Illumina RNA Prep Enrichment with Viral Surveillance Panel (VSP)

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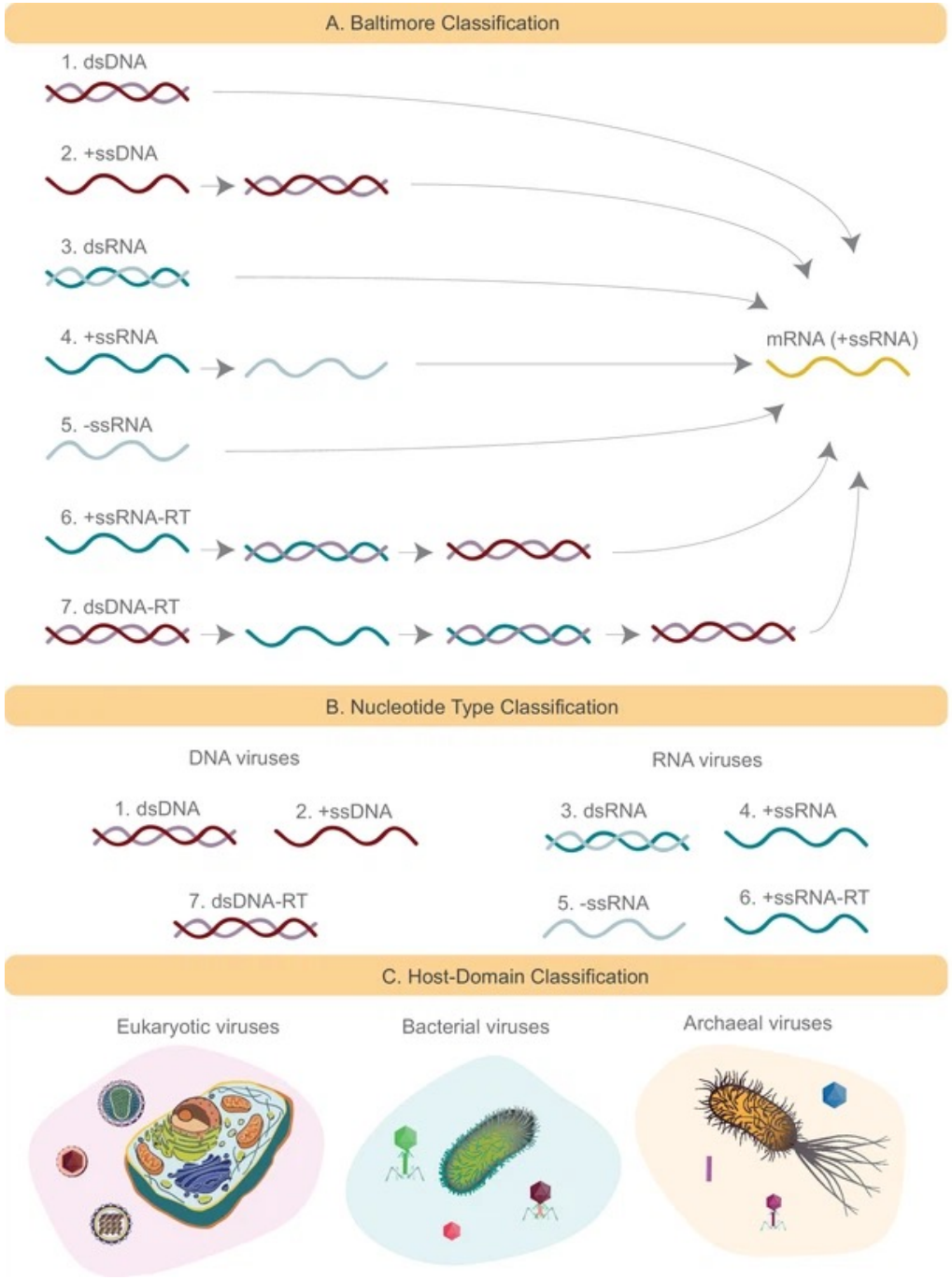
Significance of Detecting and Monitoring Viral Pathogens for Public Health and Research

- Early Detection
- Disease Surveillance
- Public Health Interventions
- Understanding Transmission Dynamics
- Epidemiological Research
- Antiviral Development
- Vaccine Development

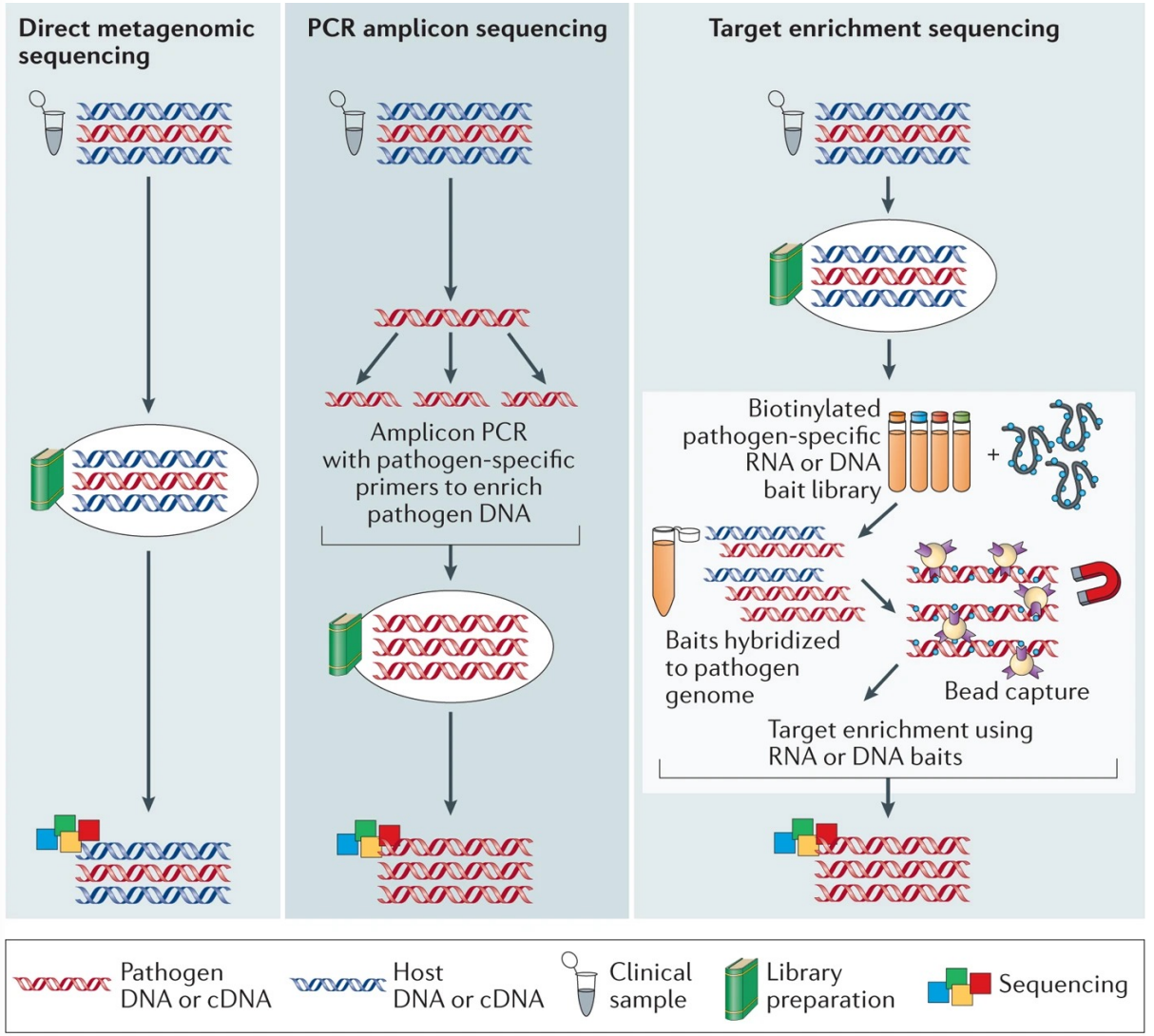
Challenges of Sequencing Viral Genomes

- Genetic Diversity
- Low Abundance
- Contamination and Background Noise
- Viral Genome Complexity
- Viral Genetic Variability
- Primer Design and Target Enrichment
- Bioinformatics Analysis



Viral Genome Sequencing Technologies

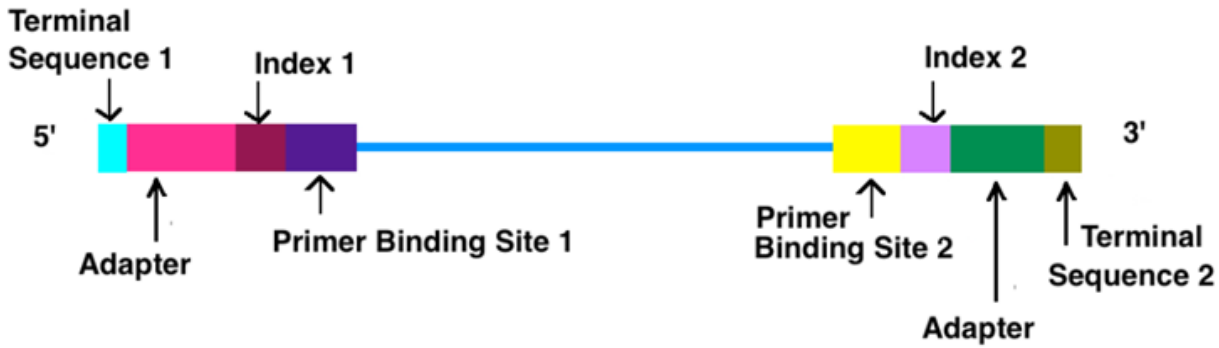
1. Amplicon Sequencing
2. **Target Enrichment/ Hybrid Capture**
3. Metagenomic Sequencing



Overview of Illumina RNA Prep Enrichment

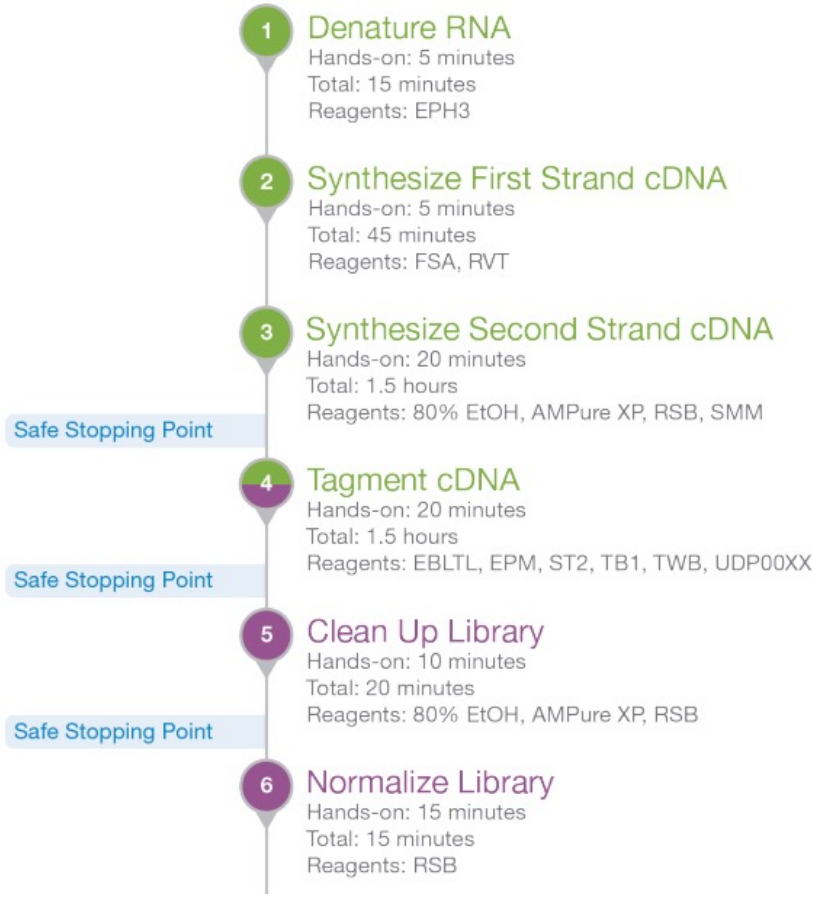
Uses **On-Bead Tagmentation technology** followed by **Hybrid-capture Target enrichment**

Exceptional capture efficiency and coverage uniformity

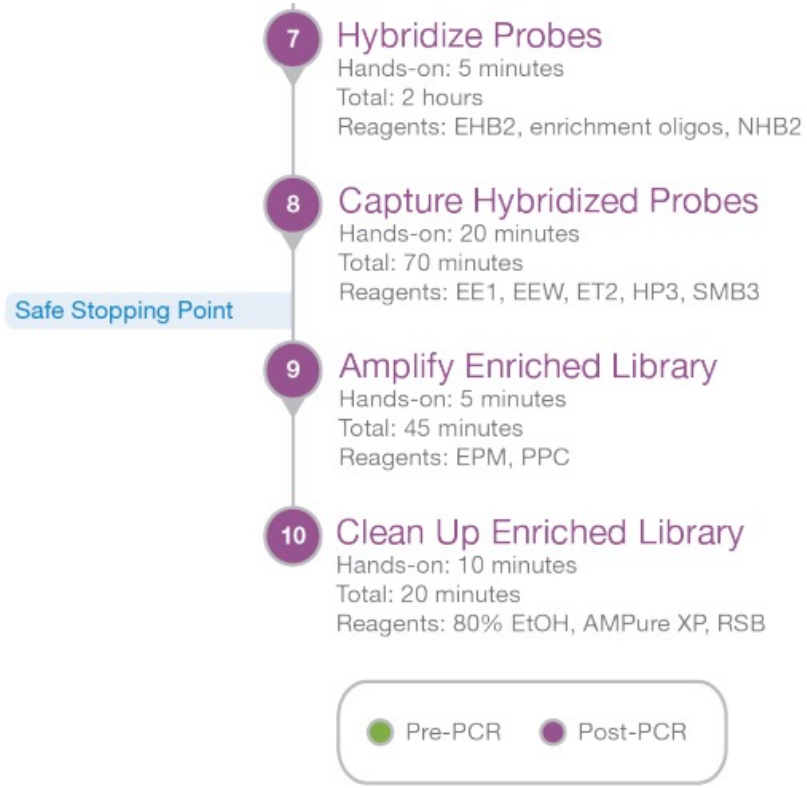


Illumina RNA Prep with Enrichment Workflow

Pre-enrichment library prep



Enrichment



cDNA Synthesis

NA Input	Total Nucleic Acid
Quantification	Fluorescent method
Qualification	Fragment analysis
DNase treatment	Omit

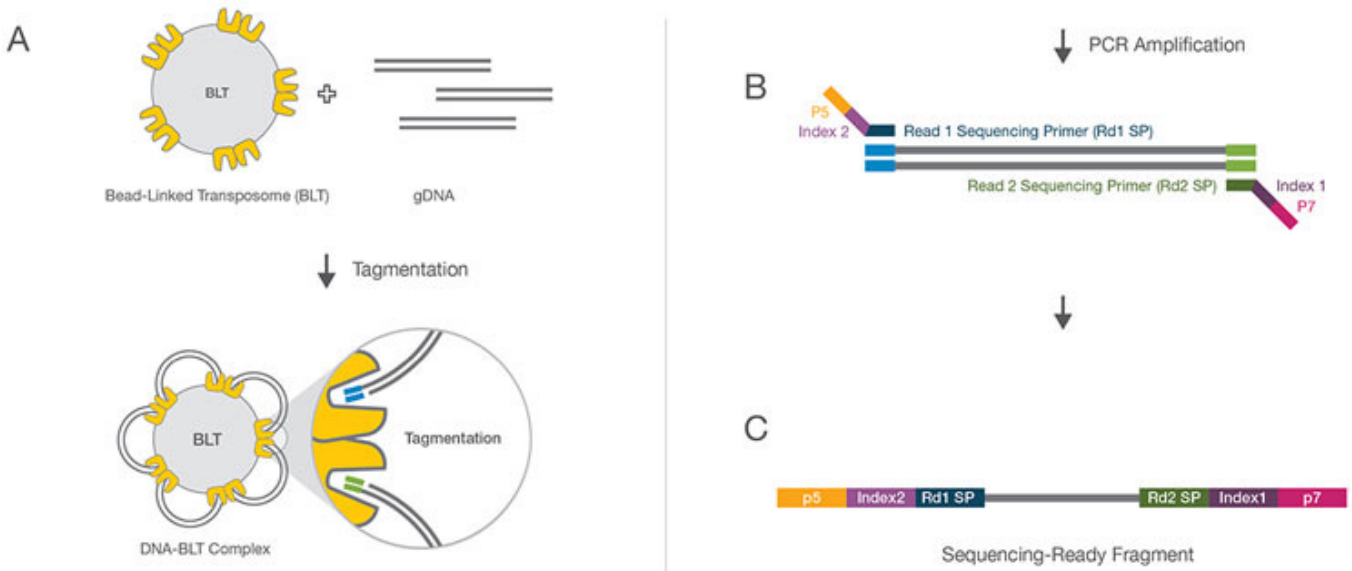
RNA Handling Best Practices

- Avoid multiple freeze-thaws
- Avoid extended poses until RNA is converted into cDNA
- Keep thawed RNA reagents on ice
- Preheat the thermocycler before each step

Tagment and Amplify cDNA

Best Practices for using beads

- Bring to room temperature before use
- Fully resuspend
- Prepare fresh 80% ETOH
- Do not allow beads to dry
- Do not freeze; store at +4°C



https://www.illumina.com/techniques/sequencing/ngs-library-prep/tagmentation.html

Normalize-Prepare For Enrichment

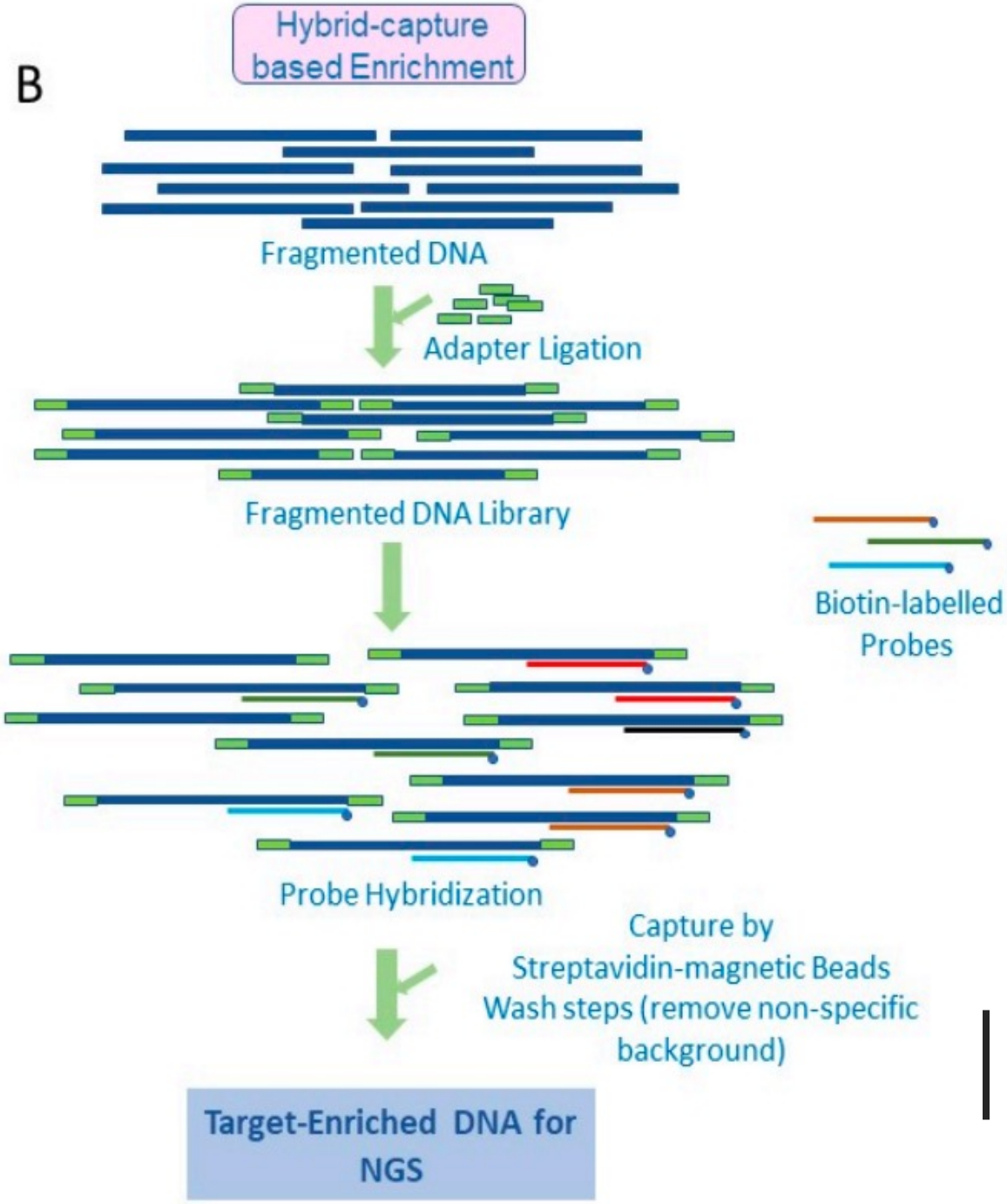
Quantification and QC

- Quantify libraries by Qubit dsDNA BR Kit
- Optional: QC libraries with Bioanalyzer
  - Average library size- ~380-450 bp

Pooling for enrichment

- Pool 1 or 3 libraries only
- 1-plex - dilute 200ng to a volume of 7.5µl
- 3-plex- dilute 200ng of each library to a volume of 2.5µl

Hybrid-capture Target enrichment



Hybridize Probes and Capture

Pooling and Hybridization Best Practices

Preheat microheating system to 50°C

Keep NHB2 buffer warm to prevent precipitation

Add Hybridization reaction reagents in listed order

Hybridization time: 24 hours

Capture and Wash Best Practices

- Ensure the use of correct beads: SMB3
- Thermal cycler runs continuously
- Prepare Fresh Elution Master Mix
- Transfer wash is critical, do not skip!

Viral Surveillance Panel

Table 1: Included on the Viral Surveillance Panel.[1]

Adenovirus	Hepatitis B virus	Parechovirus
Aichivirus	Hepatitis C virus	Parvovirus
Astrovirus	Hepatitis E virus	Poliovirus
Chapare virus	Human Immunodeficiency Virus 1	Polyomavirus
Chikungunya virus	Human Immunodeficiency Virus 2	Respiratory syncytial virus
Coronavirus-229E	Influenza A virus	Rhinovirus
Coronavirus-HKU1	Influenza B virus	Rift Valley fever virus
Coronavirus-OC43	Japanese encephalitis virus	Rotavirus
Coronavirus-NL63	Junin virus	Rubella virus
Coxsackievirus	Kyasanur Forest disease virus	Sabia virus
Crimean-congo haemorrhagic fever virus	Lassa fever virus	Salivirus
Dengue virus 1	Lujo hemorrphagic fever virus	Sapovirus
Dengue virus 2	Machupo virus	SARS-COV
Dengue virus 3	Marburg virus	SARS-COV-2
Dengue virus 4	MERS-CoV	Tick-borne encephalitis virus
Eastern equine encephalitis virus	Metapneumovirus	Torque Teno virus
Ebola virus	Monkeypox virus	Variola virus
Enterovirus	Nipah virus	Venezuelan equine encephalitis virus
Guanarito virus	Norovirus	West Nile virus
Hantavirus	Omsk hemorrhagic fever virus	Western equine encephalitis virus
Hendra henipavirus	Oncolytic human papillomavirus	Yellow fever virus
Hepatitis A virus	Parainfluenza virus	Zika virus

Thank you