
Gene Expression designs
(single-pool)



RNA
Gene Fusion designs
(multi-pool)

3 Select genome to use *



Human
(hg19)



Human
(hg38)



Mouse
(mm10)



Cow
(bosTau7)



Chicken
(galGal4)



Dog
(canfam3)



Chinese hamster
(crigr1)



Pig
(susScr3)



Sheep
(oviAri3)



Custom
Reference



Maize
(AGPv3)



Rice
(IRGSP-1.0)



Soybean
(Glyma1.1)



Tomato
(SL2.40)

Metabarcoding AmpliSeq Custom



Trzebny et al. *Parasites & Vectors* (2022) 15:26
https://doi.org/10.1186/s13071-022-05150-9

Parasites & Vectors

SHORT REPORT Open Access

Metabarcoding reveals low prevalence of microsporidian infections in castor bean tick (*Ixodes ricinus*)

Artur Trzebny^{1*}, Justyna Liberska¹, Anna Slodkiewicz-Kowalska² and Mirosława Dabert¹



in this study, they applied a recently developed DNA metabarcoding method for efficient microsporidian DNA identification to assess the role of ticks as potential vectors of microsporidian species causing diseases in humans.

They did a **DNA barcode** covering 322 bp of the COI Gene for sequencing using the **Ion Torrent system**. We totally can imagine design this panel with the help of our **AmpliSeq Designer** and our Bio – informatic Team.

Table 2 DNA sequences used in phylogenetic analysis

Microsporidium species	Host species	Host class	GenBank no	References
<i>Endoreticulatus bombycis</i>	<i>Bombyx mori</i>	Insecta	AY009115	[41]
<i>Endoreticulatus itili</i>	<i>Listronotus bonariensis</i>	Insecta	KJ755828	[42]
<i>Endoreticulatus poecilmonae</i>	<i>Poecilmon thoracicus</i>	Insecta	KJ755827	[43]
<i>Endoreticulatus schubergi</i>	<i>Lymantria dispar</i> , <i>Hyphantria cunea</i> , <i>Choristoneura fumiferana</i>	Insecta	L39109	[44, 45]
<i>Endoreticulatus</i> sp. CHW-2004 Bulgaria	<i>Lymantria dispar</i>	Insecta	AY502945	[46]
<i>Endoreticulatus</i> sp. CHW-2004 Taiwan	<i>Ocinara lida</i>	Insecta	AY502944	[46]
<i>Endoreticulatus</i> sp. CHW-2008 Austria	<i>Thaumetopoea processionea</i>	Insecta	EU260046	[47]
<i>Endoreticulatus</i> sp. isolate JMM 2007	<i>Loxostege sticticalis</i>	Insecta	M8929470	[48]
<i>Endoreticulatus</i> sp. Melnik	<i>Euproctis chrysanthoea</i>	Insecta	KU900486	[43]
<i>Endoreticulatus</i> sp. PL01	<i>Ixodes ricinus</i>	Arachnida	MT911425	This study
<i>Endoreticulatus</i> sp. Shengzhou	<i>Bombyx mori</i>	Insecta	JN792450	[49]
<i>Endoreticulatus</i> sp. Sofia	<i>Euproctis chrysanthoea</i>	Insecta	KU900485	[43]
<i>Endoreticulatus</i> sp. Zhenjiang	<i>Bombyx mori</i>	Insecta	FJ772431	[50]
<i>Endoreticulatus</i> sp. isolate EC	<i>Eilema complana</i>	Insecta	KY615713	[51]
<i>Microsporidium</i> sp. clone Chula Myositis 1	<i>Homo sapiens</i>	Mammalia	JN619406	[52]
<i>Cystosporogones legeri</i>	<i>Lobesia botrana</i>	Insecta	AY233131	[53]
<i>Cystosporogones operophterae</i>	<i>Operophtera brumata</i>	Insecta	AJ302320	[54]
<i>Cystosporogones</i> sp. GKX-2009	<i>Agrius anxius</i>	Insecta	GQ379703	[55]
<i>Cystosporogones</i> sp. KIC-2	<i>Operophtera brumata</i>	Insecta	GU299511	[56]
<i>Chytridiopsis typographi</i>	<i>Ips typographus</i>	Branchiopoda	MH728789	[57]

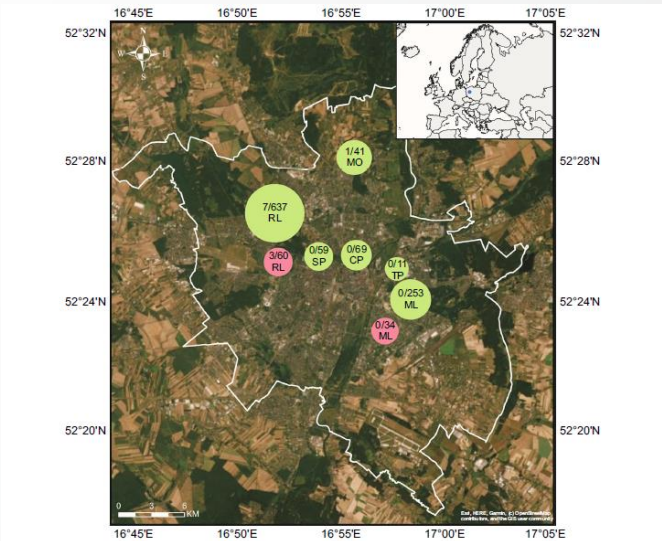


Fig. 1 Sampling places of unfed host-seeking ticks (red circles) and feeding (green circles) ticks in the city of Poznań (blue dot on the Europe map). The size of circle corresponds to the number of analyzed ticks. Numeric value inside the circles: number of microsporidian-positive ticks/number of analyzed ticks. For sampling place characteristics, see Additional file 1: Table S1 and Additional file 2: Table S2

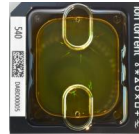
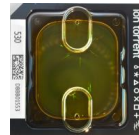
Our data suggest that *I. ricinus* is **not involved** in transmission of zoonotic microsporidian species. Moreover, we noticed a **very low prevalence** of these parasites in the tested ticks

Equipments



Combined with Novel Innovation in Library Preparation Ion Torrent Platform

ThermoFisher
SCIENTIFIC



On Demand: Flexibility



**Ion AmpliSeq™
technology**

Foundational technology
for targeted sequencing

Made to order: Sensitivity



**Ion AmpliSeq™ HD
technology***

Novel core technology for next-
generation targeted sequencing

The content provided herein may relate to products that have not been officially released and are subject to change without notice.

Flexible Portfolio Configurable to Your Needs

ThermoFisher
SCIENTIFIC

Ion GeneStudio™ S5



Fast.

Ion GeneStudio™ S5 Plus



Flexible.

Ion GeneStudio™ S5 Prime



Powerful.



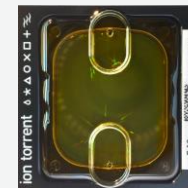
Ion 510™ Chip
2–3 M reads
Up to **400 bp**



Ion 520™ Chip
3–6 M reads
Up to **600 bp**



Ion 530™ Chip
15–20 M reads
Up to **600 bp**

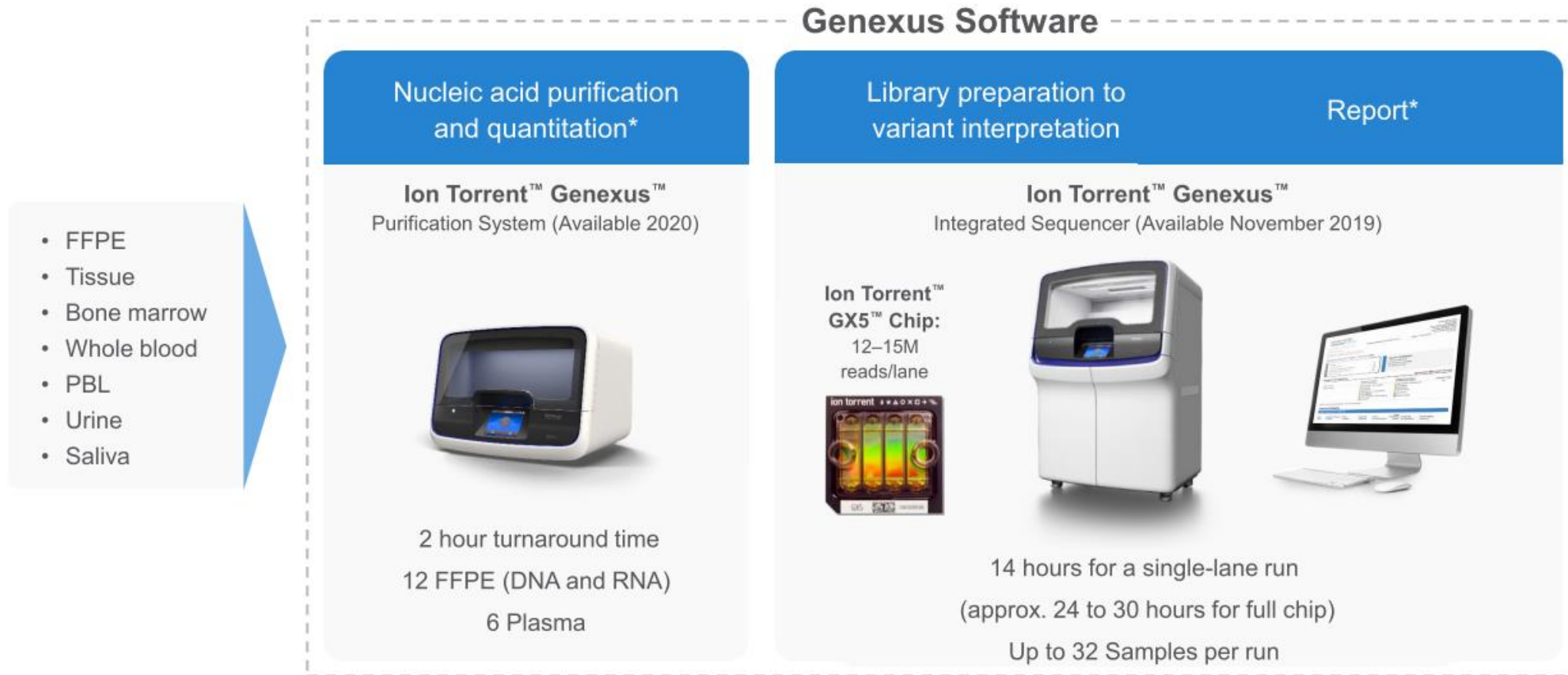


Ion 540™ Chip
60–80 M reads
Up to **200 bp**



Ion 550™ Chip
100–130 M reads
Up to **200 bp**

Genexus System End to End Workflow



*Specimen-to-report workflow will be available after the Ion Torrent™ Genexus™ Purification System and integrated reporting capabilities are added in 2020.

The content provided herein may relate to products that have not been fully validated by Thermo Fisher Scientific and is subject to change without notice.

We are here for you !!!



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Thank you

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Strain-Typing and Antibiotic Resistance Profiling

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Strain-Typing and Antibiotic Resistance Profiling From Research Samples Using Highly Multiplexed Targeted Library Construction with High Throughput Semi-Conductor Based Sequencing.

John Bishop, Loni Pickle, Miro Dudas, Guoying Liu, Melanie Baumann, and Astrid Ferlinz

Thermo Fisher Scientific, Carlsbad, CA, USA

iontorrent
by Thermo Fisher Scientific

- They want to identify **Mycobacterium** from culture and sputum samples to **genotype specific loci** and antibiotic Resistance in **M. Tuberculosis**



Sample ID Report

IonXpress_001_R_2012_03_13_10_55_20_user_C02-426-R141050_E209_2-kr

F-YGACRCRW

Allele Coverage for Sample Identification SNPs

Chrom	Position	Target ID	TaqMan Assay ID	Call	Ref	AF	Cov	A Reads	C Reads	G Reads	T Reads	Deletions	+Cov	-Cov
chr3	193207380	SNP#1	C_25748280_10	Y	T	51.67%	3833	10	1833	3	1960	27	2036	1770
chr4	169663615	SNP#2	C_11245682_10	G	T	97.94%	5828	3	0	5694	11	120	2134	3574
chr5	178690725	SNP#3	C_3153696_10	A	G	97.37%	12236	11788	319	56	44	29	5784	6423
chr7	137029838	SNP#4	C_3004178_10	C	T	98.16%	5219	2	5078	22	22	95	2463	2661
chr10	17193346	SNP#5	C_2822618	R	A	50.72%	5771	2831	1	2914	7	18	2737	3016
chr12	6945914	SNP#6	C_2184724_1	C	C	99.68%	12669	4	12613	1	11	40	6988	5641
chr16	9749879	SNP#7	C_1371205_10	R	G	50.42%	9580	4735	14	4815	13	3	4707	4870
chr22	33558508	SNP#8	C_11887110_1	W	T	51.4%	5747	2762	8	11	2921	45	2474	3228

“We demonstrated this process with a panel specific [Ion AmpliSeq™ TB Research Panel](#) to full gene coverage of eight known antibiotic resistance loci in M. tuberculosis, however this process is scalable and extensible to simultaneously assess thousands of loci across multiple organisms per sample”

