USE OF RECOMBINANT VIRUS TECHNOLOGY TO PRODUCE NON-INFECTIOUS, MULTIPLEXED RNA OR DNA VIRUS WHOLE PROCESS CONTROLS

Catherine E. Huang, Rajeswari Vemula, Jessica Dickens, Mikir Patel and Bharathi Anekella
SeraCare Life Sciences, Gaithersburg, MD

AMP 2015
November 5-7, 2015
Austin, Texas

INTRODUCTION

Non-infectious, whole process controls are needed by laboratories and test developers as they design, manufacture and validate new diagnostic assays. New assays are needed to prepare for emerging viruses, such as the recent outbreak of Ebola 2014 virus, to combat drug resistance in highly adaptive viruses such as HIV-1, and to advance diagnostics for non-culturable viruses such as HBV. SeraCare's AccuPlex™ technology uses recombinant viruses containing target sequences from the pathogen of interest and has many advantages as a NAT quality control material. First, it mimics clinical samples because it undergoes the entire extraction procedure. Second, it is non-infectious and ensures safety for lab personnel. Finally, AccuPlex™ recombinant viruses can be highly multiplexed, and have extended stability at 2 - 8 ˚C and at ambient temperature.

MATERIALS AND METHODS

Recombinant viruses are produced as in Figures 1 and 2. The AccuPlex™ rEbola was made using portions of Ebola 2014 virus nucleoprotein (NP), envelope glycoprotein (GP), and VP24 genes. Similarly, recombinant virus was produced bearing HIV-1 sequence with 48 mutations in Protease, Reverse Transcriptase, and Integrase genes, which represent the clinically relevant mutations for drug resistance. This multi-mutant virus was mixed at low levels with WT recombinant virus to form whole process controls for NGS minor variant detection. SeraCare also developed AccuPlex™ recombinant DNA virus as an internal control for HBV and CMV viral load assays.

QC of recombinant RNA viruses includes:

- Assay for contaminating DNA (incubation plus and minus DNAse and demonstrating no change in viral titer by TaqMan® assay).
- Verification that there is no non-encapsulated RNA is performed (incubation plus and minus RNAse and demonstrating no change in titer by TaqMan® assay).
- Verification that there are no viruses that do not bear the desired insert. ddPCR™ is performed using a probe that recognizes the Sindbis (or adenovirus) vector sequences as well as a second probe that recognizes the sequence of interest. Concordance demonstrates that all viral particles contain the sequence of interest.

	Vector Specific probe Copies/mL	Insert Specific Probe Copies/mL
HIV Wild Type	5.13E+05	4.32E+05
HIV Multi-Mutant	8.98E+05	7.35E+05

- QC of DNA viruses is similar

TECHNOLOGY

Sindbis Virus System – RNA Targets

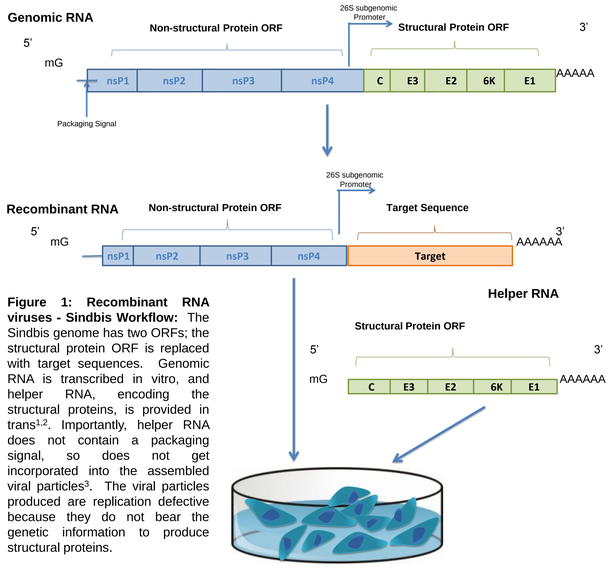

Figure 1: Recombinant RNA viruses - Sindbis Workflow: The Sindbis genome has two ORFs; the structural protein ORF is replaced with target sequences. Genomic RNA is transcribed in vitro, and helper RNA, encoding the structural proteins, is provided in trans[1,2]. Importantly, helper RNA does not contain a packaging signal, so does not get incorporated into the assembled viral particles[3]. The viral particles produced are replication defective because they do not bear the genetic information to produce structural proteins.

Adenovirus System – DNA Targets

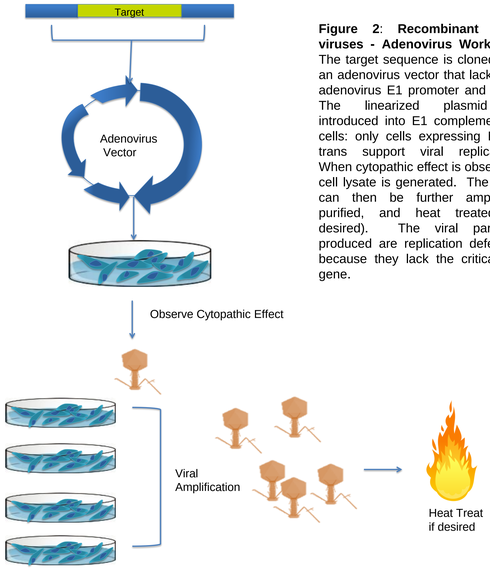

Figure 2: Recombinant DNA viruses - Adenovirus Workflow: The target sequence is cloned into an adenovirus vector that lacks the adenovirus E1 promoter and ORF. The linearized plasmid is introduced into E1 complementing cells: only cells expressing E1 in trans support viral replication. When cytopathic effect is observed, cell lysate is generated. The virus can then be further amplified, purified, and heat treated (if desired). The viral particles produced are replication defective because they lack the critical E1 gene.

APPLICATIONS

Whole Process Control

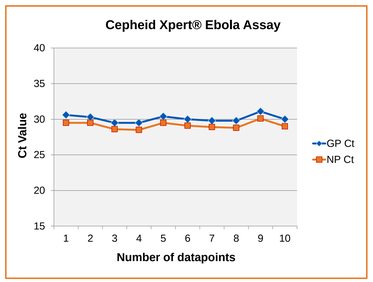

Figure 3: Whole process Control for pathogen Detection: SeraCare developed AccuPlex™ multiplex recombinant rEbola Reference Material, that targets the glycoprotein (GP) and nucleoprotein (NP) genes. Ten replicates were tested on Cepheid Xpert® Ebola Assay and all ten replicates were positive with Ct values between 28-30 for NP and 29-31 for GP.

NGS Pipeline Control

10% Mix			10% Mix			10% Mix		
Type	Mutation	Percentage	Type	Mutation	Percentage	Type	Mutation	Percentage
Protease	I47L	10.10%	NRTI	M184V	14.41%	NNRTI	M230L	11.46%
Protease	V82A	10.57%	NRTI	T69S	7.51%	NNRTI	P225H	12.63%
Protease	L90M	12.22%	NRTI	Q151M	9.51%	NNRTI	G190A	14.78%
Protease	I54M	12.30%	NRTI	D67N	11.37%	NNRTI	Y181C	15.91%
Protease	M46I	9.24%	NRTI	F77L	17%	NNRTI	K103N	9.67%
Protease	V32I	10.21%	NRTI	M41L	9.54%	NNRTI	K101E	8.45%
Protease	N88D	11.50%	NRTI	K219Q	11%	NNRTI	V108I	8.61%
Protease	I50V	13.67%	NRTI	L74V	11.38%	NNRTI	V106A	9.62%
Protease	L76V	13.74%	NRTI	K65R	12.64%	NNRTI	L100I	8.46%
Protease	L24I	6.69%	NRTI	L210W	13.18%	NNRTI	Y188L	9.73%
Protease	I84V	10.32%	NRTI	F116Y	9.21%			
Protease	D30N	9.58%	NRTI	K70R	7.30%			
Protease	G48V	9.62%	NRTI	T215Y	8.03%			
Protease	G73S	10.14%	NRTI	Y115F	9.30%			

Figure 4: Quality Control for NGS Minor Variant Detection: HIV-1 Drug Resistance and Tropism Reference Materials to aid in NGS detection of minor drug resistance mutations. A prototype formulation at 10% variant frequency was analyzed using Illumina-based NGS technology on the Protease (PI) and Reverse Transcriptase (NRTI and NNRTI) genes. All of the expected HIV-1 drug resistant variants were detected, but variation in the mutant frequency shows variation in the sequencing or analysis pipeline

Internal Control

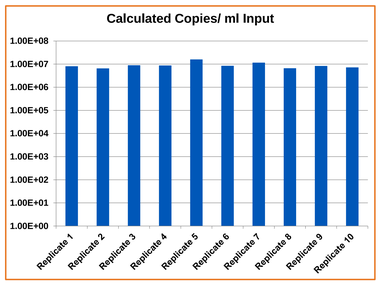

Figure 5: Internal Control in Viral Load Assay: A recombinant adenovirus was produced that bears an internal control sequence- a sequence that is amplified by the pathogen detection primers, but has a unique sequence recognized by an independent probe. Ten replicates of 1:100 diluted stock were tested via TaqMan® real time assay across multiple runs to demonstrate consistency of the internal control.

STABILITY

Real time studies were performed on AccuPlex™ recombinant virus diluted in defibrinated human plasma at room temperature, 4 ˚C and -20 ˚C. No downward trends were detected across the ten (10) months of storage, even for samples stored at ambient temperatures (Figure 6A). Stress stability was also performed at 37 ˚C (Figure 6B) and across multiple freeze thaws (Not shown), and the titer was stable. This demonstrates that the viral coat proteins form a stable protective barrier that prevents nucleases in complex clinical matrices such as plasma from degrading the target RNA sequence.

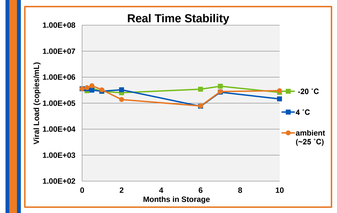

Figure 6A: Real Time Stability of recombinant Sindbis virus in defibrinated plasma at RT, 4 ˚C and -20 ˚C.

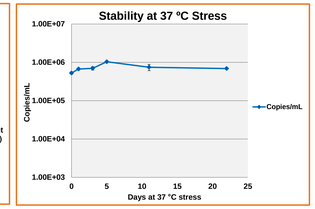

Figure 6B: Stability of AccuPlex™ rEbola GP/NP Reference at 37 ˚C

CONCLUSIONS

- AccuPlex™ recombinant technology can be used to make viruses that are non-infectious, and can be used to make reference materials that are highly stable.
- Reference materials closely mimic natural specimens: they are enveloped, mammalian viruses that require extraction.
- AccuPlex™ recombinant technology is being used to develop reference material for: Ebola, Chikungunya, Dengue, MERS CoV and Norovirus.

REFERENCES

1. Xiong, C., Levis, R., Shen, P., Schlesinger, S., Rice, C.M., & Huang, H.V.. (1989) Sindbis virus: an efficient, broad host range vector for gene expression in animal cells. *Science*, *243*(989), 1188-1191.
2. Altman-Hamamdzic, S., Groseclose, C., Ma, J.X., Hamamdzic, D., Vrindavanam, N.S., Middaugh, L.D. … Sallee, F.R. (1997). Expression of beta-galactosidase in mouse brain: utilization of a novel nonreplicative Sindbis virus vector as a neuronal gene delivery system. *Gene Therapy , 4*(8), 815–822.
3. Schlesinger, S. (2000). Alphavirus expression vectors. *Advances in Virus Research, 55*, 565-77.

ACKNOWLEDGEMENTS

We thank Jianlei Wu and Lequan Nguyen for contributions to this study and Alice Ku for help preparing the poster.