

Targeted Pathogen Sequencing



Too much host! or not enough pathogen



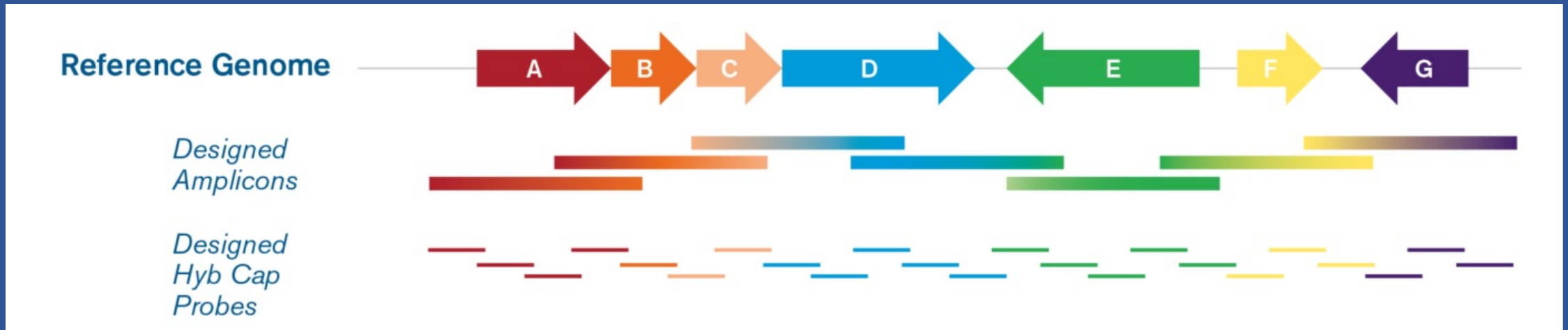
Targeted Pathogen Sequencing

Goals:

- Diagnostics
- Whole genome sequencing

Approaches:

- Hybrid capture
- Amplicon amplification



Targeted Pathogen Sequencing

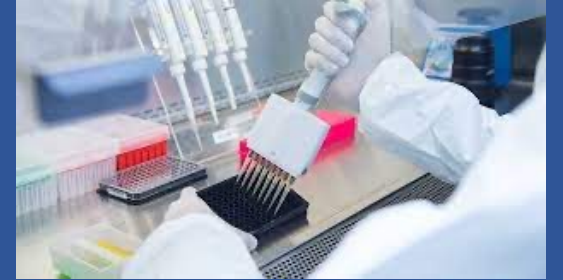
Sample
preparation



High
throughput
sequencing



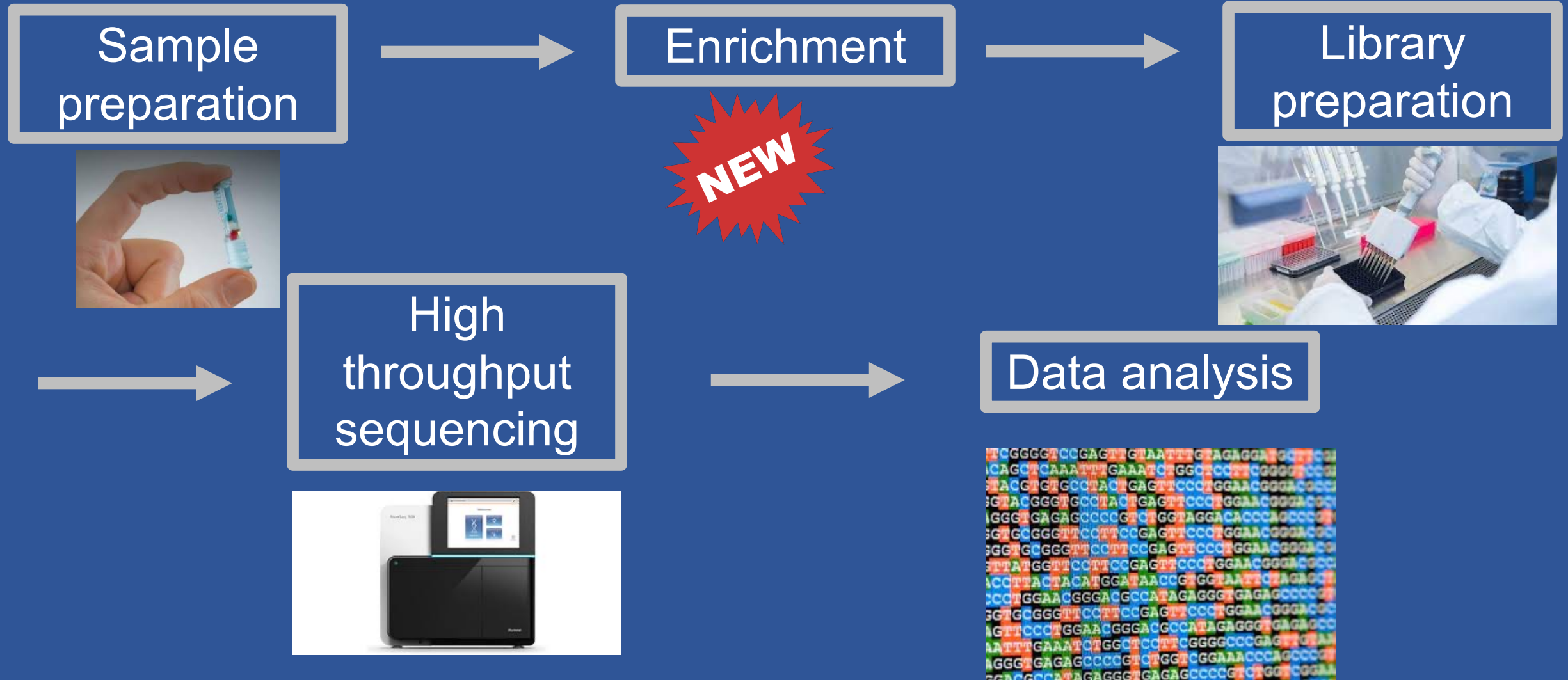
Library
preparation



Data analysis

```
TCGGGGTCCGAGTTGTAATTTGAGAGGATCCTTCG  
ACAGCTCAAAATTTGAAATCTGGCTCCCTCGGGGTC  
TACGTGTGCCTACTGAGTTCCCTGGAAACGGGACCC  
GGTACGGGTGCCCTACTGAGTTCCCTGGAAACGGGACCC  
GGGTGAGAGCCCCGTCTGGTAGGACACCCAGCCCC  
GGTGCGGGTTCCCTCCGAGTTCCCTGGAAACGGGACCC  
GGGTGCGGGTTCCCTCCGAGTTCCCTGGAAACGGGACCC  
TTATGGTTCCCTCCGAGTTCCCTGGAAACGGGACCC  
ACCTTACTACATGGATTAACCGTGGTAATTCAGAGCT  
CCCTGGAACGGGACGCCATAGAGGGTGAGAGCCCCCT  
GGTGCGGGTTCCCTCCGAGTTCCCTGGAAACGGGACCC  
AGTTCCCTGGAACGGGACGCCATAGAGGGTGAGAGCCC  
AATTTGAAATCTGGCTCCCTCGGGGCCCGAGCTTGA  
AGGGTGAGAGCCCCGTCTGGTGGAAACCCAGCCCCCT  
CGAGGCCATAGAGGGTGAGAGCCCCGTCTGGTGGAA
```


Targeted Pathogen Sequencing



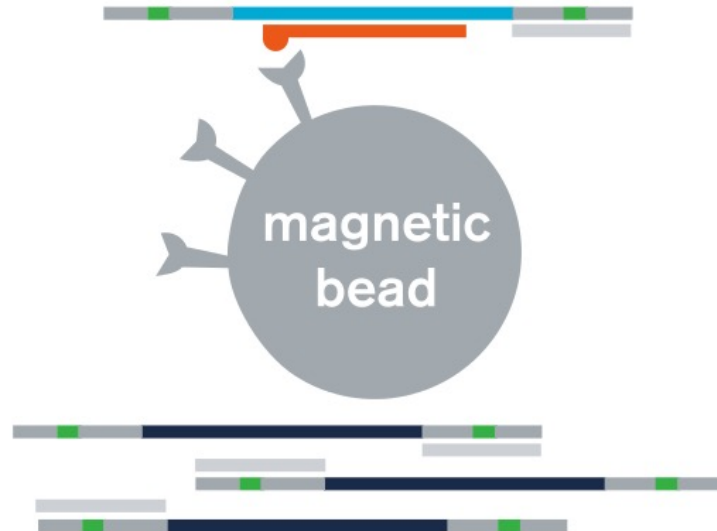
Targeted Pathogen Sequencing

- Greater sensitivity
- Increased sequencing depth of target
- Ability to recover WGS even with low copy number/poor sample quality

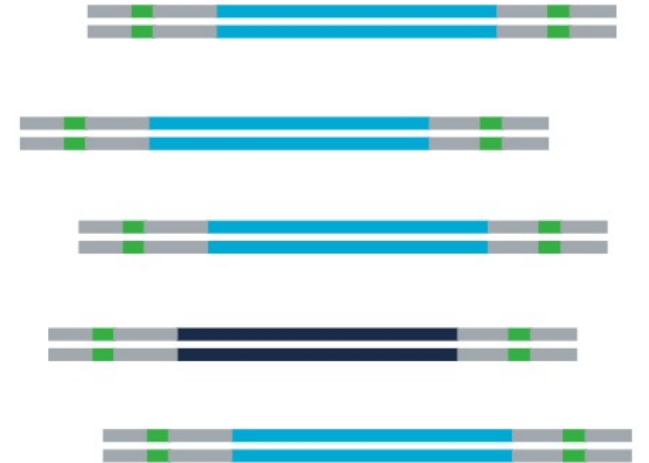
Hybrid capture/targeted capture



1 Denature library, bind to blockers and baits



2 Bind to beads, wash away off-target molecules



3 Amplify enriched library, sequence!

Hybrid capture

B

5'-GCTTAGGTGCAACGT-3'
3'-CGAATCCACGTTGCA-5'



DUPLEX formation



COMPLEMENTARY
TARGET DNA

5'-ACGTTGCACCTAAGC-3'
+



A

DNA PROBE

5'-GCTTAGGTGCAACGT-3'



C

NON-COMPLEMENTARY
DNA

+

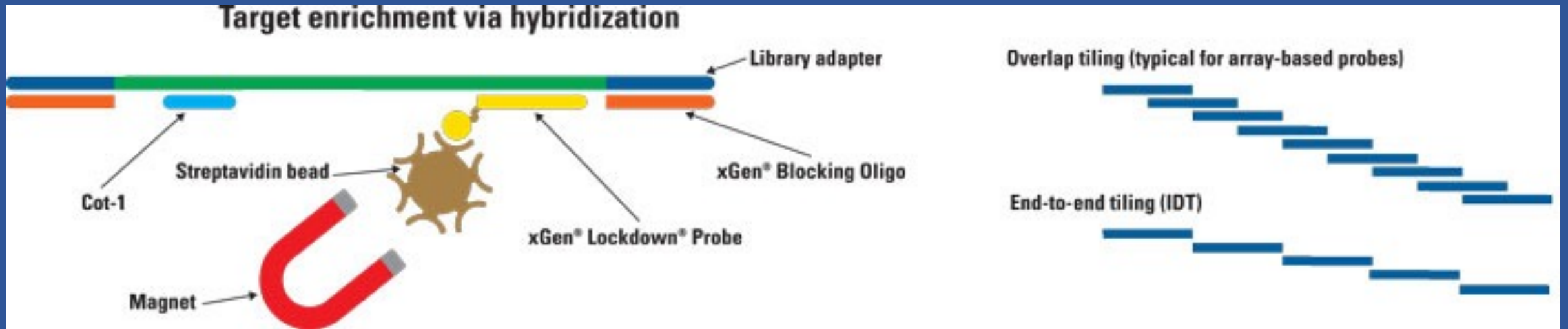
5'-ACCTTTCACGTCCGC-3'



NO DUPLEX formation

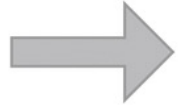


Hybrid capture

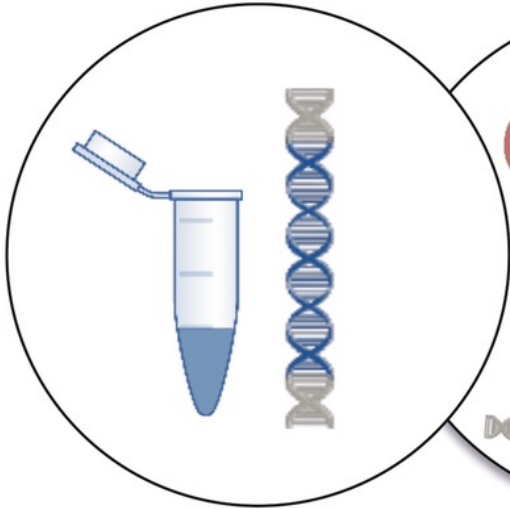


Diagnostic or Pathogen Discovery

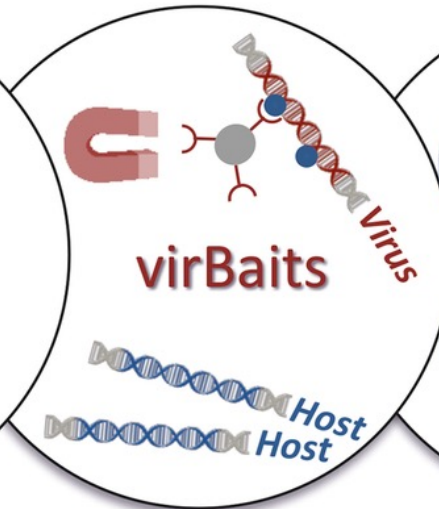
Diagnostic sample



Generic sample processing



Specific virus enrichment



Sequencing



Bioinformatic analysis



Viral pathogen

?



Developing your probes/baits

[PRODUCTS](#)[APPLICATIONS & INDUSTRIES](#)[TRAINING & EVENTS](#)[SERVICES](#)[SUPPORT & RESOURCES](#)[ORDER CENTER](#)

[Home](#) > [Products](#) > [Next Generation Sequencing](#) > [Hybridization-based Next Generation Sequencing \(NGS\)](#) > [NGS Custom Target Enrichment Probes](#) > [SureSelect Custom DNA Target Enrichment Probes](#)

NGS Custom Target Enrichment Probes

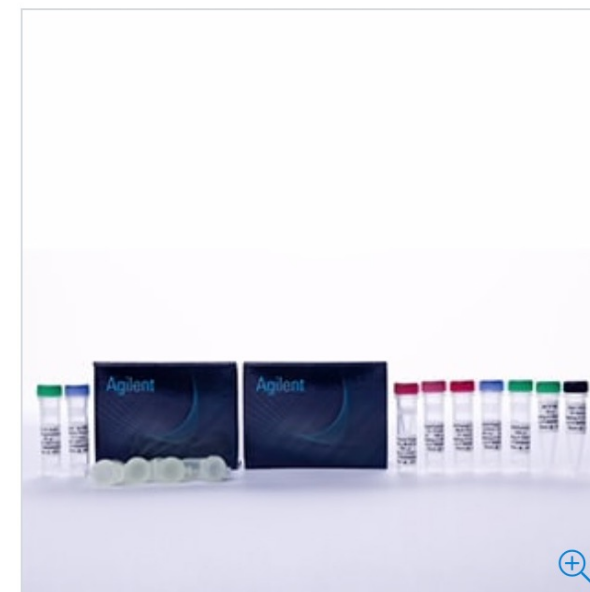
SureSelect Custom DNA Target Enrichment Probes

RUO

Agilent NGS Custom Target Enrichment Probes can be utilized to design customized hybrid capture panels to target any coding or non-coding regions of the genome or the transcriptome. Enabled by the SureDesign custom design tool, you can design and order any custom panel within minutes. Combine your custom panel with one of the OneSeq CNV backbones to achieve a comprehensive solution to detect SNVs, indels and CNVs in one workflow.

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

PRODUCT DETAILS

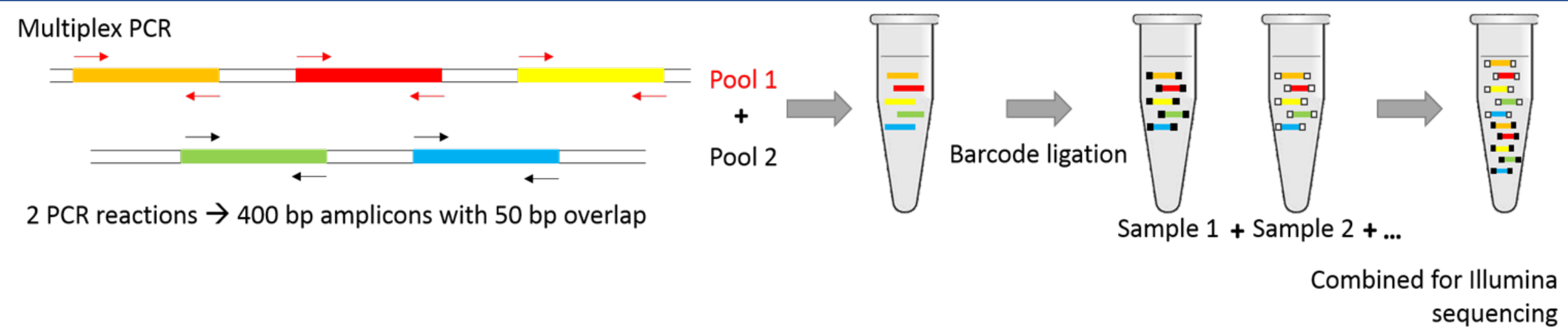


ⓘ This part number is not available for online sale. Please contact your local Agilent Representative for ordering information

Amplicon amplification



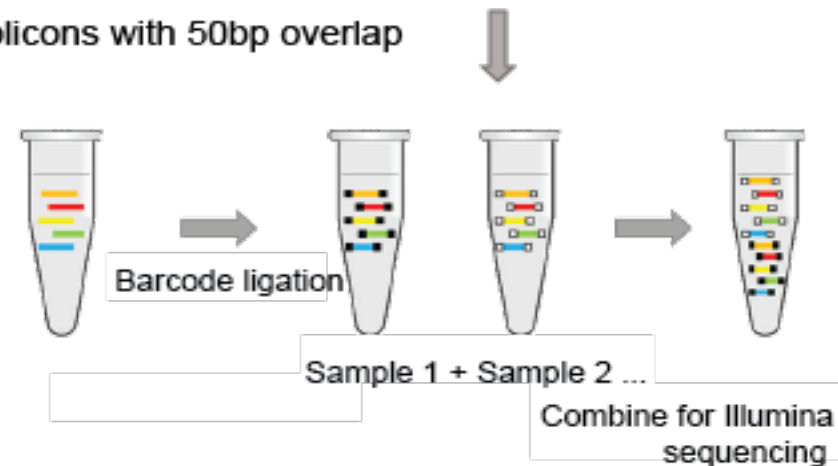
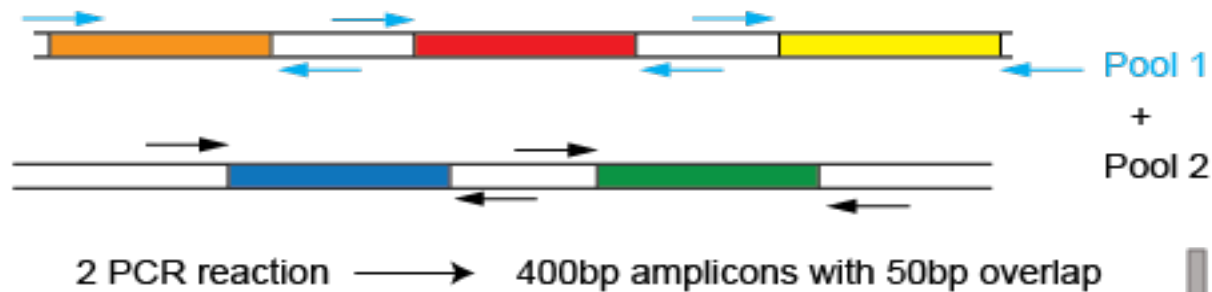
Tiled amplicon approach



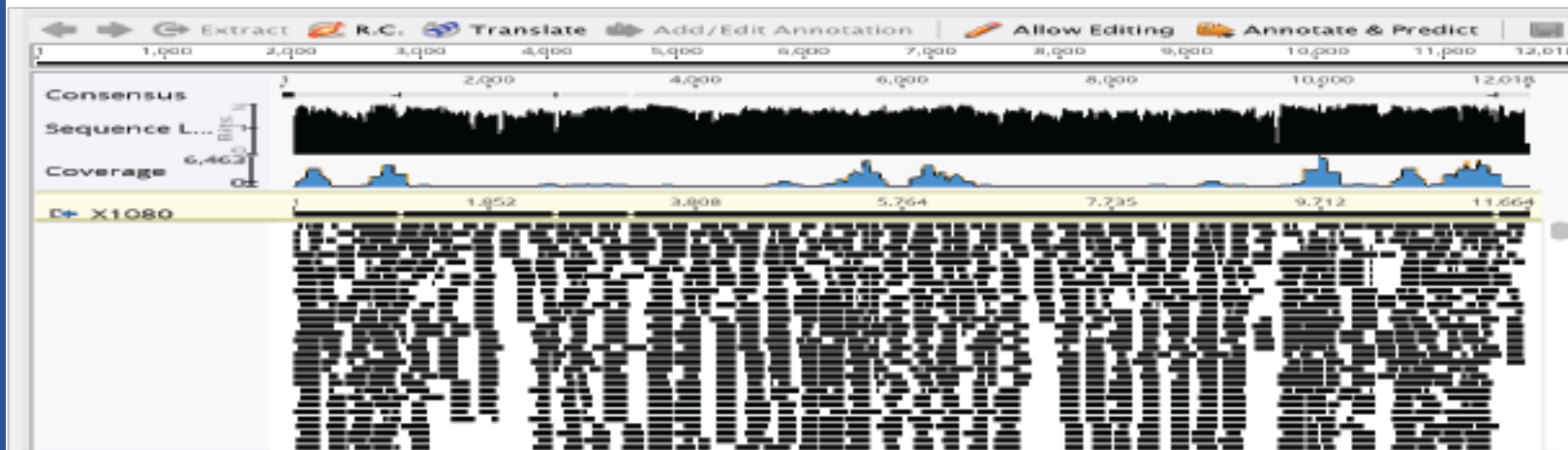
Quick et al. 2017, *Nature Protocols*

Tiled amplicon approach


Multiplex PCR



Whole genome coverage



Primer Design

 Report an issue

 Protocol

 GitHub

primalscheme

primer panels for multiplex PCR



Design a new scheme

Reset defaults



FASTA file...

Select a file...

Options



High-GC mode



Pinned

 Use the [standard protocol](#) for these settings.

Amplicon size

400

Min/max will be set at 5% either side of target.

Scheme name

e.g., nCov-400

A short name/prefix for your scheme, no spaces.

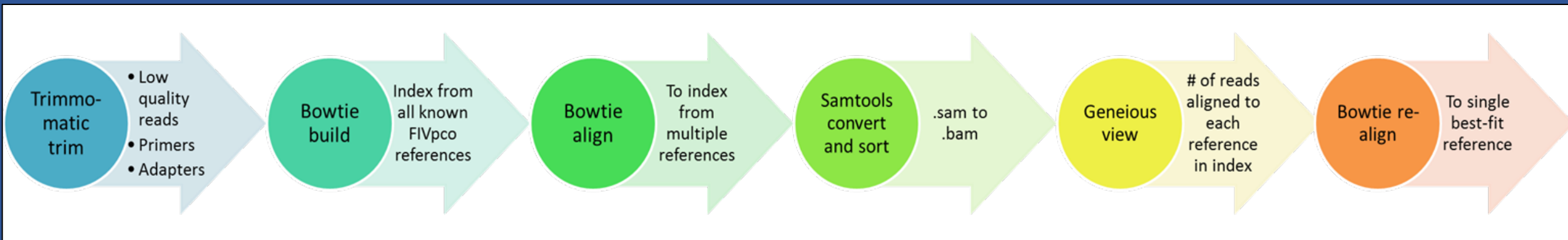
 Design scheme

View [demo inputs](#)

ARTIC primers

Name	Pool	Sequence
nCoV-2019_7_LEFT_alt0	nCoV-2019_1	CATTTGCATCAGAGGCTGCTCG
nCoV-2019_7_RIGHT_alt5	nCoV-2019_1	AGGTGACAATTTGTCCACCGAC
nCoV-2019_9_LEFT_alt4	nCoV-2019_1	TTCCCACAGAAGTGTTAACAGAGG
nCoV-2019_9_RIGHT_alt2	nCoV-2019_1	GACAGCATCTGCCACAACACAG
nCoV-2019_15_LEFT_alt1	nCoV-2019_1	AGTGCTTAAAAAGTGTAAGTGCCT
nCoV-2019_15_RIGHT_alt3	nCoV-2019_1	ACTGTAGCTGGCACTTTGAGAGA
nCoV-2019_21_LEFT_alt2	nCoV-2019_1	GGCTATTGATTATAAACACTACACACCCT
nCoV-2019_21_RIGHT_alt0	nCoV-2019_1	GATCTGTGTGGCCAACCTCTTC
nCoV-2019_45_LEFT_alt2	nCoV-2019_1	AGTATGTACAAATACCTACAACCTTGTGCT
nCoV-2019_45_RIGHT_alt7	nCoV-2019_1	TTCATGTTGGTAGTTAGAGAAAGTGTGTC
nCoV-2019_89_LEFT_alt2	nCoV-2019_1	CGCGTTCCATGTGGTCATTCAA
nCoV-2019_89_RIGHT_alt4	nCoV-2019_1	ACGAGATGAAACATCTGTTGTCACT

From sequences to genomes





Quick et al. 2017, *Nature Protocols*

Variant analyses

Keys for variant analyses is including a technical replicate and achieving sufficient depth of coverage (~400x)

An amplicon-based sequencing framework for accurately measuring intrahost virus diversity using PrimalSeq and iVar

[Nathan D. Grubaugh](#) , [Karthik Gangavarapu](#) , [Joshua Quick](#), [Nathaniel L. Matteson](#), [Jaqueline Goes De Jesus](#), [Bradley J. Main](#), [Amanda L. Tan](#), [Lauren M. Paul](#), [Doug E. Brackney](#), [Saran Grewal](#), [Nikos Gurfield](#), [Koen K. A. Van Rompay](#), [Sharon Isern](#), [Scott F. Michael](#), [Lark L. Coffey](#), [Nicholas J. Loman](#) & [Kristian G. Andersen](#)

[Genome Biology](#) **20**, Article number: 8 (2019) | [Cite this article](#)

12k Accesses | **68** Citations | **52** Altmetric | [Metrics](#)

Summary

Hybridization - benefits

- Multiple pathogens
- Diagnostics
- Baits can be generated commercially

Target amplicon -benefits

- Poor sample quality
- Relatively inexpensive
- High depth of coverage
- Can use cheaper sequencing approach

Summary

Hybridization - Challenges

- Needs to be known or close to known pathogen (vs. metagenomics)
- Relatively expensive

Target amplicon - Challenges

- Issue of PCR artifact
- No “true” haplotypes