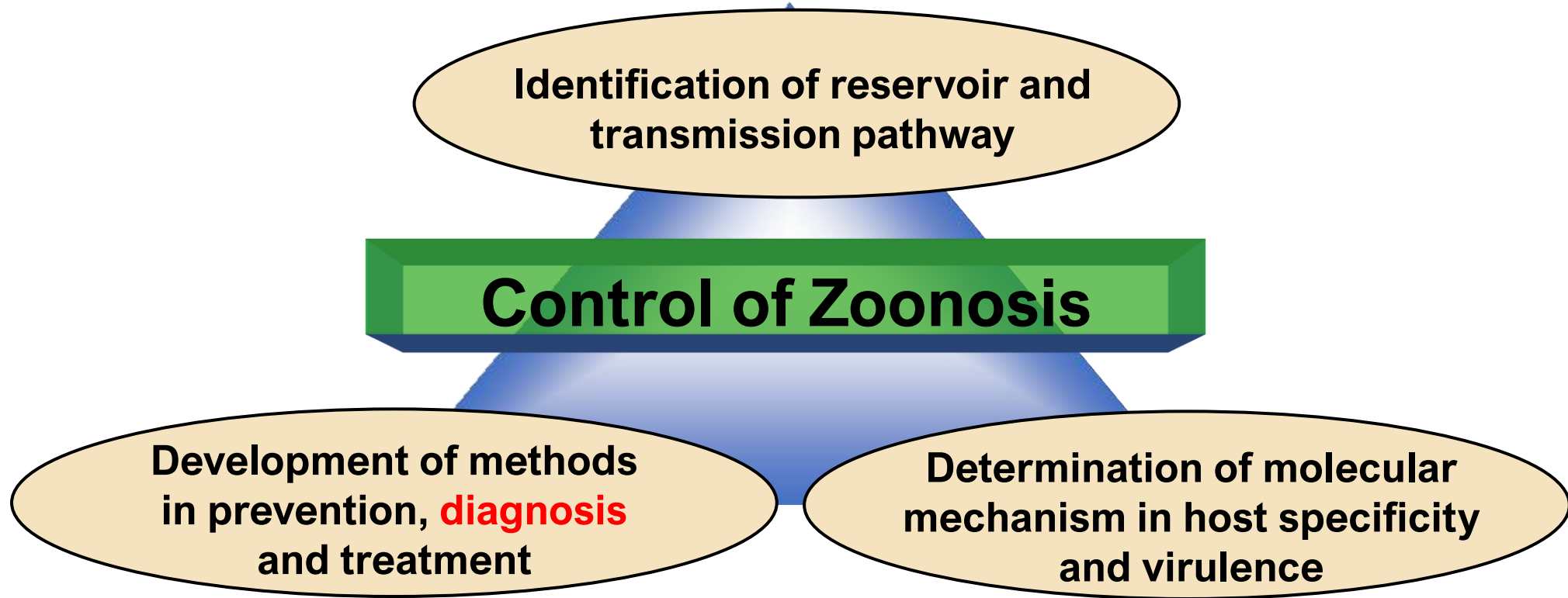


mNGSを用いた感染症診断法の開発 ～ "disease X" のコントロールへ向けて ～

**人獣共通感染症国際共同研究所
山岸潤也**

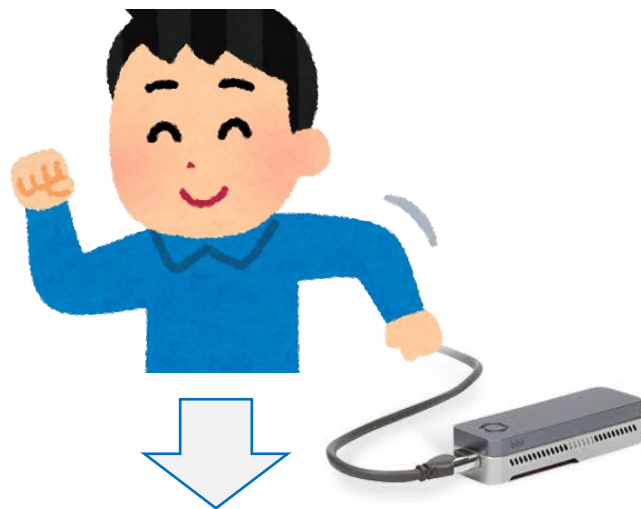
Introduction

mission of the IIZC, since 2005

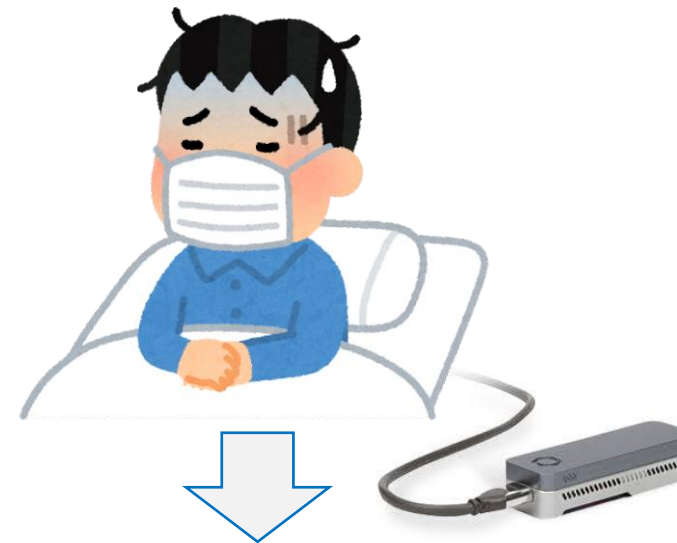


Concept of diagnosis by metagenomic NGS

Read everything!



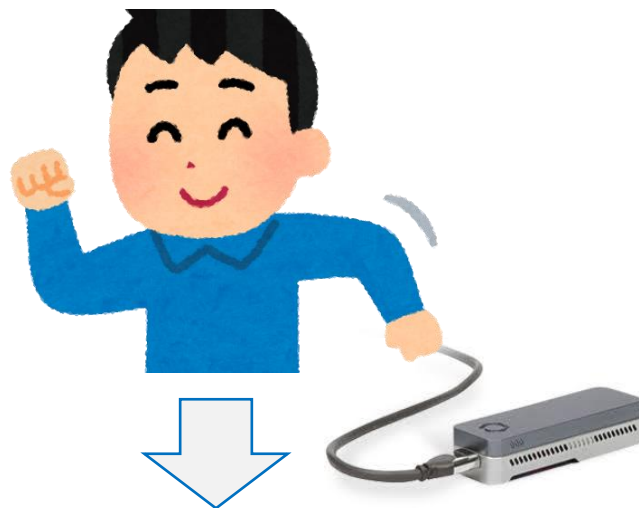
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GACGATGCAAATGACGTCACGACGCACGGCGTTAA  
CGGTCGCCGCGGAGGCGTGGCCTAGCCCGGAAGCA  
AGTCGCGGGGCTGATGACGTATAAAAAAGCGGACT  
TTAGACCCGGAAACGGCCGATTTTCCCGCGGCCAC  
GCCCGGATATGAGGTAATTCTGGGCGGATGCAAGT
```



```
GAAATTAGGTCATTTTGGCGCGAAACTGAATGAG  
GAAGTGAAAAGCGAAAAATACCGGTCCCTCCCAGG  
GCGGAATATTTACCGAGGGCCGAGAGACTTTGACC  
GATTATCGTGGGGGTTTCGATTGCGGTGTTTTTTC  
GCGAATTTCCGCGTCCGTGTCAAAGTCCGGTGTTT  
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CAGCGAGTAGAGATTTCTCTGAGCTCCGCTCCCAG
```

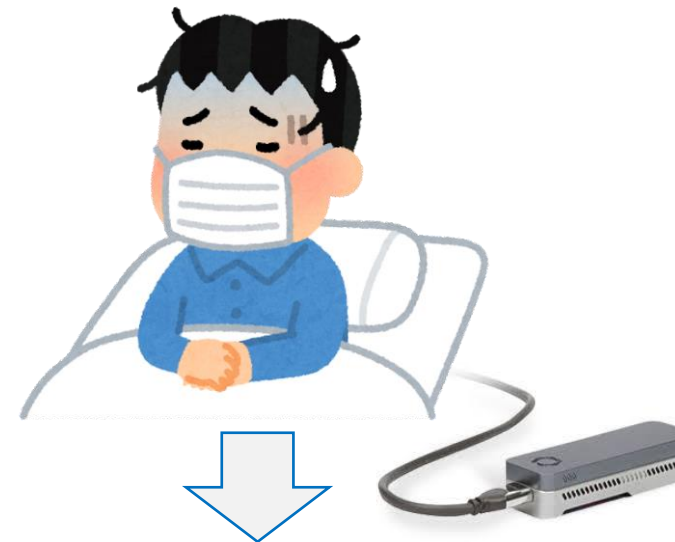
Concept of diagnosis by metagenomic NGS

Most of them should be originated from human



CATCATCAATAATATACCCACAAAGTAAACAAAA
GTTAATATGCAAATGAGTTTTTGAATTTTAACGGT
TTCGGGGCGGAGCCAACGCTGATTGGACGAGAGAA
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CGGTCGCCGCGGAGGCGTGGCCTAGCCCGGAAGCA
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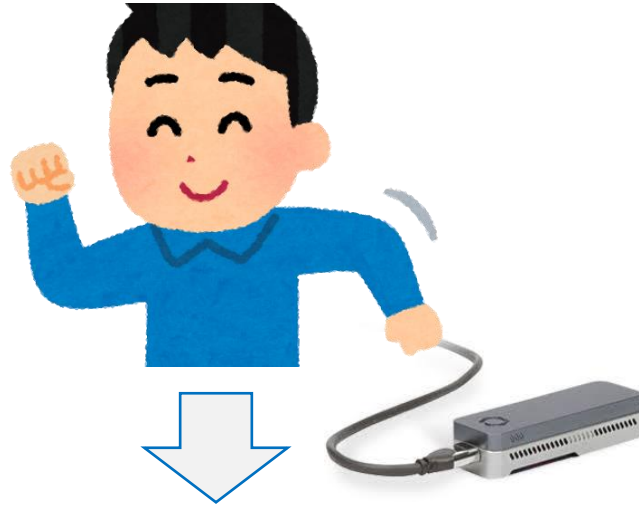
Human sequence



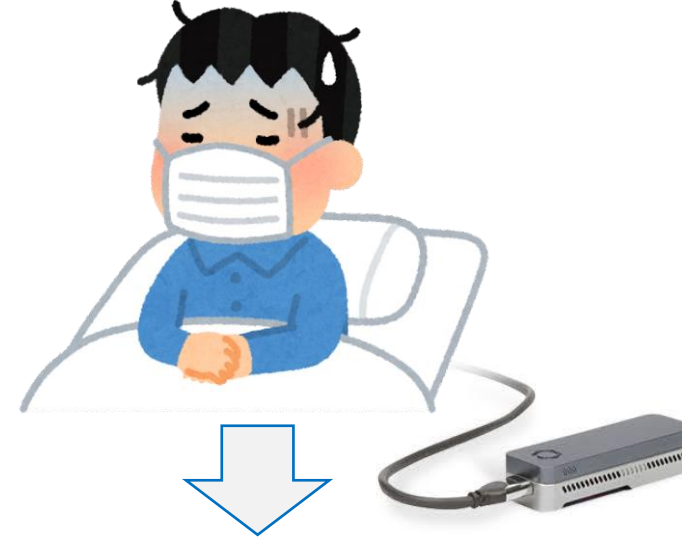
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GCGAATTTCCGCGTCCGTGTCAAAGTCCGGTGTTT
ATGTCACAGATCAGCTGATCCGCAGGGTATTTAAA
CCAGTCGAGTCCGTCAAGAGGCCACTCTTGAGTGC
CAGCGAGTAGAGATTTCTCTGAGCTCCGCTCCCAG

Concept of diagnosis by metagenomic NGS

Most of them should be originated from human



CATCATCAATAATATACCCACAAAGTAAACAAAA
GTTAATATGCAAATGAGTTTTTGAATTTTAACGGT
TTCGGGGCGGAGCCAACGCTGATTGGACGAGAGAA
GACGATGCAAATGACGTCACGACGCACGGCGTTAA
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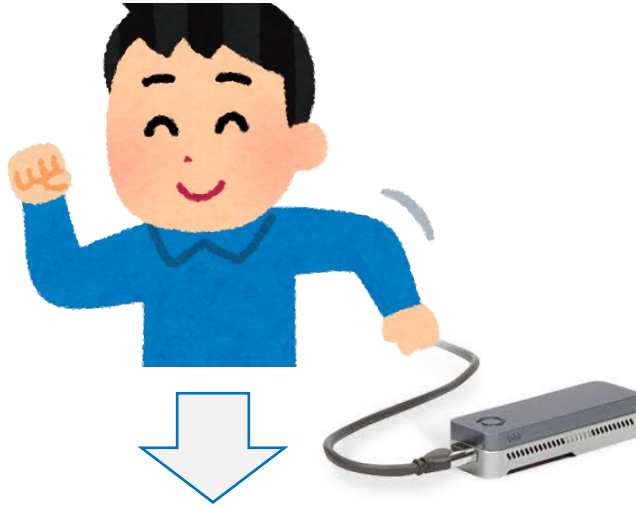


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CAGCGAGTAGAGATTTCTCTGAGCTCCGCTCCCAG

Human sequence

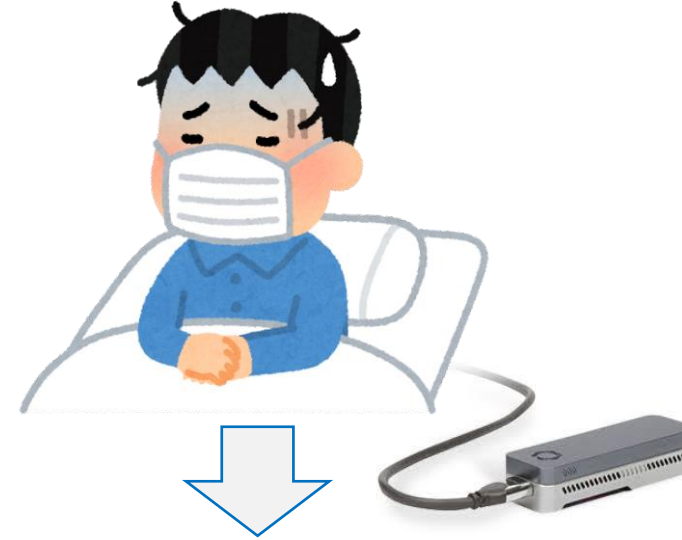
Concept of diagnosis by metagenomic NGS

Pathogen detection by sequence identification



CATCATCAATAATATACCCACAAAGTAAACAAAA
GTTAATATGCAAATGAGTTTTTGAATTTTAACGGT
TTCGGGGCGGAGCCAACGCTGATTGGACGAGAGAA
GACGATGCAAATGACGTCACGACGCACGGCGTTAA
CGGTCGCCGCGGAGGCGTGGCCTAGCCCGGAAGCA
AGTCGCGGGGCTGATGACGTATAAAAAAGCGGACT
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GCCCGGATATGAGGTAATTCTGGGCGGATGCAAGT

Human sequence



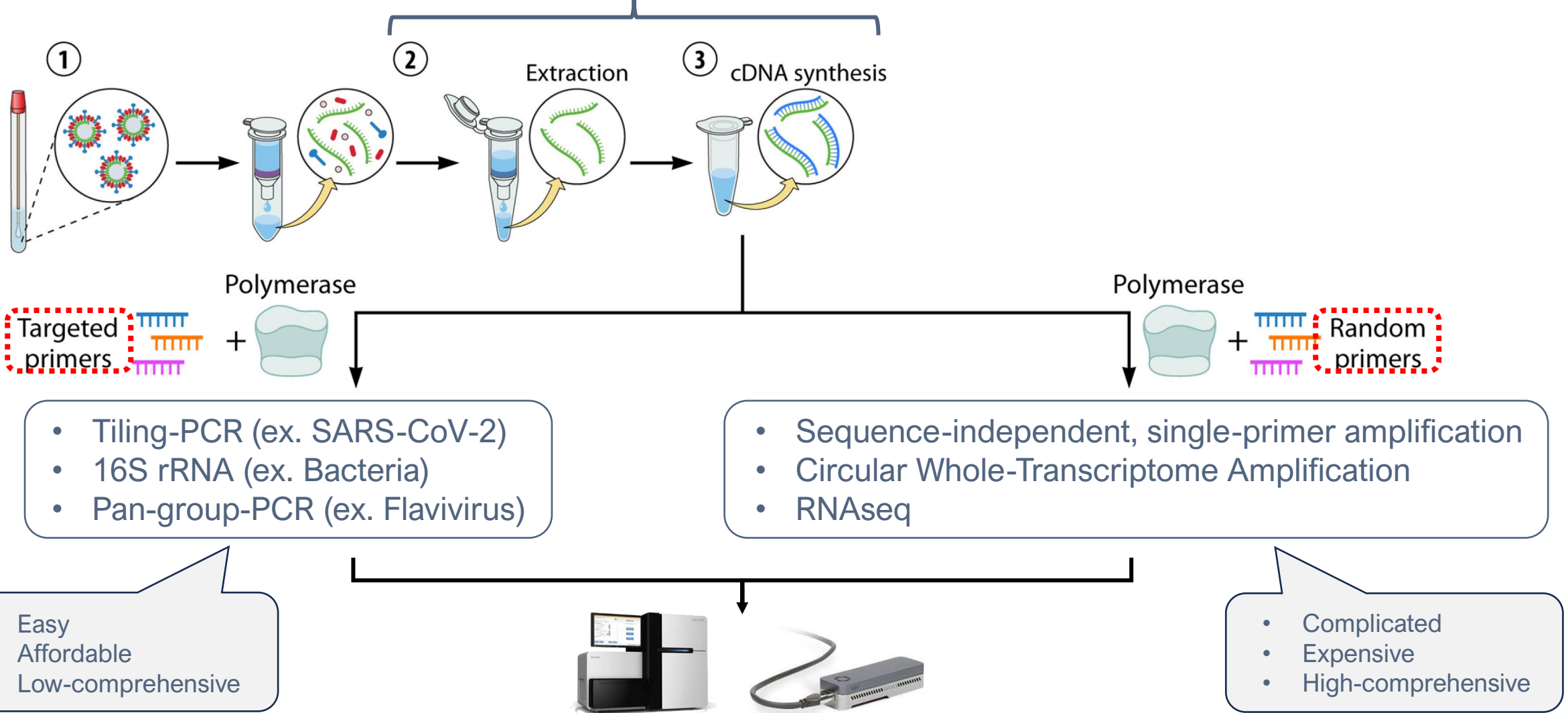
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GCGAATTTCCGCGTCCGTGTCAAAGTCCGGTGTTT
ATGTCACAGATCAGCTGATCCGCAGGGTATTTAAA
CCAGTCGAGTCCGTCAAGAGGCCACTCTTGAGTGC
CAGCGAGTAGAGATTTCTCTGAGCTCCGCTCCCAG

**DENV1
sequence!**

Strategy of pathogen detection using mNGS

Targeted or Untargeted

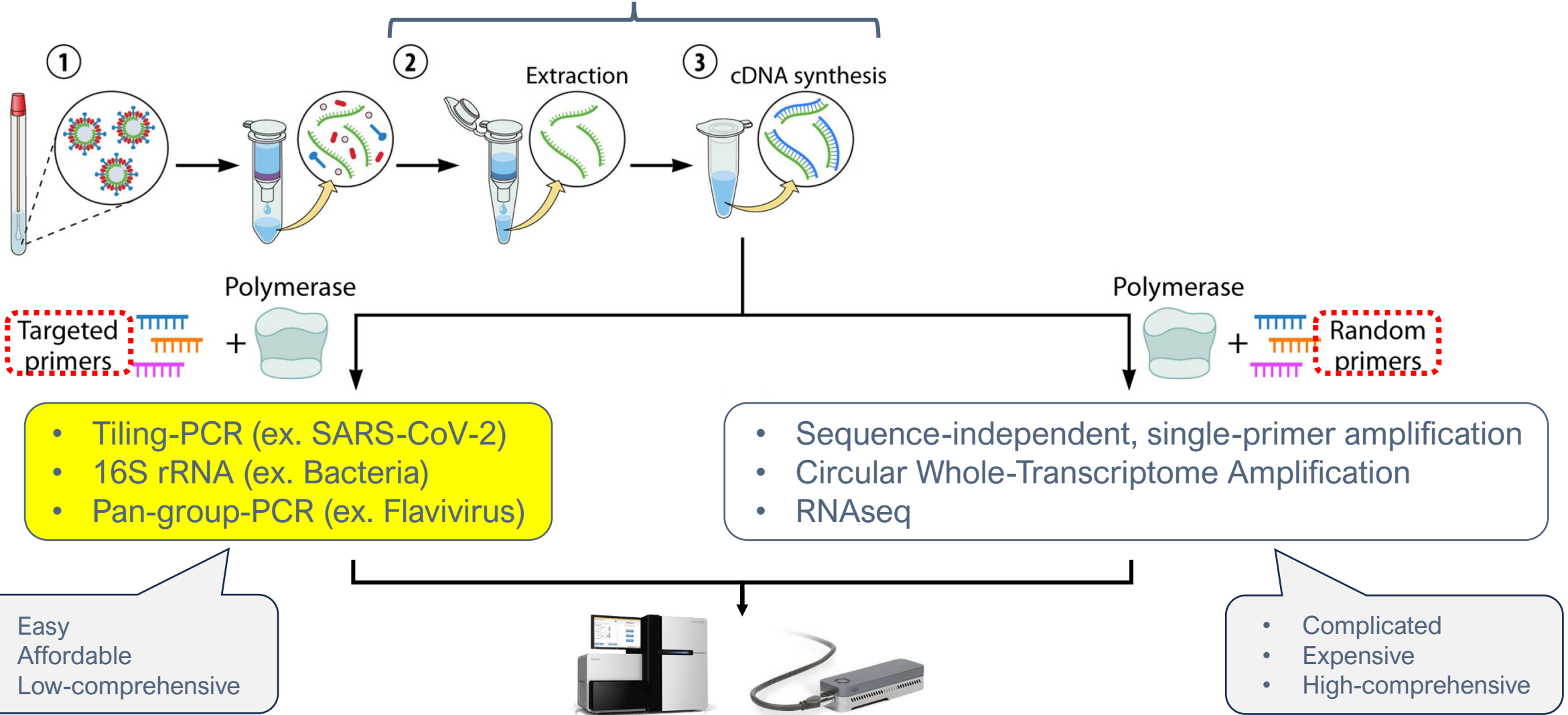
RNA is preferable



Strategy of pathogen detection using mNGS

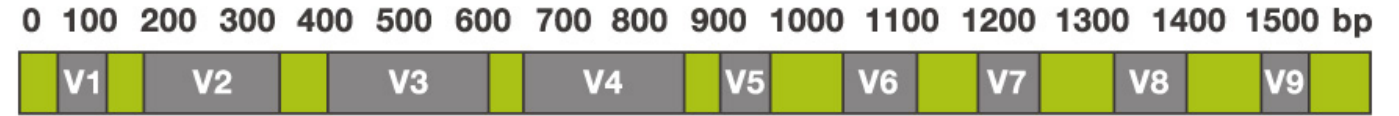
Targeted or Untargeted

RNA is preferable



Pan-group-mNGS system

The concept

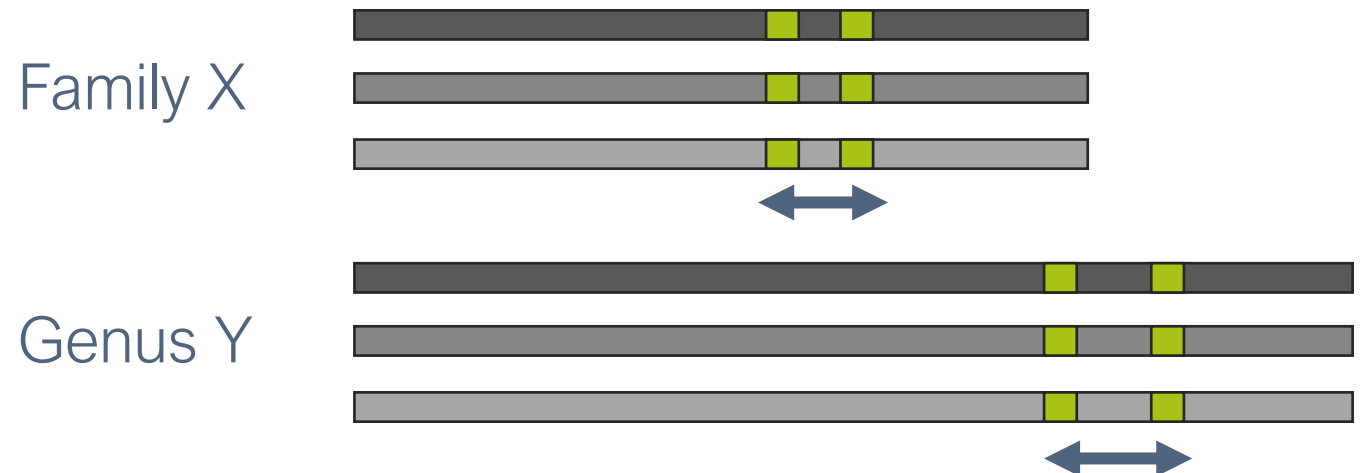


Conserved regions, Variable regions

- 16S rRNA metagenomics



- Viral metagenomics?



pan-flavi-nanopore

outline of the method

scientific reports



A targeted approach with nanopore sequencing for the universal detection and identification of flaviviruses

Patrick Reteng¹, Linh Nguyen Thuy², Tam Tran Thi Minh², Maria Angélica Monteiro de Mello Mares-Guia³, Maria Celeste Torres³, Ana Maria Bispo de Filippis³, Yasuko Orba⁴, Shintaro Kobayashi⁵, Kyoko Hayashida^{1,6}, Hirofumi Sawa^{4,6,7}, William W. Hall^{6,7,8,9}, Lan Anh Nguyen Thi² & Junya Yamagishi^{1,6}✉

Scientific Reports | (2021) 11:19031

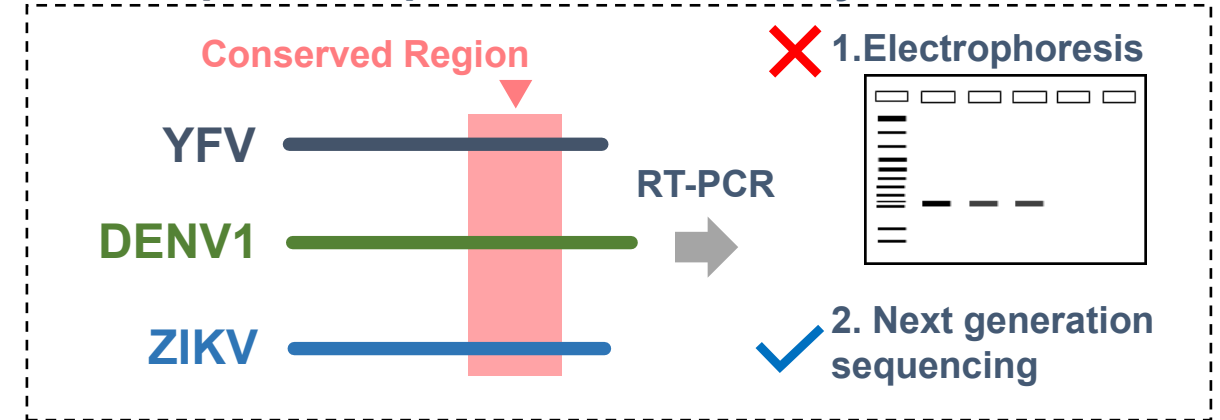
Protocol outline

1. Get amplicon by pan-flavi PCR with index-primers
2. Pool the amplicons.
3. Read with NGS at once to identify flavivirus spp.

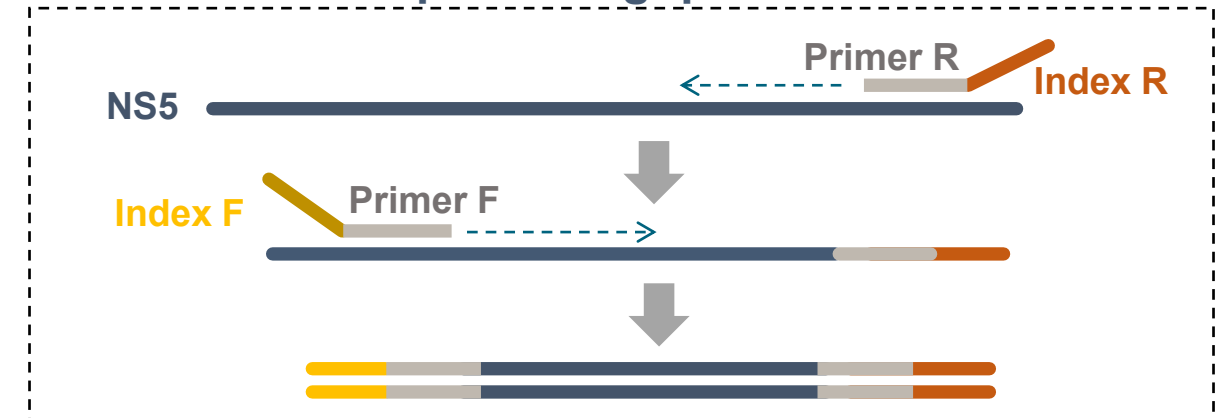
Advantage of the methods

1. Identify any flavivirus at once (comprehensiveness)
2. Low cost by multiplex sequencing enabled by index-PCR

Broad spectrum pan-flavivirus assay

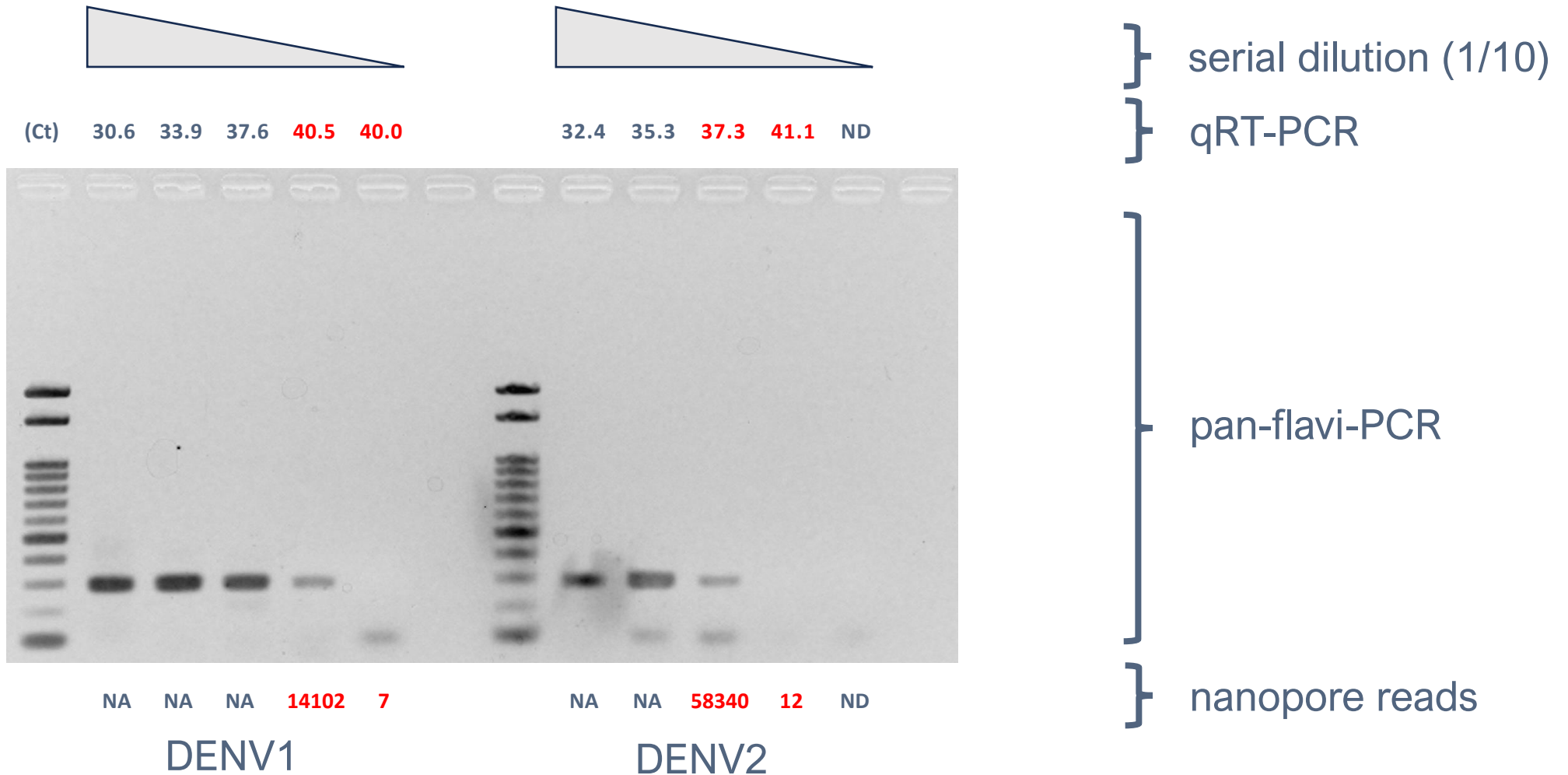


Increase the sample throughput with index



pan-flavi-nanopore

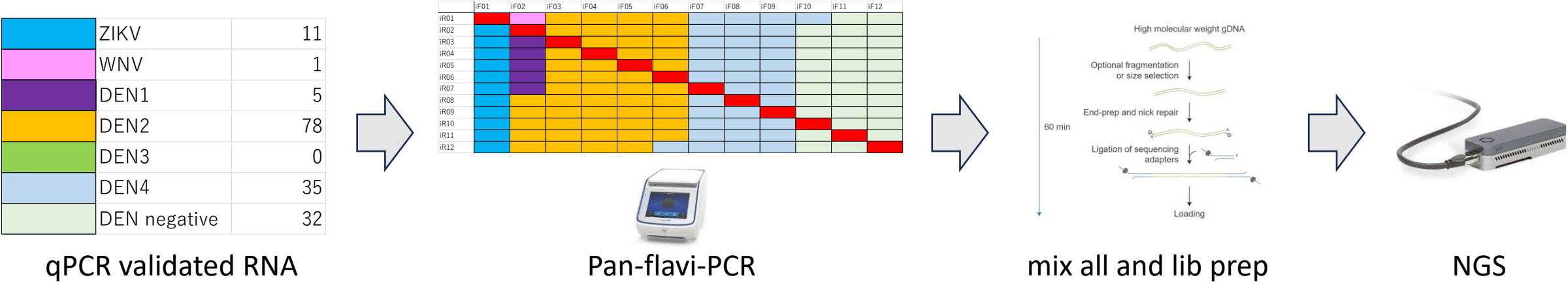
Limit of detection



Ct value around 40 can be detected by the Pan-flavi-nanopore

pan-flavi-nanopore

performance validated using clinical samples



DEN1	true P	true N
test P	4	0
test N	1	127
sensitivity	0.800	
specificity	1.000	

DEN2	true P	true N
test P	45	1
test N	3	83
sensitivity	0.938	
specificity	0.988	

DEN3	true P	true N
test P	0	2
test N	0	130
sensitivity	#DIV/0!	
specificity	0.985	

DEN4	true P	true N
test P	29	0
test N	6	97
sensitivity	0.829	
specificity	1.000	

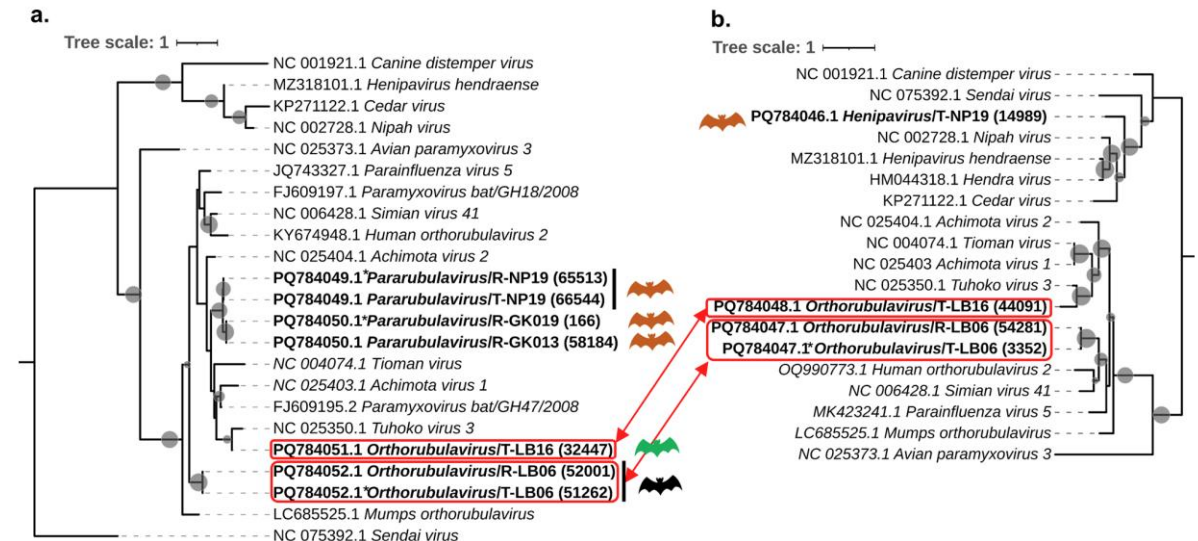
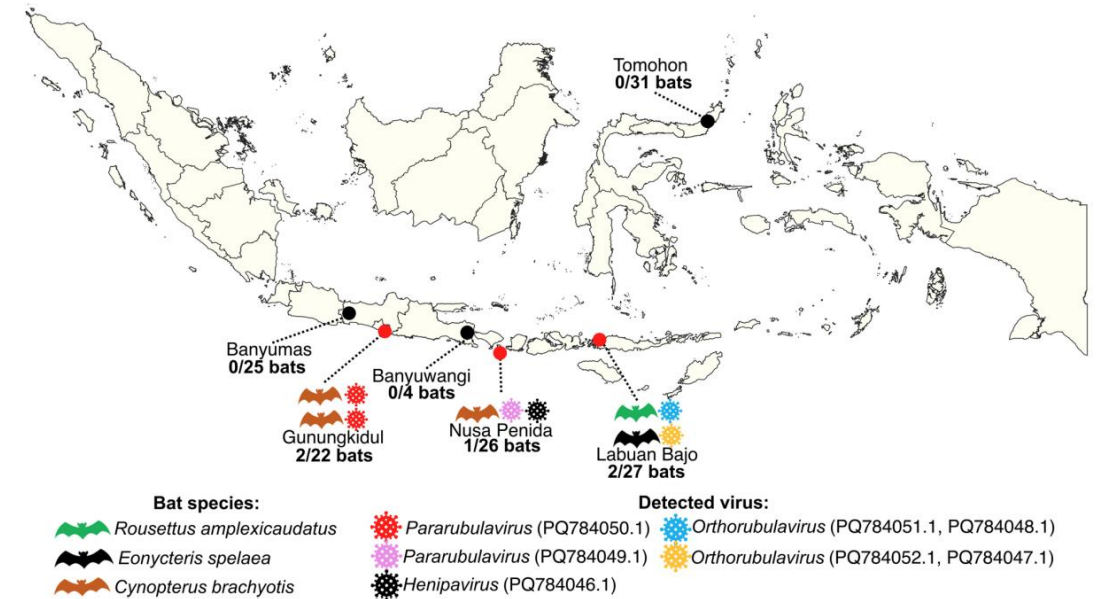
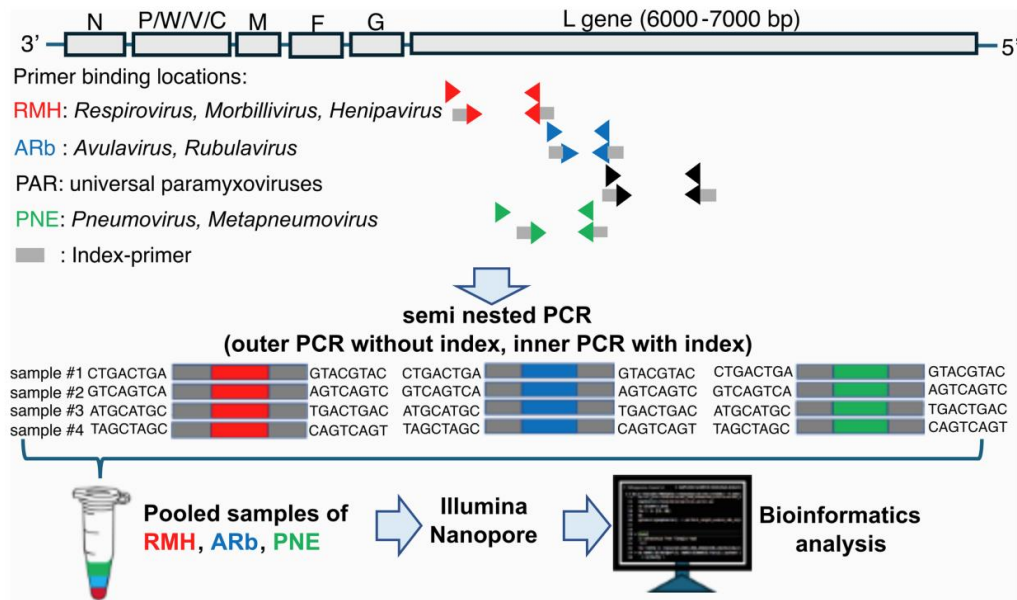
ZIKV	true P	true N
test P	2	0
test N	9	121
sensitivity	0.182	
specificity	1.000	

pan-paramyxo-mNGS

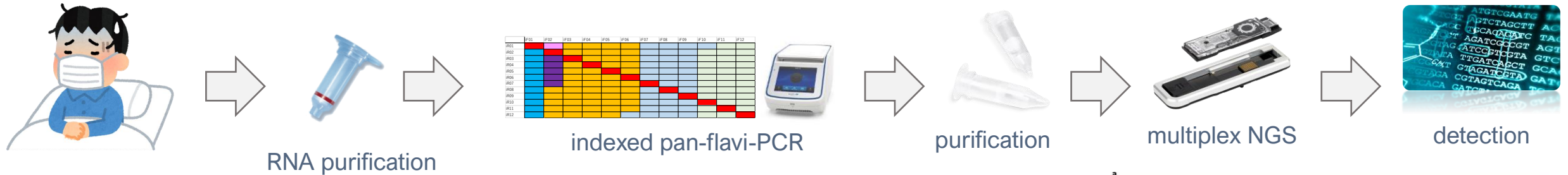
scientific reports

Development of a semicomprehensive detection method for paramyxoviruses and its validation using Indonesian bats

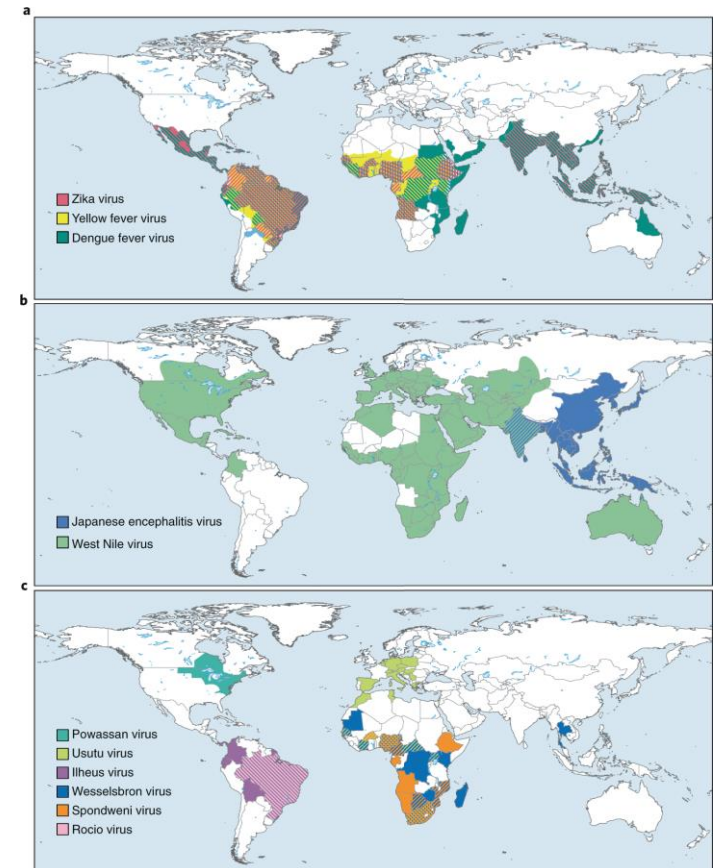
Dela Ria Nesti^{1,2}, Kyoko Hayashida¹, Tatsuki Sugi¹, Wayan T. Artama³, Hery Wijayanto⁴, Naoko Kawai¹ & Junya Yamagishi^{1✉}



まとめ：pan-flavi-mNGS

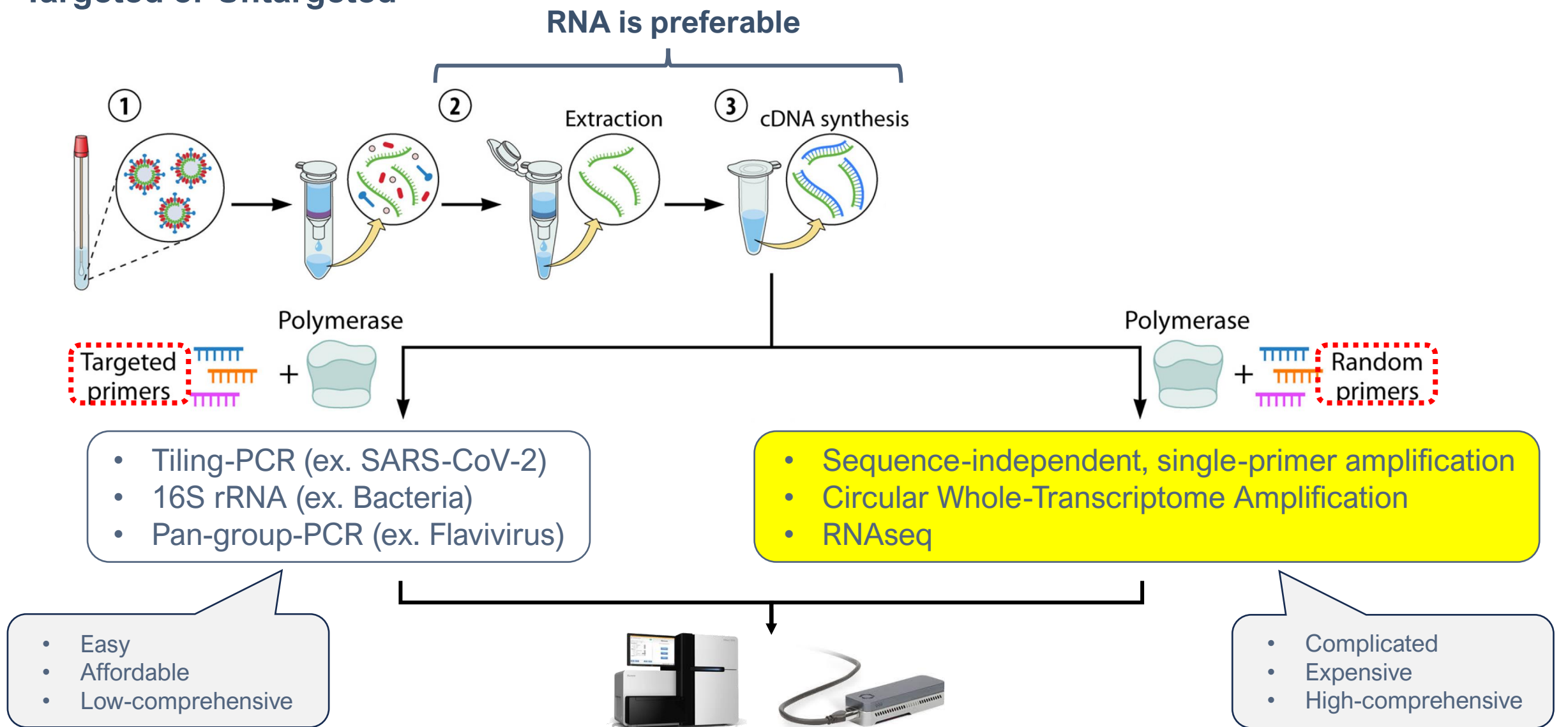


- qPCRに比肩する感度・特異度を達成
- コスト推定：RNA精製500円＋PCR 400円＋NGS 200円
- フラビウイルス多重流行地で有効
- 他pan-group-mNGSとのmultiplex化も可能
- 社会実装に向け企業パートナー求む



Strategy of pathogen detection using mNGS

Targeted or Untargeted



Unknown pathogens can be found by mNGS



SARS-CoV-2

Article

A pneumonia outbreak associated with a new coronavirus of probable bat origin

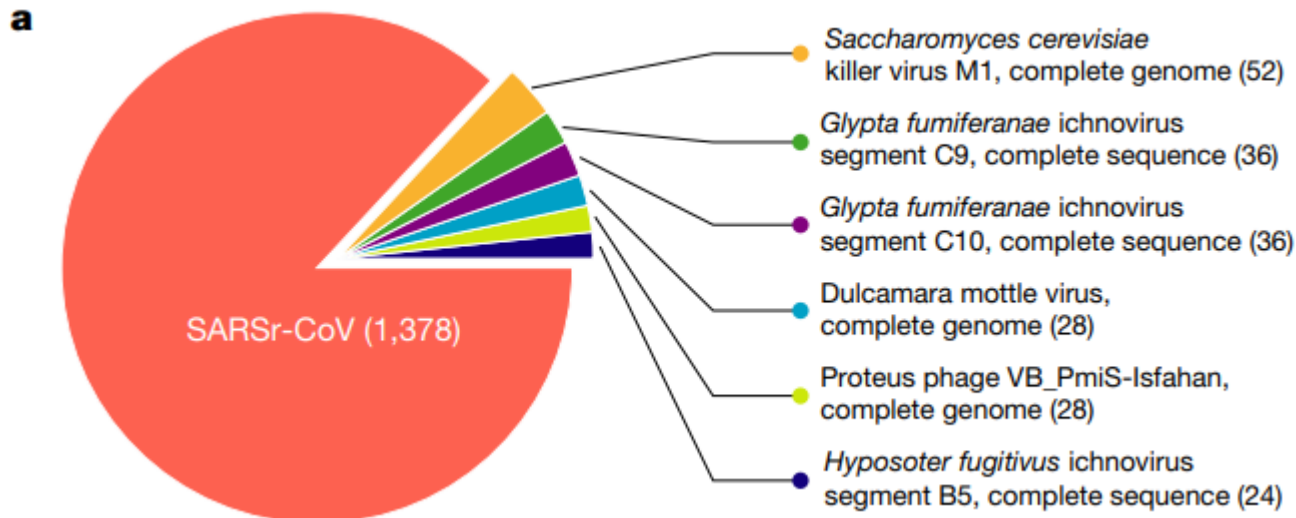
<https://doi.org/10.1038/s41586-020-2012-7>

Received: 20 January 2020

Accepted: 29 January 2020

Published online: 3 February 2020

Peng Zhou^{1,5}, Xing-Lou Yang^{1,5}, Xian-Guang Wang^{2,5}, Ben Hu¹, Lei Zhang¹, Wei Zhang¹, Hao-Rui Si^{1,3}, Yan Zhu¹, Bei Li¹, Chao-Lin Huang², Hui-Dong Chen², Jing Chen^{1,3}, Yun Luo¹, Hua Guo^{1,3}, Ren-Di Jiang^{1,3}, Mei-Qin Liu^{1,3}, Ying Chen^{1,3}, Xu-Rui Shen^{1,3}, Xi Wang^{1,3}, Xiao-Shuang Zheng^{1,3}, Kai Zhao^{1,3}, Quan-Jiao Chen¹, Fei Deng¹, Lin-Lin Liu⁴, Bing Yan¹, Fa-Xian Zhan⁴, Yan-Yi Wang¹, Geng-Fu Xiao¹ & Zheng-Li Shi^{1,5}

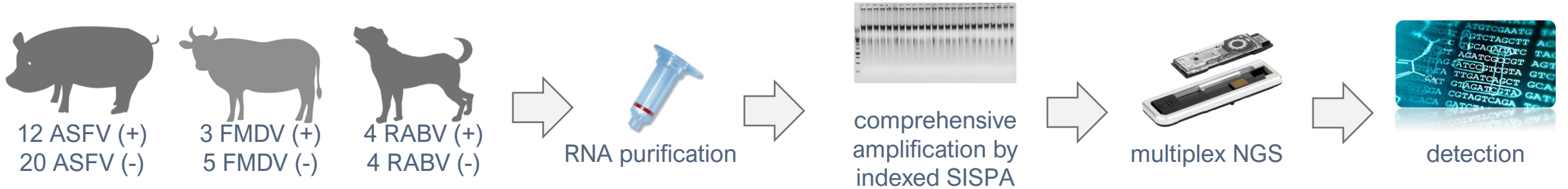


Data Table 1). As a laboratory investigating CoV, we first used pan-CoV PCR primers to test these samples¹³, given that the outbreak occurred in winter and in a market—the same environment as SARS infections. We found five samples to be PCR-positive for CoVs. One sample (WIV04), collected from the bronchoalveolar lavage fluid (BALF), was analysed by metagenomics analysis using next-generation sequencing to identify potential aetiological agents. Of the 10,038,758 total reads—of which 1,582 total reads were retained after filtering of reads from the human genome—1,378 (87.1%) sequences matched the sequence of SARSr-CoV (Fig. 1a). By de novo assembly and targeted PCR, we obtained a 29,891-base-pair CoV genome that shared 79.6% sequence identity to SARS-CoV BJ01 (GenBank accession number AY278488.2). High

It costs just 300 USD
but expensive for diagnosis...

SISPA based mNGS for agnostic diagnosis

POC study using clinical samples



ASFV		qPCR	
		+	-
mNGS	+	12	0
	-	0	20

180 Kb

FMDV		qPCR	
		+	-
mNGS	+	0	0
	-	3	5

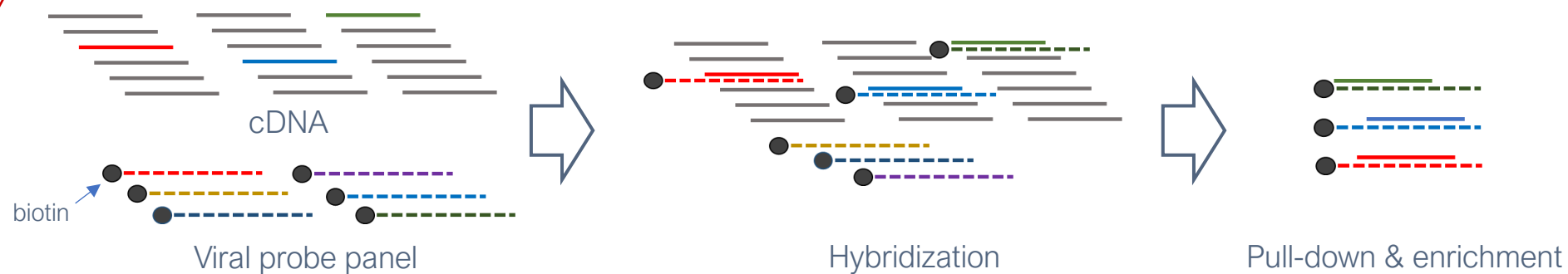
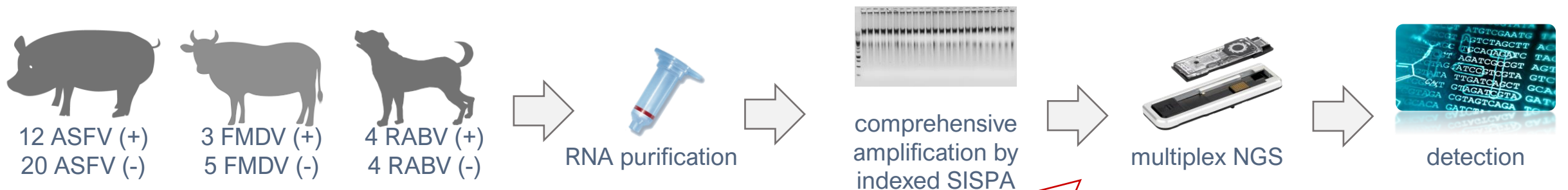
8.3 Kb

RABV		qPCR	
		+	-
mNGS	+	0	0
	-	4	4

11.9 Kb

SISPA based mNGS for agnostic diagnosis

POC study using clinical samples



- Provided by TWIST bioscience
- Targeting 3,135 viral species
- Accepting some mismatches then virtually comprehensive
- Expensive

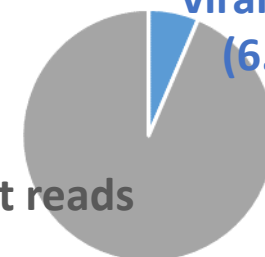


viral reads (0.0035%)



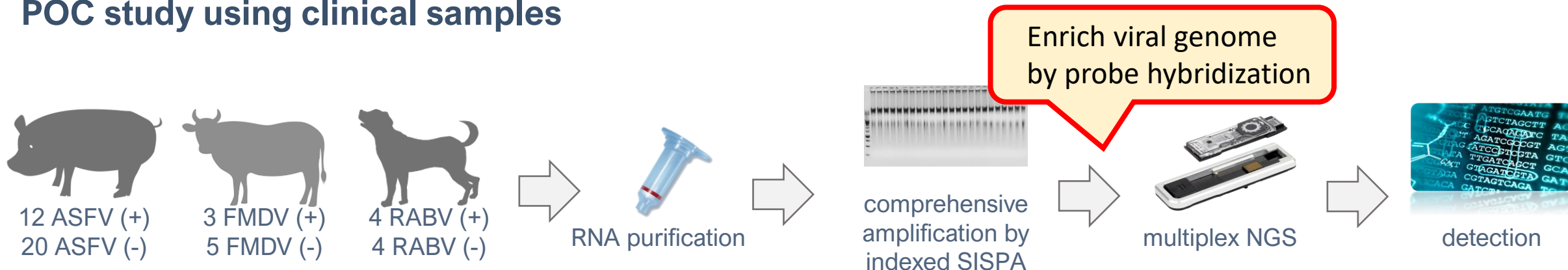
viral reads (6.4%)

host reads



SISPA based mNGS for agnostic diagnosis

POC study using clinical samples



ASFV		qPCR	
		+	-
mNGS	+	12	0
	-	0	20

FMDV		qPCR	
		+	-
mNGS	+	0	0
	-	3	5

RABV		qPCR	
		+	-
mNGS	+	0	0
	-	4	4

ASFV		qPCR	
		+	-
mNGS	+	2	0
	-	10	20

FMDV		qPCR	
		+	-
mNGS	+	0	0
	-	3	5

RABV		qPCR	
		+	-
mNGS	+	3	0
	-	1	4

SISPA based mNGS for agnostic diagnosis

further validation using clinical samples



with Twist											without Twist						
sample ID	inex	organ	Ct value	Lyssavirus	adenovirus	papilloma	parvovirus	morbillivirus	Salivirus	kobuvirus	Lyssavirus	adenovirus	papilloma	parvovirus	morbillivirus	Salivirus	kobuvirus
1	iF01	siliva	37.7	0	0	113	5	0	0	1	0	0	3	6	0	0	0
2	iF02	siliva	29.5	5	0	0	2	1	0	0	0	0	0	6	0	0	0
3	iF03	siliva	25.7	149	0	0	6	0	0	0	0	0	0	1	0	0	0
4	iF04	siliva	29.4	2	0	0	2	0	0	0	0	0	0	3	0	0	0
5	iF05	siliva	ND	2	0	5	3	3	0	0	0	0	22	0	0	0	0
6	iF06	siliva	ND	0	0	0	2	2	0	0	0	0	0	2	0	0	0
7	iF07	siliva	ND	1	37	0	4	3	0	0	0	0	1	1	0	0	0
8	iF08	siliva	ND	0	0	0	0	0	0	0	0	0	0	1	0	0	0
9	iF09	brain	25.4	65	0	0	2	0	0	0	1	0	0	1	0	0	0
10	iF10	brain	24.9	22	0	0	2	1	0	0	0	0	0	3	0	0	0
11	iF11	brain	21.4	60	0	0	1	0	0	0	0	0	0	1	0	0	0
12	iF12	brain	20.3	35	0	0	1	0	0	0	0	0	0	1	0	0	0
13	iR01	siliva	26.7	6	0	0	3	1	0	0	0	0	0	2	0	0	0
14	iR02	siliva	29.6	5	0	0	2	0	0	0	0	0	0	0	0	0	0
15	iR03	siliva	37.3	6	0	5	8	0	0	0	0	0	0	2	0	0	0
16	iR04	siliva	31.4	30	0	0	3	0	42	0	0	0	0	1	0	0	0
17	iR05	brain	ND	0	0	0	1	1	0	0	0	0	0	1	0	0	0
18	iR06	brain	ND	0	0	0	17	3873	0	0	0	0	0	0	5	0	0
19	iR07	brain	ND	0	0	0	1	0	0	0	0	0	0	2	0	0	0
20	iR08	brain	ND	0	0	0	171	0	0	0	0	0	0	24	0	0	0
21	iR09	siliva	ND	1	0	0	0	0	0	0	0	0	0	1	0	0	0
22	iR10	siliva	ND	0	0	0	0	3	0	0	0	0	0	0	0	0	0
23	iR11	siliva	ND	1	0	0	1	0	0	0	0	0	0	0	0	0	0
24	iR12	siliva	ND	1	3	0	33417	4	0	61	0	0	0	184	0	0	0

Challenge for unknown pathogen identification

nature

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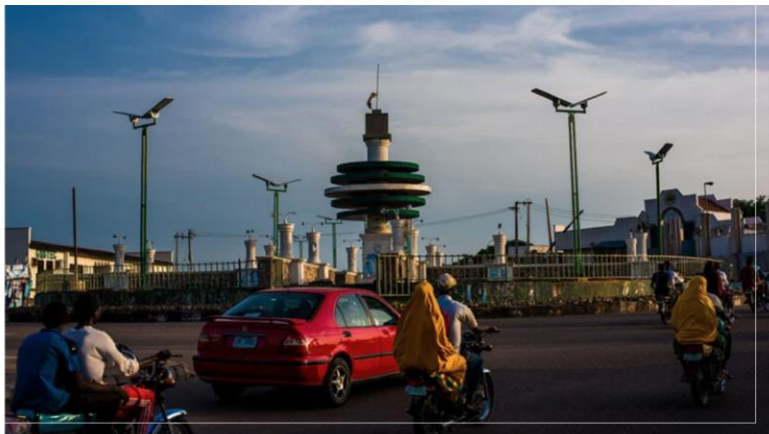
[nature](#) > [news](#) > article

NEWS | 05 March 2024 | Correction [07 March 2024](#)

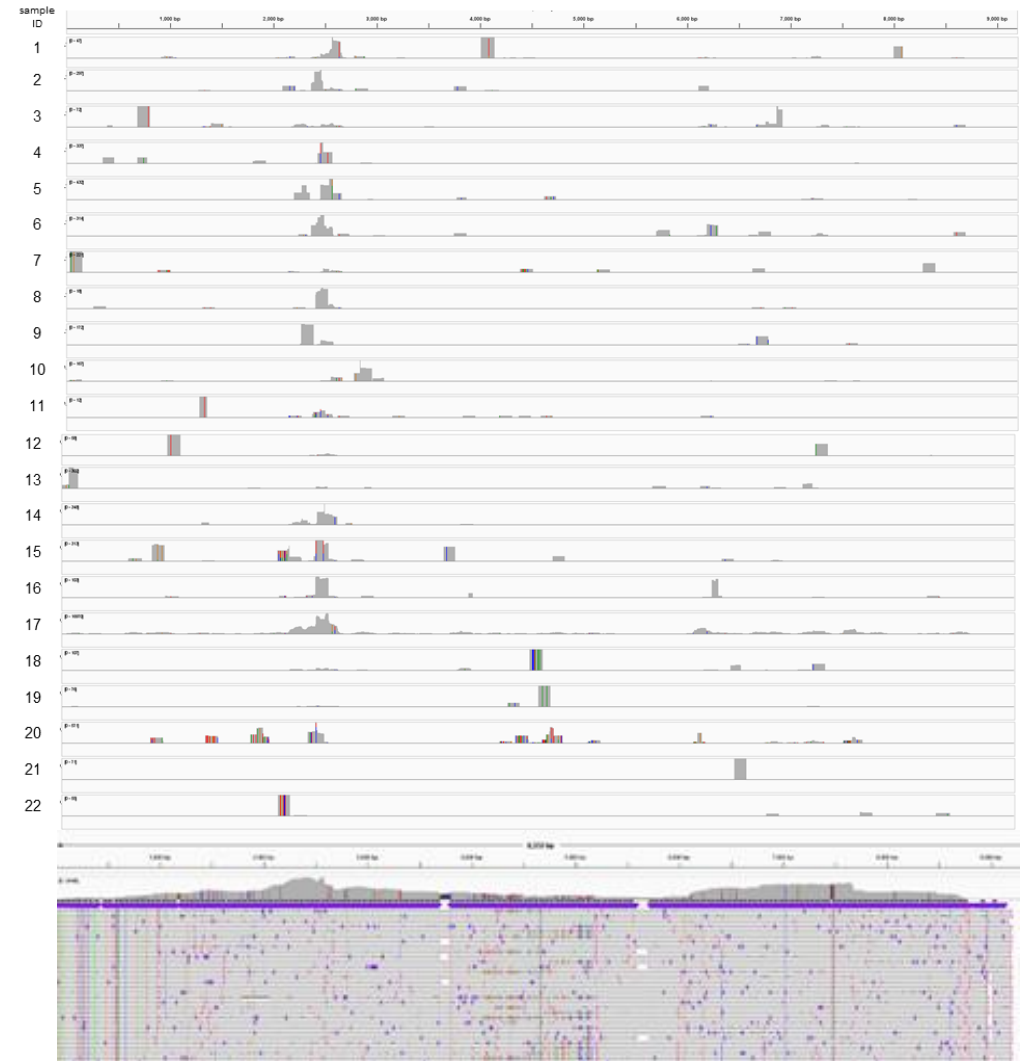
Meningitis could be behind ‘mystery illness’ reports in Nigeria

The WHO confirms three meningitis deaths, which it says might have triggered rumours of an outbreak of an unknown disease.

By [Sarah Wild](#)



The Nigerian health ministry has been told to investigate reports of deaths in the northeastern state of Gombe (pictured). Credit: Tolu Owoeye/Shutterstock



whole genome (# 17)

22/22 were positive for pegivirus-like by mNGS

Challenge for unknown pathogen identification

nature

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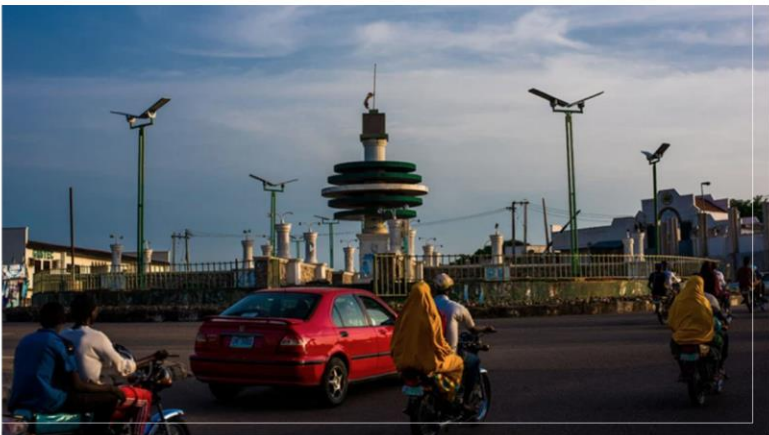
[nature](#) > [news](#) > article

NEWS | 05 March 2024 | Correction [07 March 2024](#)

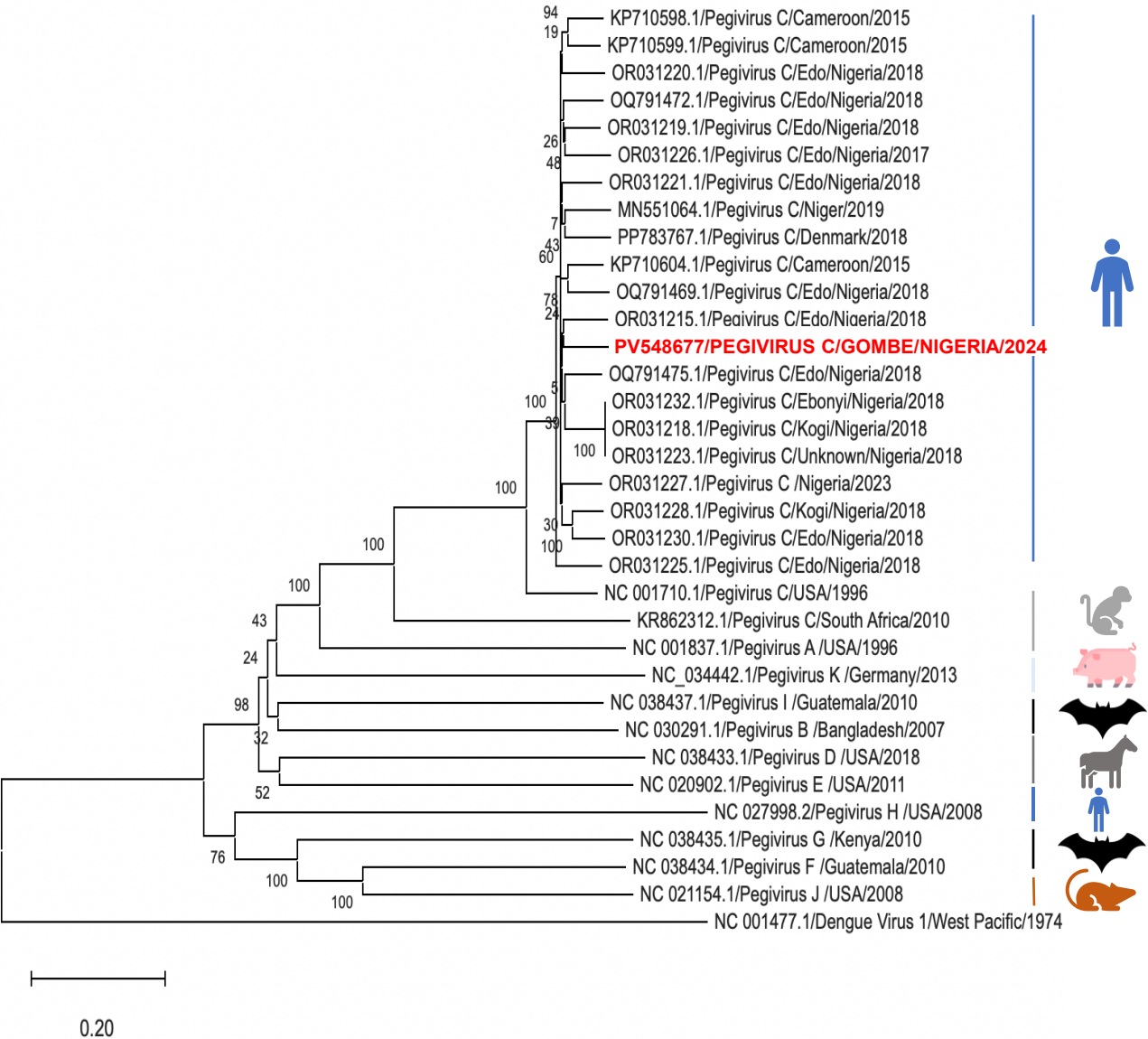
Meningitis could be behind ‘mystery illness’ reports in Nigeria

The WHO confirms three meningitis deaths, which it says might have triggered rumours of an outbreak of an unknown disease.

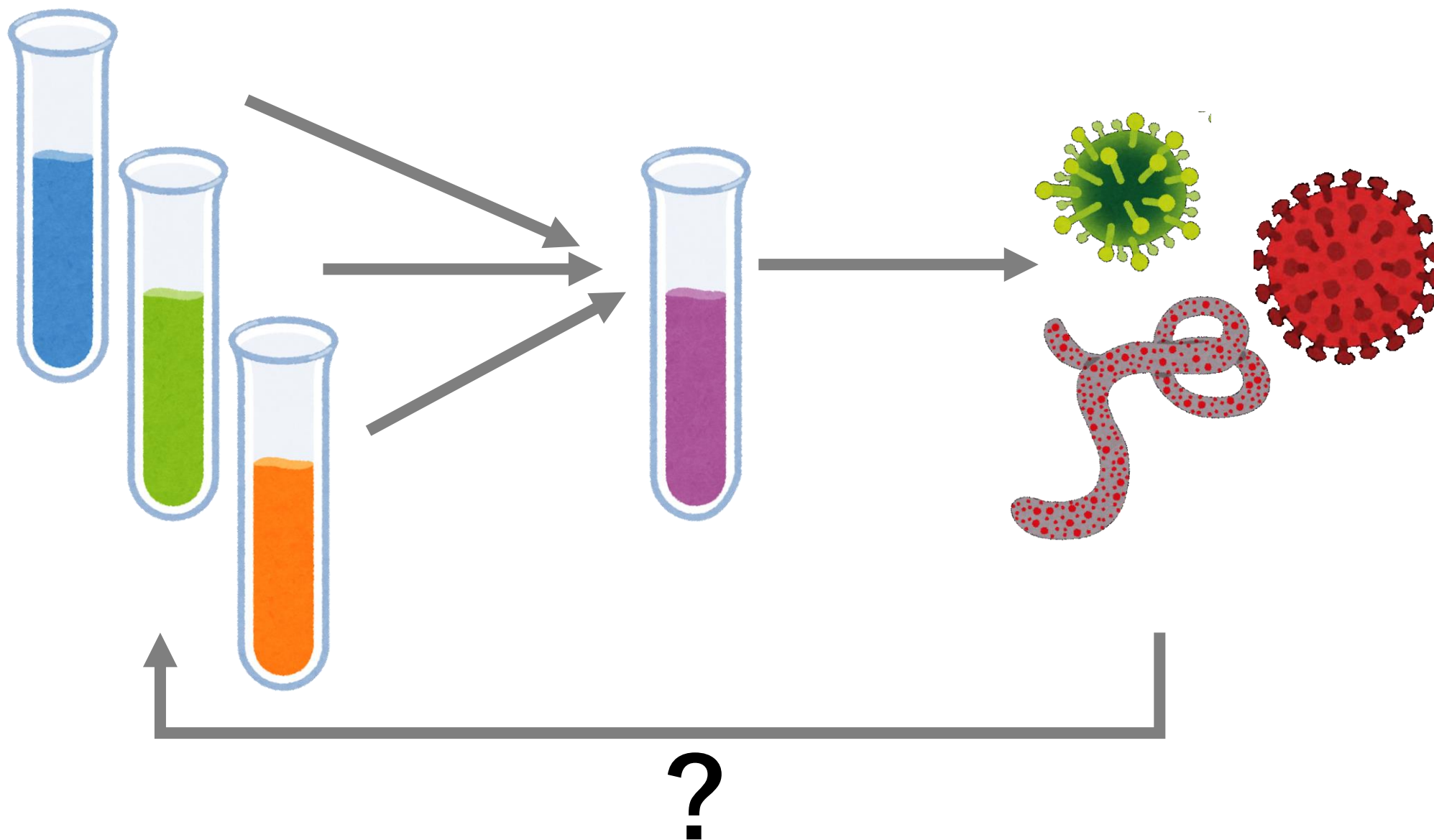
By [Sarah Wild](#)



The Nigerian health ministry has been told to investigate reports of deaths in the northeastern state of Gombe (pictured). Credit: Tolu Owoeye/Shutterstock



How to reduce cost? maybe pool them but...



Ultra parallel **full**-comprehensive mNGS

mNGS Screening Enhanced by a Group Testing Algorithm (mEGA)

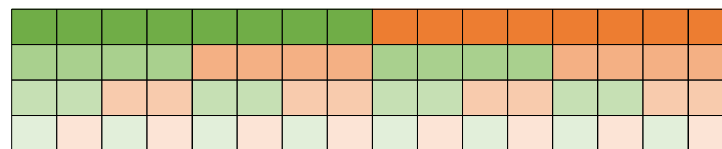


Diagnosis by mNGS

○ comprehensiveness

△ multiplicity

+



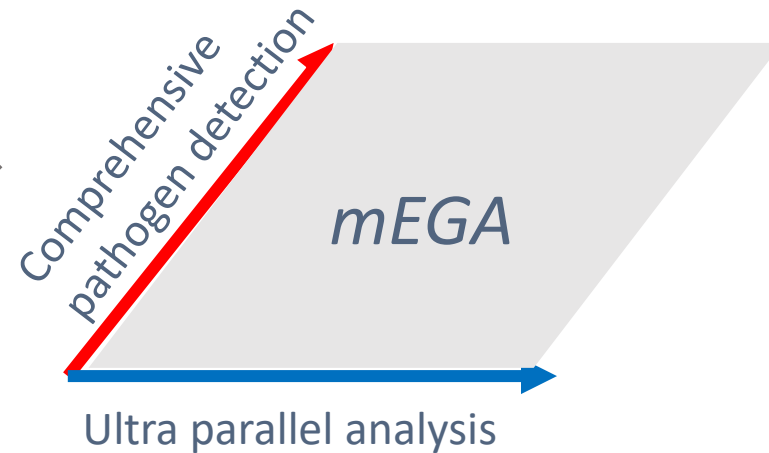
test $2n$ pools instead of 2^n samples



Group testing algorithm

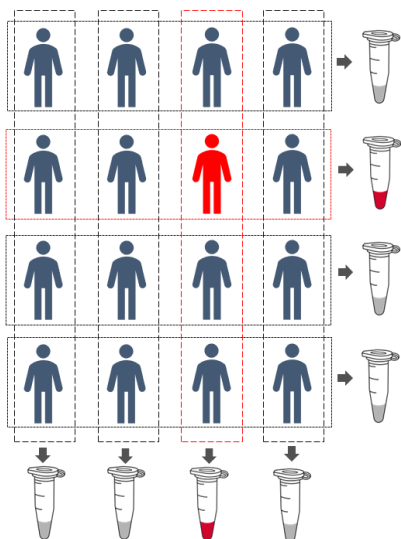
× comprehensiveness

○ multiplicity



Group testing algorithm

test 8 pools instead of 16 samples



The concept has been known since 1974



RESEARCH ARTICLE

Circular Whole-Transcriptome Amplification (cWTA) and mNGS Screening Enhanced by a Group Testing Algorithm (mEGA) Enable High-Throughput and Comprehensive Virus Identification

Patrick Reteng,^a Linh Nguyen Thuy,^b Mizanur Rahman,^c Ana Maria Bispo de Filippis,^d Kyoko Hayashida,^{a,e} Tatsuki Sugi,^{a,e} Gabriel Gonzalez,^f William W. Hall,^{g,h} Lan Anh Nguyen Thi,^b Junya Yamagishi^{a,e}

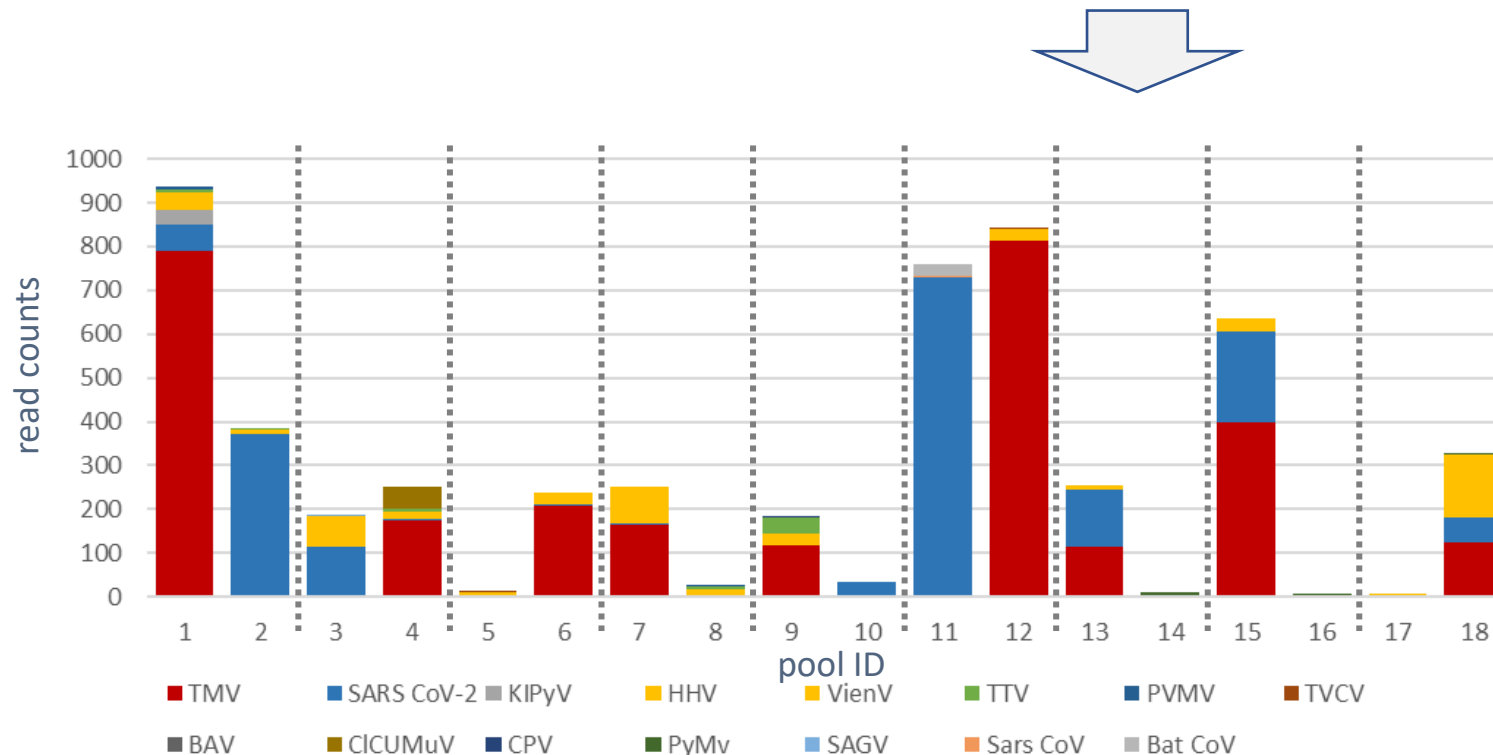
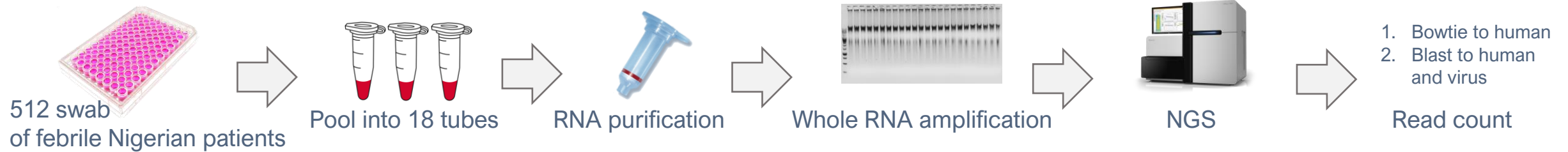
September/October 2022 Volume 7 Issue 5

- 44 samples of fever of unknown origin were analyzed
- DEN2, HBV and B19 were detected at once without prior knowledge

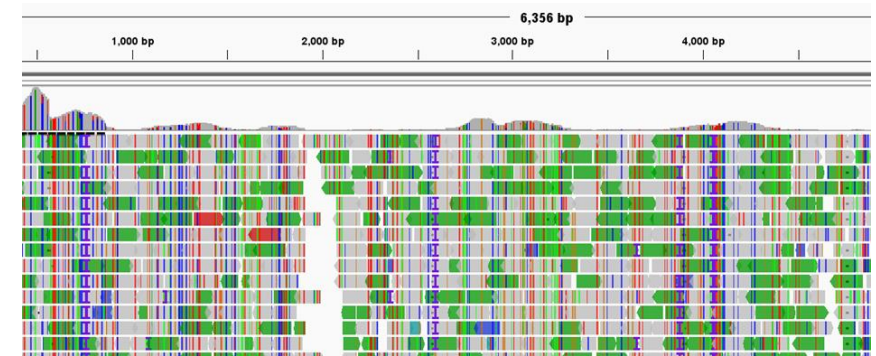
➡ Scale up is needed for social implementation.

Up-scaling of mNGS (n=512)

to enable 5\$/sample and **2** problems found



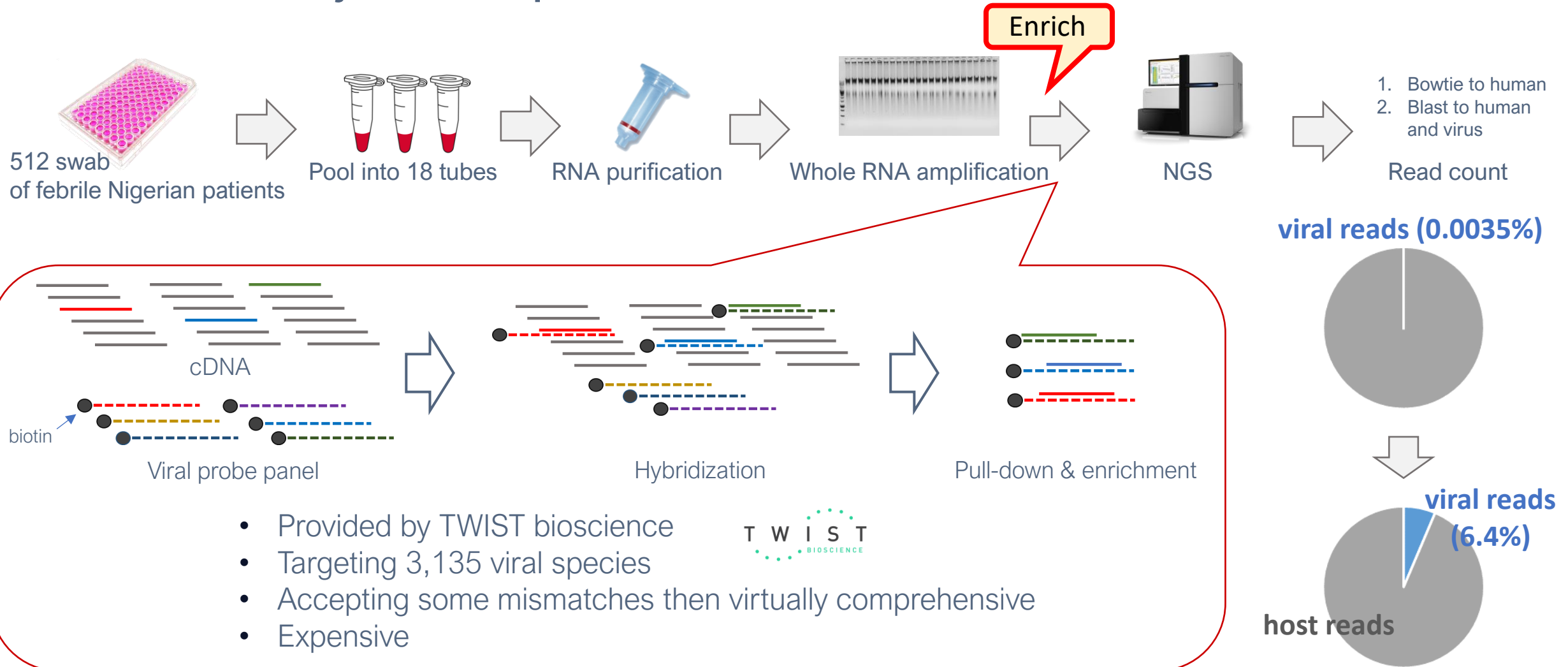
- Tobacco Mosaic Virus (TMV) was successfully traced back (confirmed by RT-PCR).



- Obtained number of **viral reads were limited** (5337/290M).
- There was a **large bias** in the number of reads obtained in each pool, probably caused by uneven PCR.

Improving sensitivity of mEGA

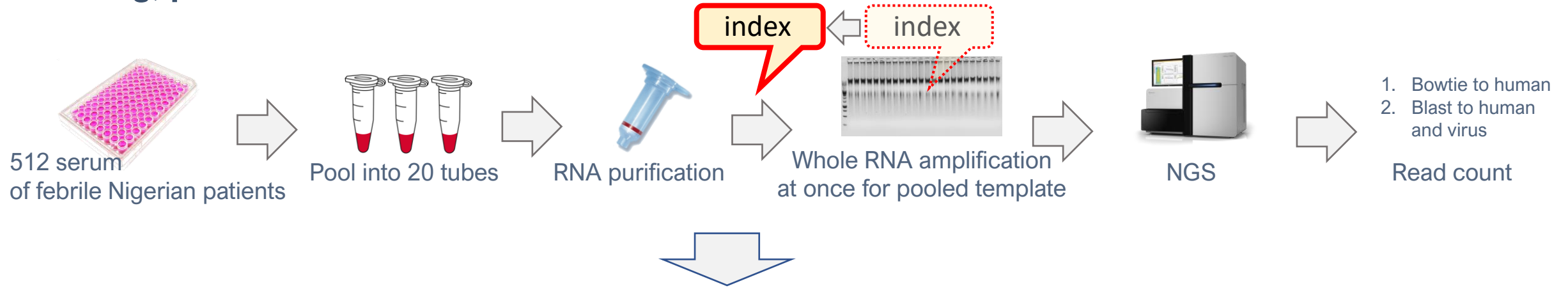
with a commercial hybridization panel



Viral reads were **x1850** enriched by TWIST viral read enrichment panel.

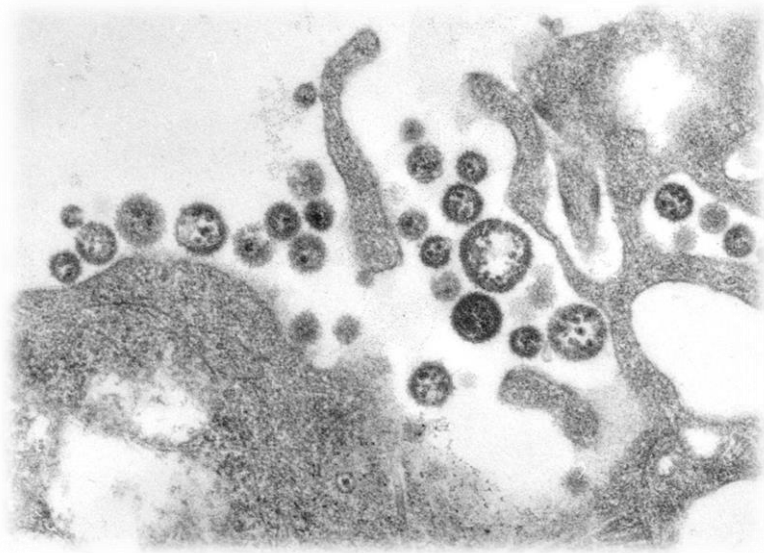
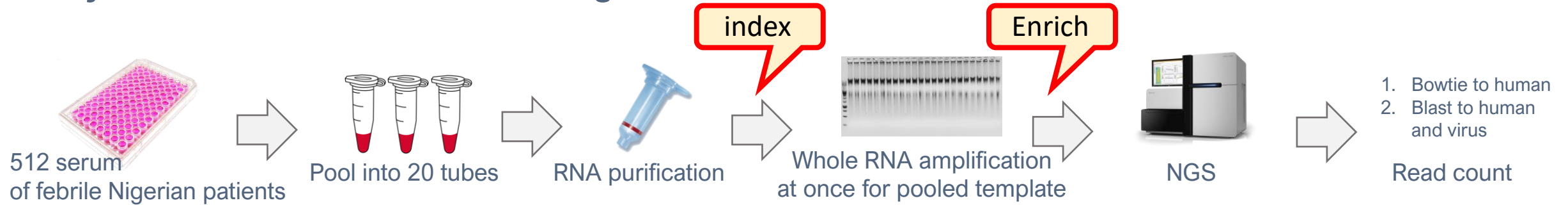
Improving PCR bias

indexing, pool then PCR



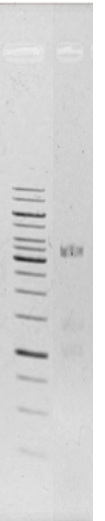
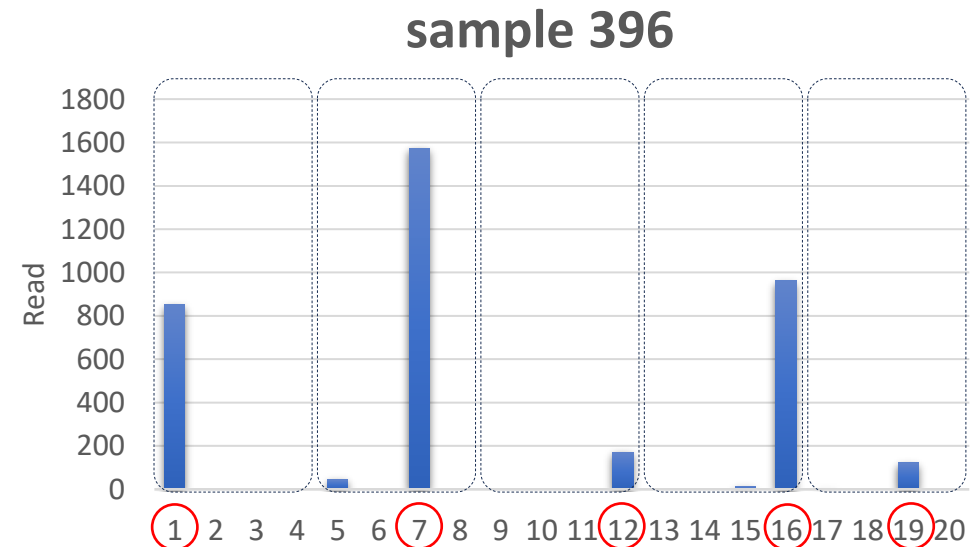
Improved mEGA

Analysis of **512** fever of unknown origin human serum



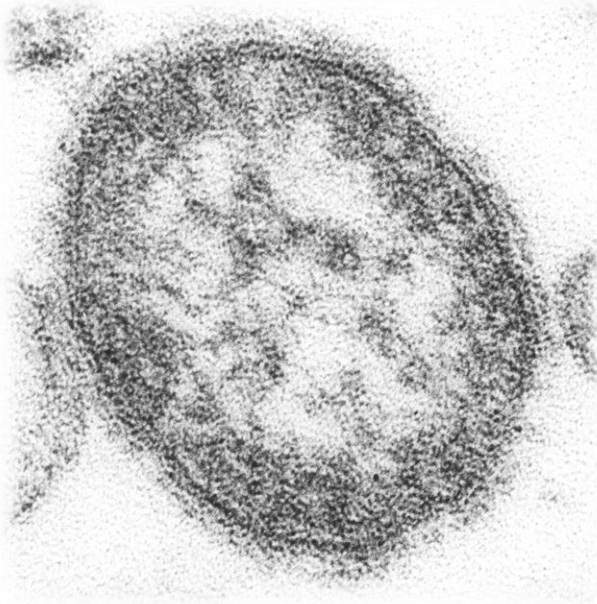
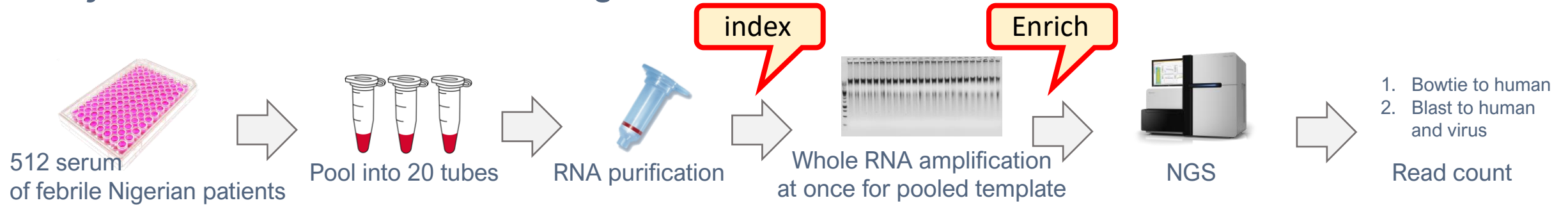
CDC

Lassa Virus



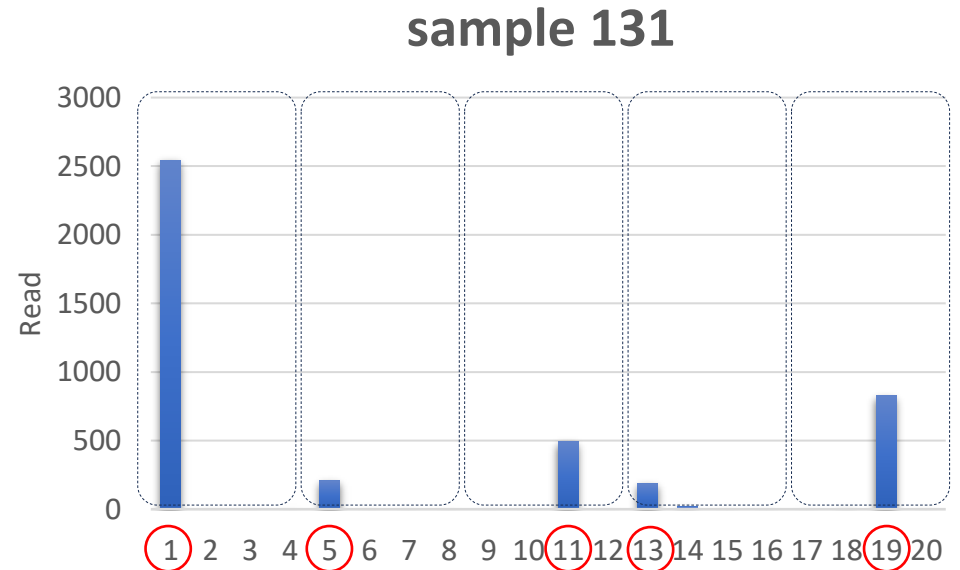
Improved mEGA

Analysis of **512** fever of unknown origin human serum



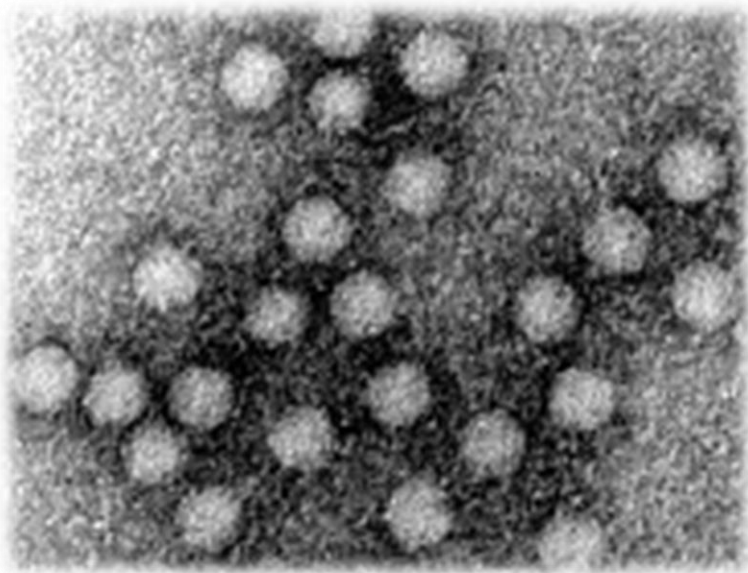
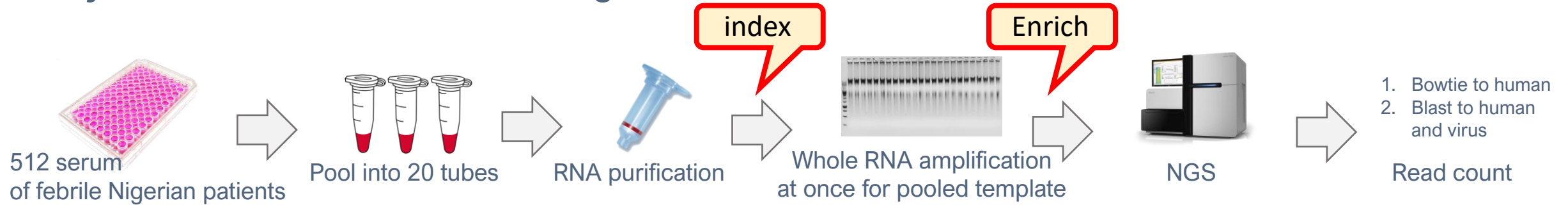
CDC

Measles Virus



Improved mEGA

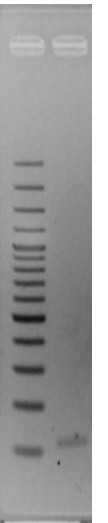
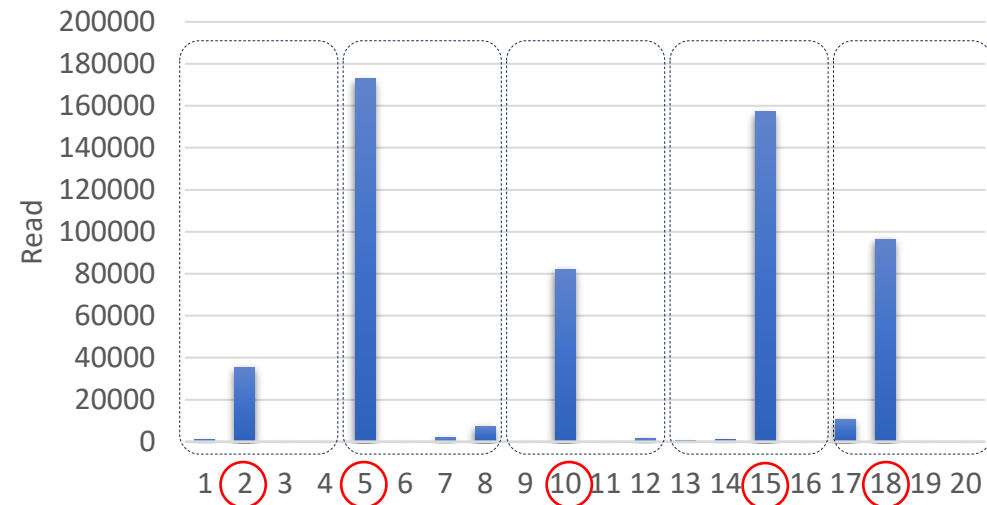
Analysis of 512 fever of unknown origin human serum



Linda M. Stannard, University of Cape Town

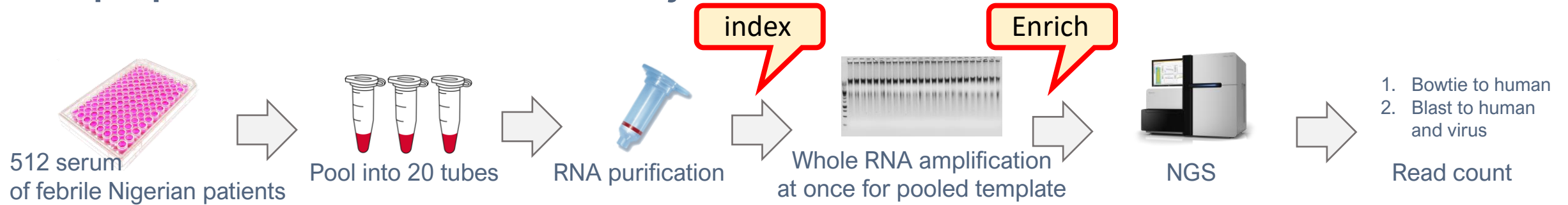
Human Parvovirus B19

sample 338



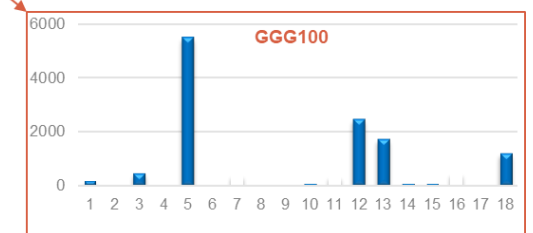
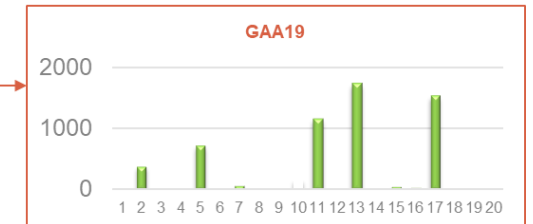
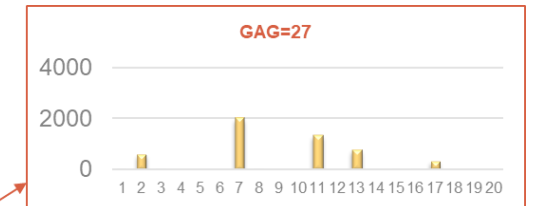
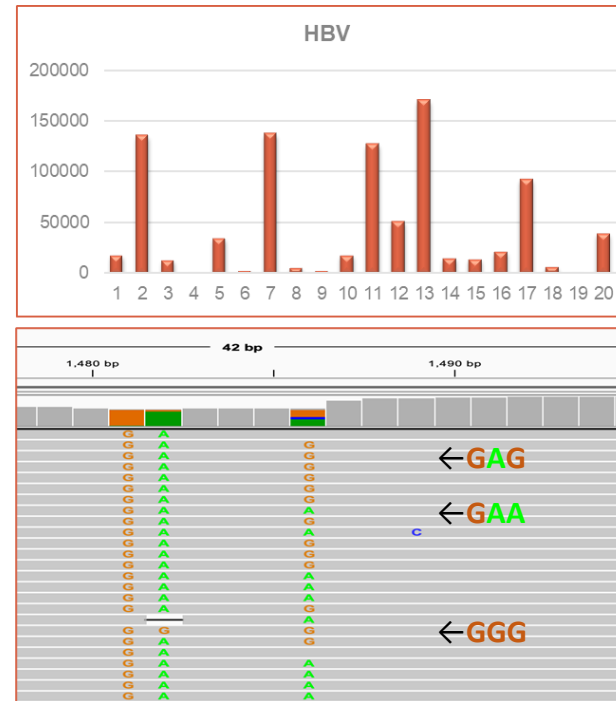
Improved mEGA

Multiple positives can be differentiated by **SNPs**



CDC

Hepatitis B Virus



まとめ：超並列網羅的検出法 mEGA

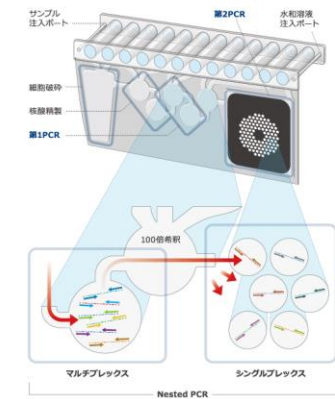
- 512 検体の同時解析でLassa, Measles, B19の検出に成功
- SNPにより、HBVは複数の陽性検体の同時検出にも成功
- コスト推定：1000検体で30万円（300円/検体）
- 社会実装？
- 感度の向上と評価が課題

DETECTING MICROBES THAT IMPACT HUMAN HEALTH.

By leveraging the power of clinical metagenomics, our vision is to create a world where every patient gets a clear and timely diagnosis.



KARIUS®



空港での水際対策の代替

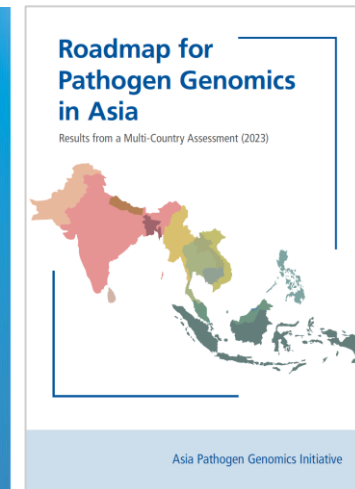
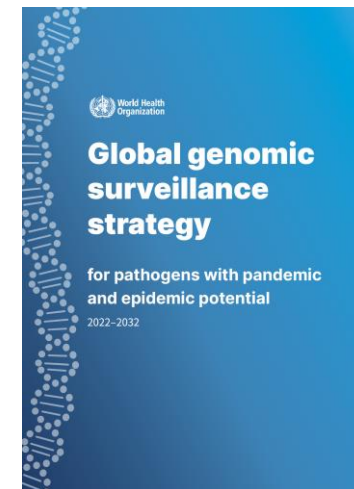
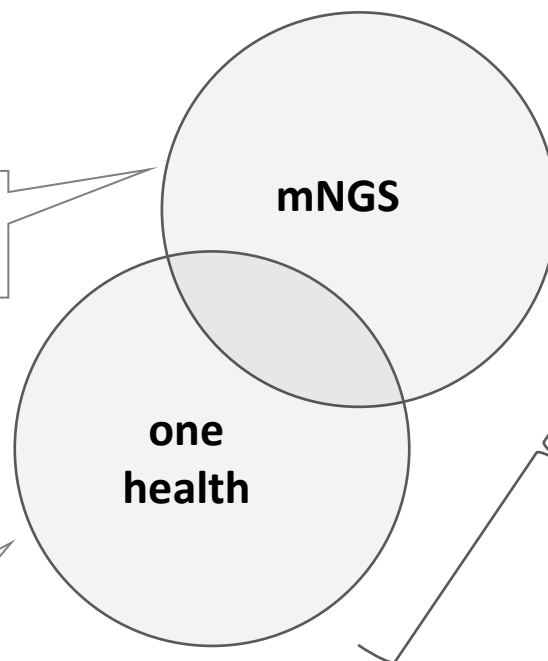
感染症診断におけるmNGSの標準法化へ向けて

-- もう一つの目的 --

2. mNGSによる網羅的解析は未知の病原体にも対応可能だが、高コスト。

1. disease X は新規かつ動物由来の人獣共通感染症である可能性が高い。

3. 野生動物・環境を対象とした大規模疫学調査の実施は容易ではない。



Pandemic Prevention, Preparedness and Response (PPPR)

one health + mNGSによるアプローチは有望だが、対象範囲が膨大でmNGSも高コストなため、現状では非現実的。

"disease X" によるパンデミックの防止？

感染症**診断**におけるmNGSの標準法化へ向けて

-- もう一つの目的 --

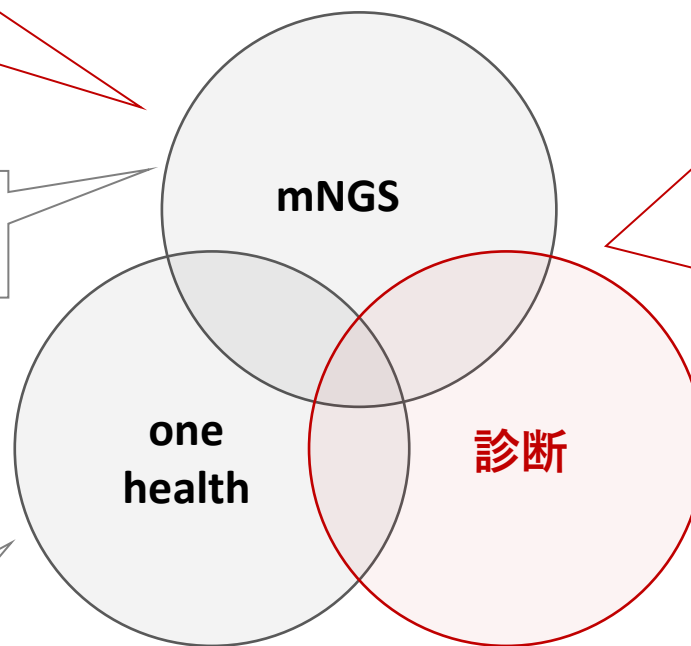
5. mNGSの低コスト化は既に検討済み

- pan-group mNGS
- mEGA
- 16S/18S rRNA mNGS
- SISPA-panel AMR enrich nanopore

2. mNGSによる網羅的解析は未知の病原体にも対応可能だが、高コスト。

1. disease X は新規かつ動物由来の人獣共通感染症である可能性が高い。

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6. mNGSが感染症診断のスタンダードになることで...

- アウトブレイク直後に病原体を検出できるようになる（早期発見）
- アウトブレイク前の、再生産数 $R < 0$ の病原体の検出と、進化過程がモニター可能になる（超早期発見）
- 患者のQOLに資する不明熱の原因を特定できるようになる
- 検査費用は患者負担 → 大規模疫学が無料で可能！

4. これまでqPCR等で行われている感染症診断をmNGSで置き換えることが出来れば、既存の検査システムを流用でき、莫大な数の検体の網羅的解析が可能になる。ただし、mNGSのコストを十分下げる必要がある。

"disease X" によるパンデミックの防止！