

AccuPlex™ Recombinant Virus Technology – A tool to generate reference material for pathogenic virus

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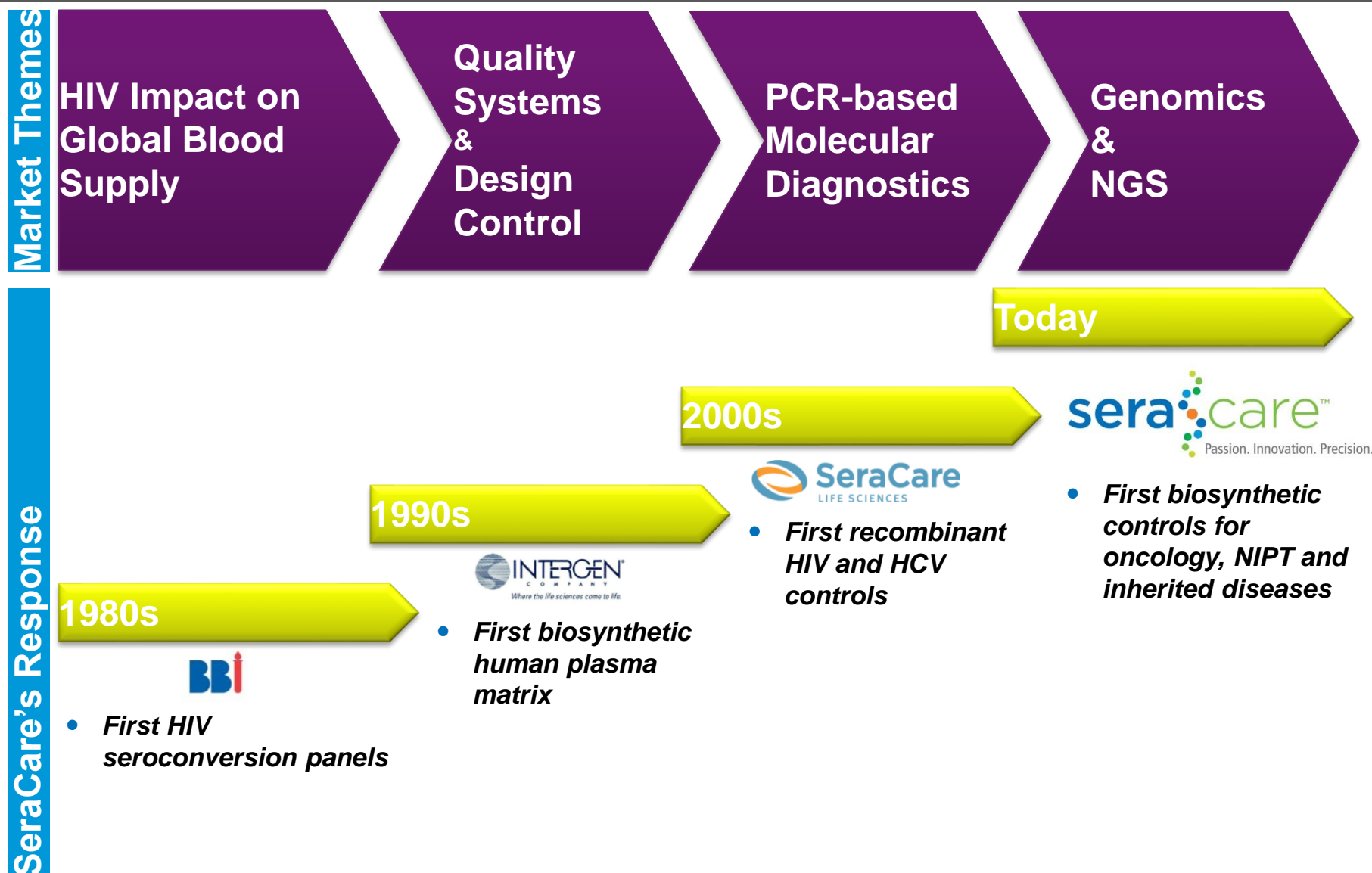
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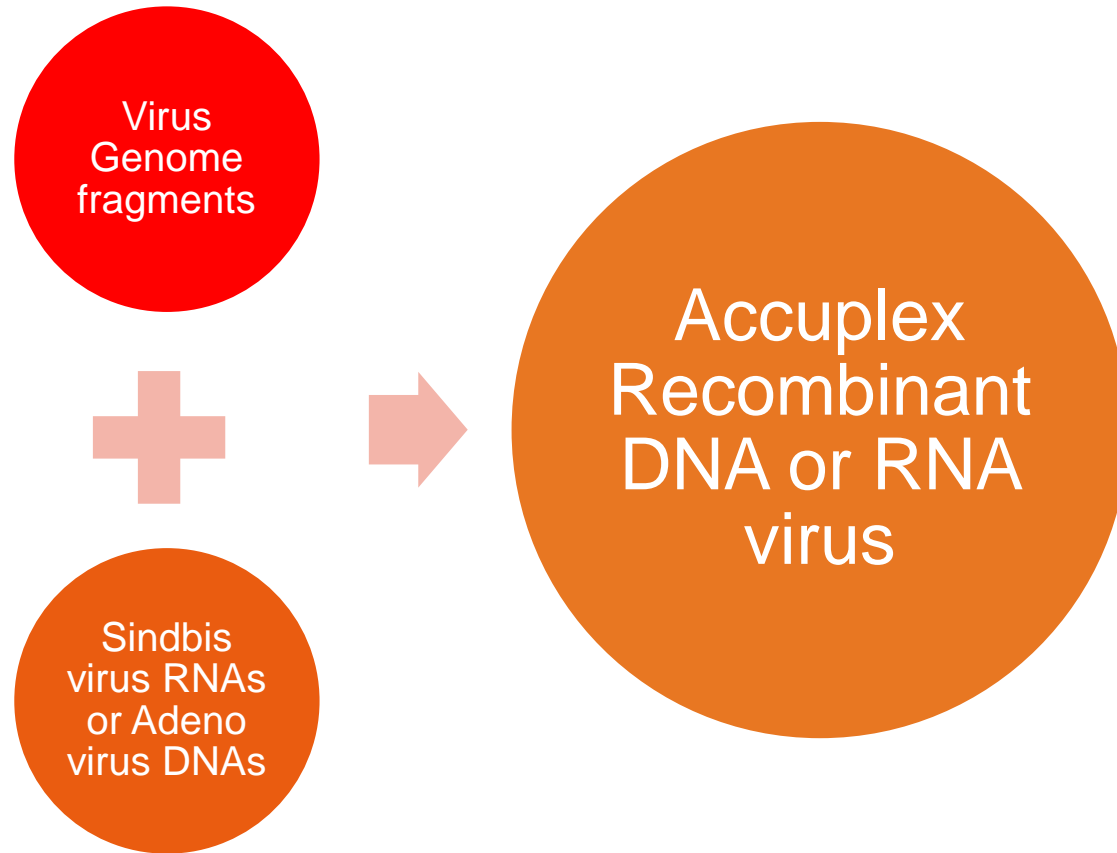
Advancing Diagnostics for More Than 30 Years



Accuplex™ - Recombinant Virus Technology

- LGC-SeraCare developed proprietary AccuPlex recombinant virus technology that is customizable and scalable
- This technology can be used to develop products for infectious disease applications for both RNA and DNA viruses.
- Sindbis AccuPlex technology is used for manufacture of RNA viruses
- Adeno AccuPlex technology is used for manufacture of DNA viruses
- This technology can be utilized to make viral reference material that can be used for assay development, internal calibrator and daily run controls.
- This technology can be used to generate viruses that are hard to source, highly infectious and viruses that cannot be cultured easily in the lab

Accuplex™, the recombinant DNA or RNA virus

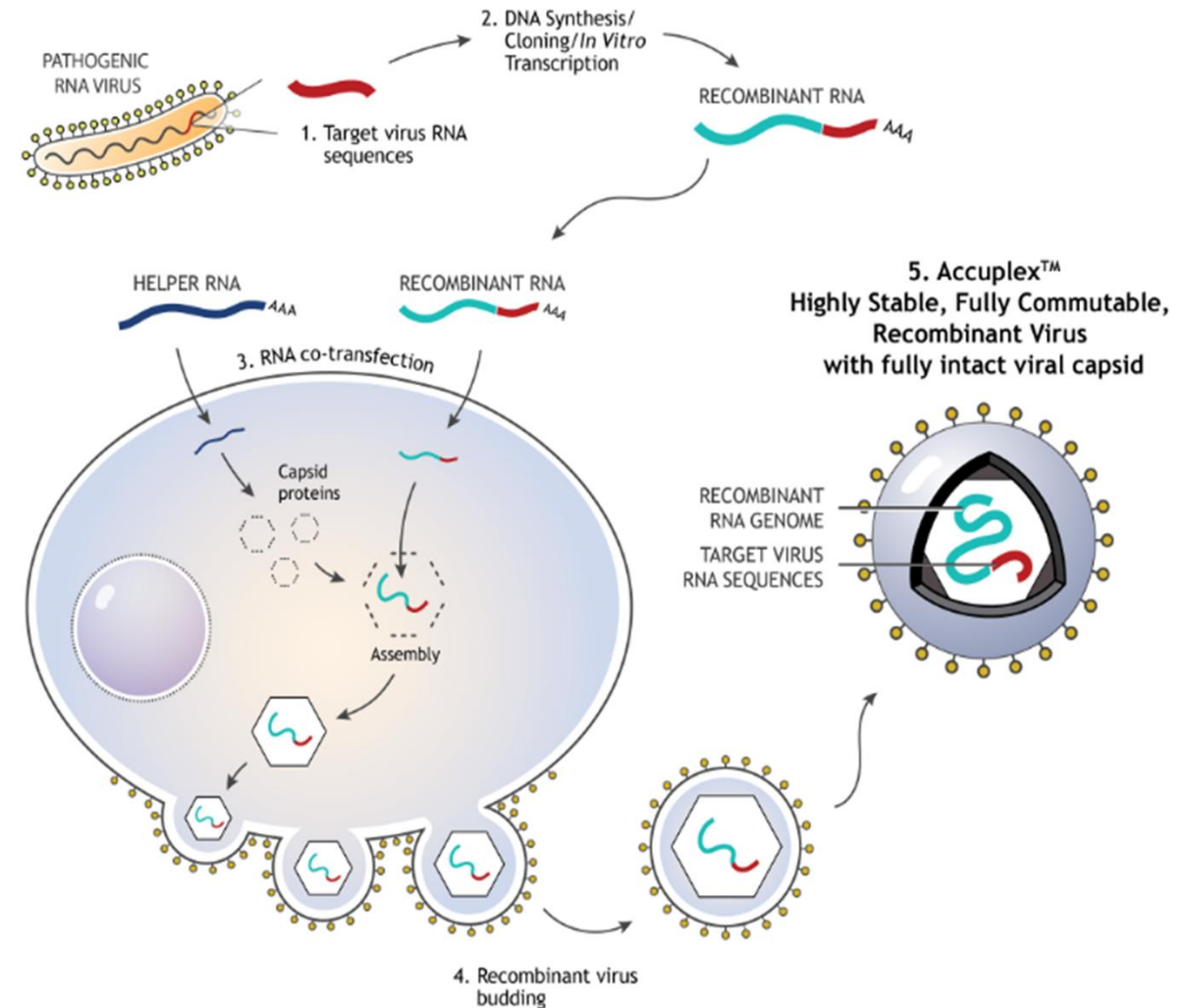


Replication Defective Recombinant RNA Viruses

- Sindbis virus is an Alpha virus and is used for generating recombinant RNA virus
- Contains a ~11.7kb single-stranded positive sense genomic RNA
- RNA encodes both structural proteins (for capsid assembly and viral budding) and nonstructural proteins (such as the replication enzymes).
- Separation of the viral genome into two ORF allows for relatively easy manipulation of the viral genome
- The structural protein ORF can be replaced with gene of interest.
- Genes for the 4 structural proteins required for packaging of the Sindbis viral genome can be moved into a helper RNA transcript.

Accuplex™ Technology Overview

- Recombinant and helper RNAs are introduced into BHK-21 cells
- The helper RNA does not contain a packaging signal, so does not get incorporated into the assembled viral particles.
- The viral particles are replication defective because they do not bear the genetic information to produce the structural proteins.
- Virus buds from cells and is collected in the culture media
- Virus is heat treatment for additional safety
- Copy number is assigned to the recombinant virus using a sequence specific dPCR assay
- This technology yields high titer virus: ~1.0e8 cp/mL



Replication Defective Recombinant DNA Viruses

- Adenovirus vector system is used for making recombinant DNA viruses
- E1 promoter and ORF is deleted in recombinant DNA Vector
- Recombinant virus will only replicate in E1 complementing cell line only
- Proprietary vector is generated into which the sequence of interest is cloned
- The linearized DNA is introduced into HEK-293 cells, viral particles are assembled
- Virus is collected from the cell culture
- Virus is heat treatment for additional safety
- Copy number is assigned to the recombinant virus using a sequence specific dPCR assay

Essential Benefits to AccuPlex Recombinant Viruses

- **Full Process Control**
 - Evaluates entire molecular assay PROCESS from extraction thru amplification/detection
 - Can be used as Internal control/calibrator
- **Mammalian and molecular complexity**
 - Mimics patient sample
 - Size of the genome is large so represents the target virus genome complexity
- **Noninfectious**
 - Sindbis Vector - Deletion of genes in the Sindbis genomic RNA coding for the structural proteins generates a replication defective virus
 - Adenovirus Vector- Deletion of E1 promoter and ORF means the virus can only replicate in E1 complementing cell lines
 - Heat treated to further assure noninfectious
 - Additional stop codons can be introduced as safety feature during the design phase
- **Stable**
 - Stable - frozen, 4C and dry storage
- **Accepts up to 4kb insert**
 - Allows significant multiplex-ability
- **No licensing fees or royalties**

Recombinant Virus Process

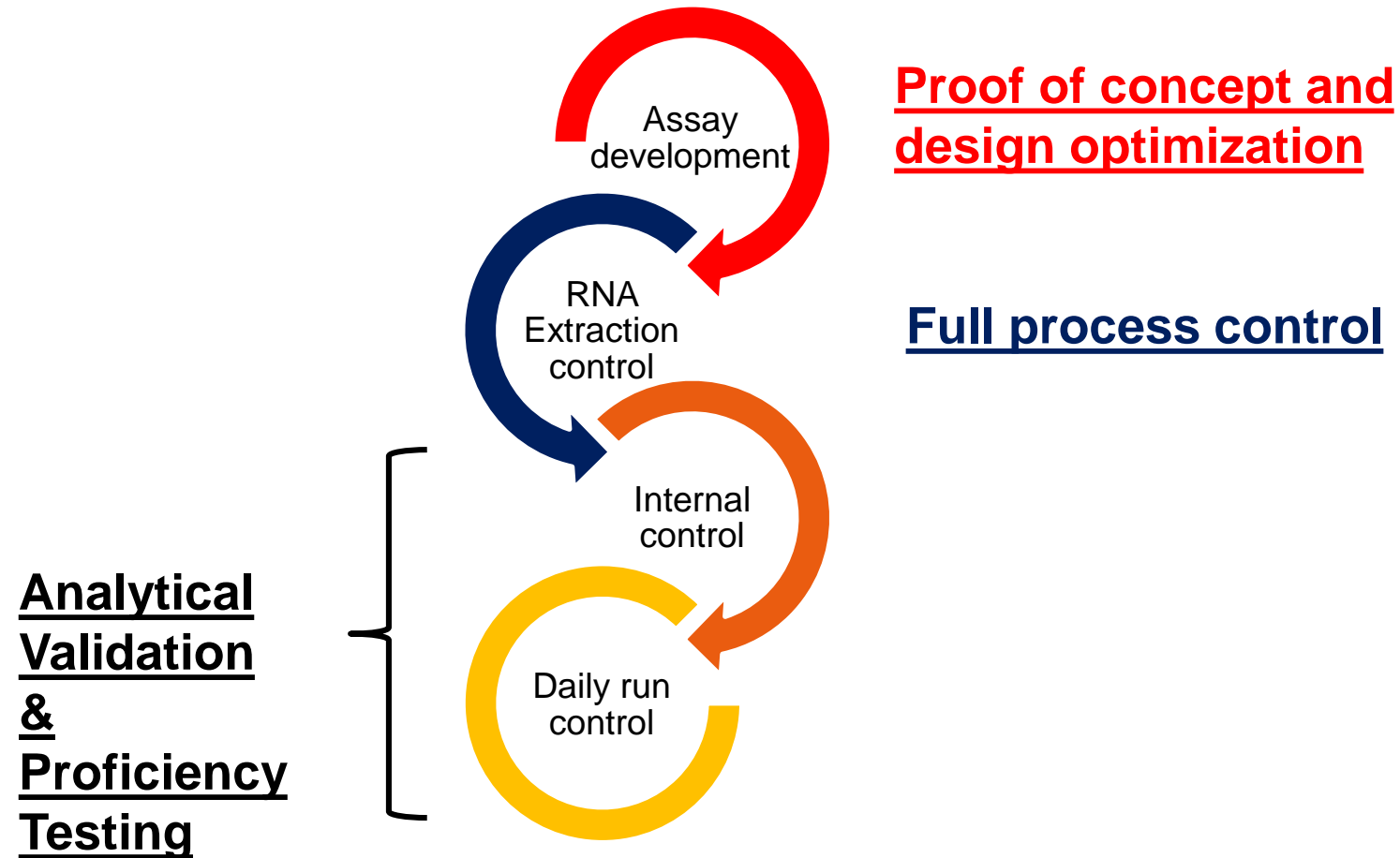
Phase I:

- Sequence *design*
- Sequence synthesis
- Small scale plasmid DNA

Phase II:

- Recombinant virus production
- Purification (Optional)
- Quantitation
- Bulk/dispense

What is Accuplex Recombinant Virus Used for?



AccuPlex™ is the closest to patient-like QC material

Technology	Packaged Insert Size	Analyte	Morphology/ Protein Coat	Complex Viral Structure	True Process Control
SeraCare AccuPlex	-4kb	Mammalian virus	Lipid bilayer	✓	✓
Armored RNA™	-1kb	Bacterial virus	Protein, no lipid		
MS-2	-1kb	Bacterial virus	Protein, no lipid		
Plasmid DNA	-10kb	DNA	No protein coat		
Naked RNA	-5kb	RNA	No protein coat		
Human Sample	Complete genome	Virus	Lipid bilayer	✓	✓

Accuplex™ Recombinant Virus Products

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Products Using AccuPlex Technology

- AccuPlex™ Zika Virus
- Accuplex™ Ebola virus
- Accuplex™ Norovirus
- Accuplex™ Dengue virus
- Accuplex™ MERS virus
- Accuplex™ Chikungunya virus
- SeraSeq HIV DR and Tropism – 47 mutations
- Accuspan HCV Linearity Panel
- Accuplex™ Rare Genotypes – HIV and HCV
- Accuplex™ FluA/B and RSV

AccuPlex Zika Reference Material

- Four recombinant AccuPlex viral particles are manufactured
- The copy number is assigned based on an inhouse developed dPCR assay
- All 4 recombinant virus are mixed, normalized and formulated in Basematrix at 1.0E+05cp/mL (dPCR assay)
- Positive on Roche Cobas and Aptima assays
- Fill volume - 250uL
- Product format
 - All 4 constructs in one tube
 - Individual constructs will be available as needed

Recombinant HIV Virus - HIV Drug Resistant

- Seraseq HIV-1 Drug Resistance and Tropism Reference Material utilizes AccuPlex RNA technology.
- The reference material contains PRO/RT/INT regions which correspond to nucleotides 1900-5400 in HXB2 reference strain (Genbank K03455) and envelope regions which correspond to 6300-7825.
- The reference material is a mixture of recombinant viruses and contains 47 variants in the PRO/ RT/ INT and Envelope regions at 3 different mutation frequencies (target of 1%, 5% and 20%).
- The variants in the reference material were selected based on their key role in antiretroviral drug resistance.
- Used as a reference material for NGS and sanger sequencing based assays

HIV DR and Tropism Mutations

Gag Region	Protease Gene	RT Gene (NRTI)	RT Gene (NNRTI)	Integrase Gene
H219Q (CAT to CAA)	L24I (TTA to ATA)	M41L (ATG to TTG)	L100I (TTA to ATA)	T66I (ACA to ATA)
G381S (GGC to AGC)	D30N (GAT to AAT)	K65R (AAA to AGA)	K101E (AAA to GAA)	L74M (CTG to ATG)
	V32I (GTA to ATA)	D67N (GAC to AAC)	K103N (AAA to AAC)	E92Q (GAA to CAA)
	M46I (ATG to ATA)	T69S (ACT to TCT) insert 2 Serines	V106A (GTA to GCA)	T97A (ACA to GCA)
	I47L (ATA to CTA)	K70R (AAA to AGA)	V108I (GTA to ATA)	E138A (GAA to GCA)
	G48V (GGG to GTG)	L74V (TTA to GTA)	Y181C (TAT to TGT)	G140S (GGA to TCA)
	I50V (ATT to GTT)	F77L (TTC to CTC)	Y188L (TAT to TTA)	Y143R (TAC to CGC)
	I54M (ATC to ATG)	Y115F (TAT to TTT)	G190A (GGA to GCA)	Q148H (CAA to CAC)
	G73S (GGT to GCT)	F116Y (TTT to TAT)	P225H (CCT to CAT)	N155H (AAT to CAT)
	L76V (TTA to GTA)	Q151M (CAG to ATG)	M230L (ATG to CTG)	
	V82A (GTC to GCC)	M184V (ATG to GTG)		
	I84V (ATA to GTA)	L210W (TTG to TGG)		
	N88D (AAT to GAT)	T215Y (ACC to TAC)		
	L90M (TTG to ATG)			

Accuplex Recombinant Virus - Stability

- Accuplex Recombinant virus is stable at 4C and -20C for 25 months
- Stable under stress conditions
 - 22 days at 37 C
 - 5 freeze thaw cycles
 - Dry storage

Summary

- AccuPlex technology offers a safe and flexible alternative for creation of reference material
- This enables packaging of either full viral genomes or specific target amplification sequences for molecular testing.
- Viral particles are enveloped in a lipid bilayer
- AccuPlex technology generates full-process controls that monitor assay performance from extraction to detection.
- AccuPlex reference material is non-infectious. The viral particles are replication defective and heat treated to ensure safety.
- AccuPlex reference material is highly stable, and is particularly amenable to diagnostic applications in settings where cold storage is challenging

Thank you!

