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Generation and in *vitro characterization* of reverse genetics influenza B system based on a swine *polI* promoter

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Introduction

Influenza A (IAV) and Influenza B (IBV) are enveloped viruses with a negative-sense, segmented, single-stranded RNA genome, with eight viral RNA (vRNA) segments that encode for at least 12-13 proteins (1). After receptor-mediated endocytosis, the viral ribonucleoprotein complex composed of vRNA, the nucleoprotein (NP), and three polymerase proteins (PB2, PB1, and PA) is released into cytoplasm and transported into the nucleus for transcription and replication (2).

Influenza viruses (IV) are considered a major pathogen in avian, swine and humans. Influenza infection results in significant economic loss for agricultural species, particularly in swine. Influenza infections in swine rank among the three major health problems in the industry. A previous report has shown that pigs can get infected with B/Brisbane/60/2008 (B/Bris) and develop influenza-like clinical signs and lung lesions, seroconverted, and successfully transmitted virus to direct contact pigs, therefore demonstrating that pigs are susceptible to infection with B/Bris (3).

Reverse genetics (RG) is the process of generating infectious virus using cDNA containing the viral genome of the desired strain. It is currently one of the most powerful tools to study IV. Based on the species specificity of the polymerase I promoter (*polI*), we sought to assess the efficiency of an RG plasmid carrying the swine *polI* (*spolI*) promoter to rescue IBV viruses in vaccine approved swine and in human cells.

General goal:

To assess the viability of a reverse genetics vector carrying the *spolI* promoter to rescue recombinant IBVs in swine and human origin cells.

Experimental Design

Cells: Human embryonic kidney 293T (HEK293T), pig kidney (PK-15) and Madin-Darby canine kidney (MDCK) cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS) (Sigma, St. Louis, MO), 2mM of L-glutamine (Sigma, St. Louis, MO) and 1% antibiotic/antimycotic solution (Sigma-Aldrich, St. Louis, MO). The cells were cultured at 37°C under 5% CO2.

Cloning: All eight B/Bris gene segments were amplified by PCR and then cloned into the RG vectors by performing ligation reactions using Quick ligation kit (New England BioLabs, MA). Ligation products were transformed into One Shot TOP10 chemically competent *E. coli* cells (Life Technologies). Colonies were screened by colony PCR to confirm the presence of the gene segments. Positive colonies were cultured in Luria Bertani media supplemented with ampicillin. Plasmids were then purified to be used for virus rescue.

Figure 1. The *hpolI* promoter in pDP2002, located between the *polII* promoter and the bovine growth hormone polyadenylation signal (BGH), was replaced by the *spolI* promoter in order to generate the pPig2012, the spol1 reverse genetic system.

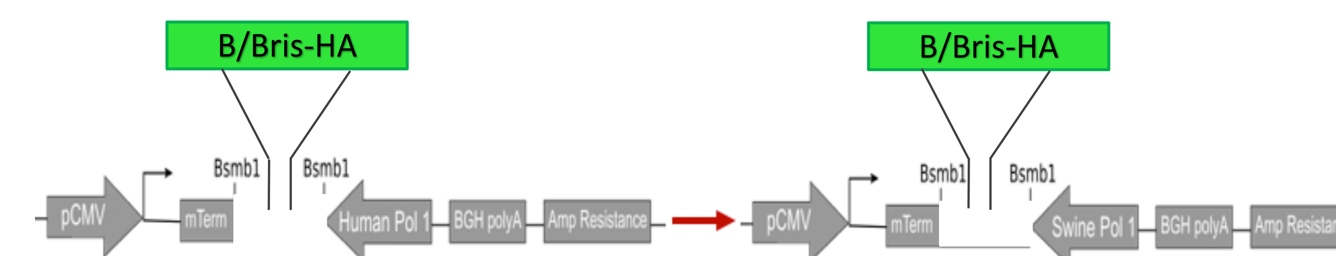

Figure 2. The B/Bris gene segments were subcloned between a RNA *polII* promoter (pCMV) and *hpolI* or the *spolI* to construct the full reverse genetics plasmid.

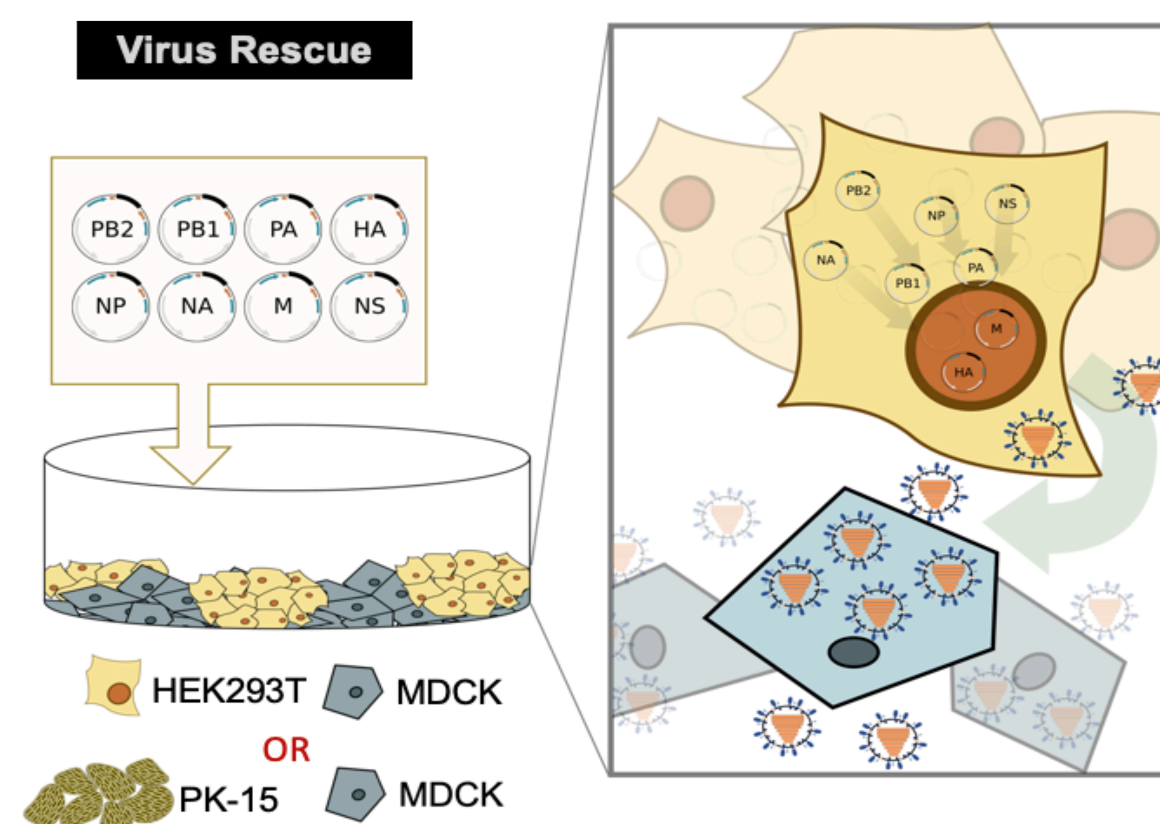

Figure 3. As shown in the Figure, the 8 plasmids containing the 8 segments of IV are transfected into the HEK293T cell, which produce a virus. Following, trypsin is added and needed, so the virus can infect the MDCK and continue with mass reproduction. While the use of the bi-directional vector is efficient in generation IVs de novo, previous reports showed that the *polI* is species specific, thus requiring the species of the *polI* to match the cell type.

Results

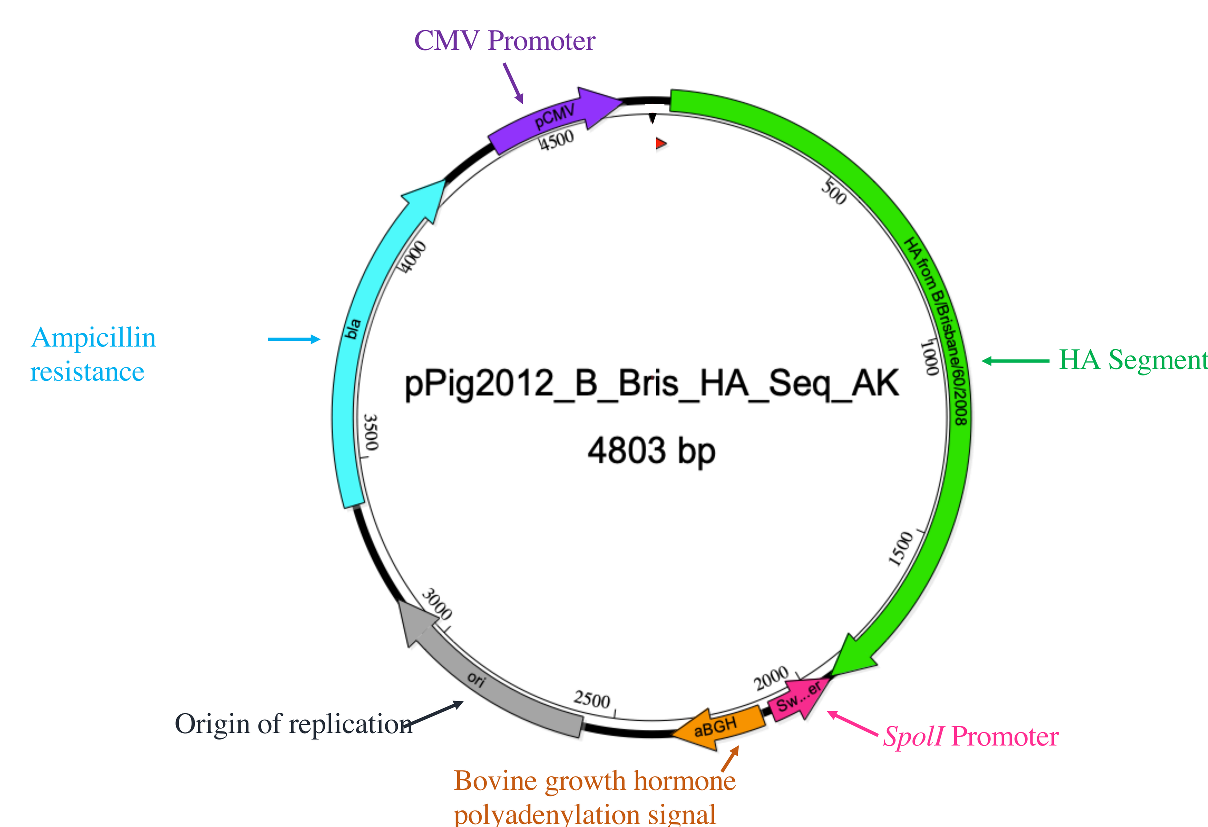

Figure 4. Plasmid map shows the HA segment cloned into the pPig2012 plasmid. We modeled our plasmid containing the *spolI* promoter, called pPig2012, in similar arrangement as pDP2002 with a major difference being the *spolI* promoter.

Transfection Rescue					Passage 1		
Plasmid Vector	Cell Type	hpt	HAU	$TCID_{50}$/mL (Log10)	hpt	HAU	TCID50/mL (Log10)
pPIG2012	PK-15/MDCK	120	64 - 256	6.39 ± 0.16^{a}	-	-	-
pDP2002	PK-15/MDCK	120	8 - 128	7.23 ± 0.28^{b}	-	-	-
pPIG2012	HEK293T/MDCK	120	0	$2.93 \pm 0.63^{\dagger}$	120	128 - 256	5.73 ± 1.73
pDP2002	HEK293T/MDCK	120	128 - 256	$6.42 \pm 0.72^{\ddagger}$	-	-	-

Superscript of letters or symbols indicate that the mean comparison between those two groups is signicantly different

Table 1. Hemagglutinin assay (HA) and virus titers by $TCID_{50}$/mL for the rescue of B/Bris in PK-15/MDCK and HEK293T/MDCK at 120 hours post transfection. HA and $TCID_{50}$ are shown for the blind passage of pPIG2012 in HEK293T/MDCK on the right.

Conclusions

- Our results establish that the *spolI* can be used to rescue IBV in vaccine-approved swine PK-15 cells and human origin HEK293T cells.
- Compared to the *hpolI*, the *spolI* was less efficient in HEK293T/MDCK cocultures but showed CPE after one passage in MDCK cells. This confirms our previous observations where the *spolI* is more efficient in swine cells in comparison with *hpolI* but less efficient in human cells.
- While the *spolI*-based reverse genetics system could be used as a new platform to produce LAIVs for swine, additional research is needed to explore the potential of rescuing recombinant viruses for vaccine seed stocks in the pPIG2012 (*spolI*) vector.
- In addition to potentially serving as reverse-genetics platform for swine vaccines, the *spolI* could be used to explore the potential for in vivo IV reverse genetics in swine.

References

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