MERCK ANIMAL HEALTH
TECHNICAL SERVICES BULLETIN
1004 Merck

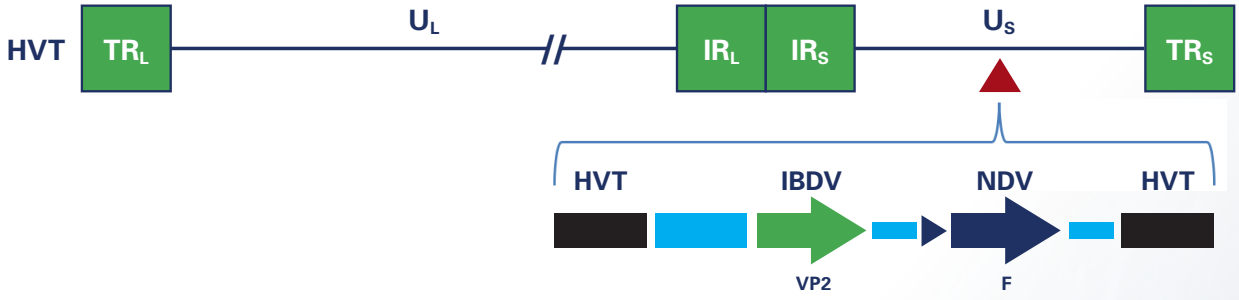
Construction of rHVT Vaccines: Questions and Answers reveal that Different Approaches can achieve Effective Constructs

INTRODUCTION

Recombinant HVT (rHVT) vaccines developed from the familiar HVT (herpesvirus of turkeys) vaccine virus have demonstrated excellent safety and efficacy in the field. From the original single-insert rHVT vaccines to the new multiple insert vaccines, rHVT vaccines from several biological companies have become well-accepted and frequently used in the poultry industry.

Lacking field efficacy data, some newly launched products have featured details of vaccine construction as a way to distinguish themselves from one another. The following questions and answers clarify key concepts about the construction of rHVT vaccines.

Figure 1. Schematic of the construction of rHVT vaccine



TR_L - terminal repeat long region
U_L – unique long region
IR_L – internal repeat long region
IR_S – internal repeat short region
U_S – unique short region
TR_S- terminal repeat short region

KEY POINTS

- There are many ways to construct an rHVT vaccine. Successful candidates will have the correct level of antigen expression, good replication in the chickens and genetic stability.
- The size of a promoter or the location of a promoter-ORF insert in the HVT genome does not make a vaccine candidate better or worse.
- The use of an IRES sequence in addition to a promoter-ORF sequence is simply another way to achieve a successful construct. Like those that use two promoter-ORF sequences, the candidates must be tested for antigen expression, replication in the chickens and genetic stability.
- The true success of an rHVT dual construct vaccine is determined by its ability to protect flocks against challenge with long duration of immunity. Protection against challenge indicates the correct balance and amount of antigen expression, while long duration of immunity indicates stability of the virus construct in the chicken.

WHEN CONSTRUCTING AN rHVT VACCINE, WE HEAR ABOUT "US" AND "UL" AND "ORF". WHAT ARE THEY?

- The U_S stands for "unique short" region of HVT
- The U_L stands for "unique long" region of HVT
- ORF stands for Open Reading Frame - an area of the genome capable of encoding for a protein. The ORF is recognized as the sequence of genetic material between a "start" and a "stop" site that a ribosome will recognize when it is creating a protein.
  - Some ORFs are essential for viral replication, but some are not.
  - A non-essential ORF can be replaced with an ORF that generates a protein of interest, like the F (fusion) protein of the Newcastle Disease virus.
    - The new protein will be recognized by the immune system, generating an immune response against that protein.
  - It is also possible to add an ORF between other ORFs to code for a desired protein.

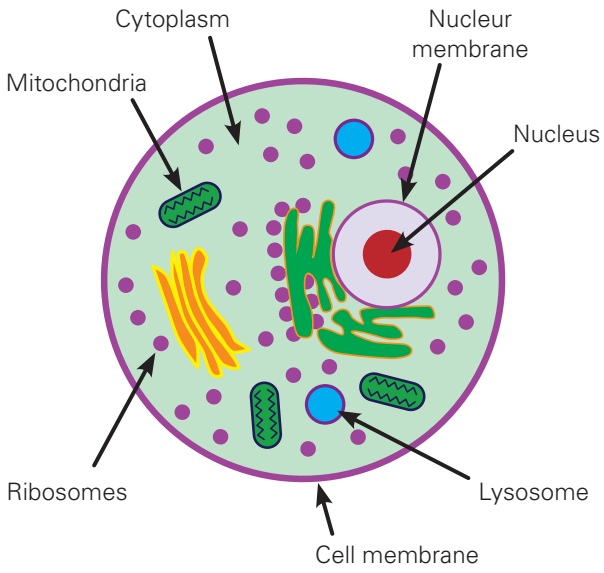
ORFs can be replaced or added in either the US region or the UL region to produce a genetic code that directs production of proteins of interest to stimulate a desired immune response.

HOW DOES AN ORF PRODUCE AN ANTIGENIC PROTEIN?

The DNA in the ORF is unzipped and a complementary strand of messenger RNA (mRNA) is created by an RNA polymerase enzyme. The mRNA has the blueprint for the protein, which is used by a cellular structure called a ribosome (Figure 2) to create the protein.

The process of creating the mRNA strand is started by the binding of RNA polymerase to a specific place on the original DNA strand called a "promoter". Everything downstream of the promoter is converted into mRNA until a stop site is reached. A "cap" is added to the start of the mRNA sequence by another enzyme (guanyl transferase) that plays a role in the recognition of the mRNA by the ribosome. The mRNA is now the code that the ribosome can read to produce the antigenic protein that we need.

Figure 2. Cell diagram



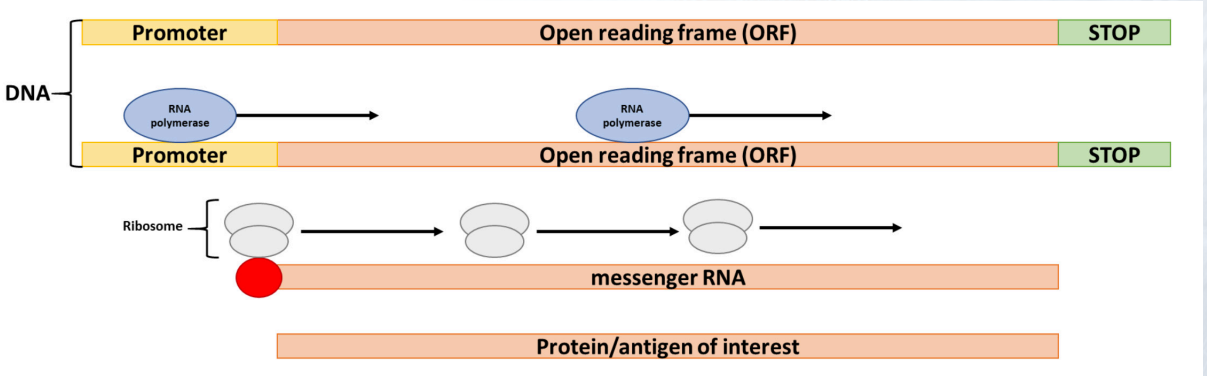
Ribosomes can recognize the cap-structure or an internal RNA structure in the mRNA called an IRES or "internal ribosome entry site" that will allow the ribosome to construct a protein from the coding in the mRNA without the promoter sequence.

Either of these methods can result in effective creation of the antigenic protein by the ribosome.

WHAT IS A PROMOTER AND HOW DOES IT IMPACT THE PRODUCTION OF ANTIGENIC PROTEIN?

A promoter is a sequence of DNA that is recognized by the RNA polymerase as a binding

Figure 3: Sequence of steps to create a protein or antigen of interest



site to start making mRNA. The promoter strength functions like the accelerator pedal in an automobile to determine how much antigenic protein will be made. The size of this promoter is not significant: a small promoter can be very strong or a large promoter can be very weak. More is not always better: too much antigen expression may result in an genetically unstable recombinant virus while insufficient antigen expression results in poor immune response.

WHAT ABOUT THE SOURCE OF THE DNA SEQUENCE INSERTED? FOR EXAMPLE, IF THE F-PROTEIN COMES FROM A CLONE 30 VACCINE ND OR A TEXAS GB VIRULENT ND?

This is specific to the immune response generated against the protein induced by the DNA code. In the case of ND fusion genes, there is 96% homology between the F-protein of Nobilis® ND Clone 30 and Texas GB strain. The Clone 30 F insert has a different cleavage site -GRQGR- compared to the one in Texas GB -RRQKR-, and this cleavage site partly determines the pathogenicity of the wildtype NDV. Yet, in the context of an HVT vaccine there is no effect of this cleavage site on pathogenesis. In fact, it has been proposed that the lack of this cleavage site in Clone 30 might even increase F protein stability, which could increase immunogenicity.

HOW ARE MULTIPLE ANTIGEN rHVT VACCINES MADE?

Multiple antigen vaccines can be made just like the single-antigen vaccines, with a promoter plus an ORF that codes for one protein and a different promoter- ORF pair that codes for the second protein. These can be located side-by-side or they can be in completely different positions in the original genome of the HVT virus. The construction of the new recombinant virus must have the correct balance of antigenic expression of the two proteins, it must be genetically stable and the virus should still replicate well in the chickens.

Multiple antigen vaccines can also be constructed with a single promoter-ORF to start, followed by an IRES-ORF in sequence. This is a different way to achieve a stable recombinant virus that produces two antigens, but it does not have an advantage over the individual promoter-ORF sequence for each individual protein blueprint. This construction method must still produce a recombinant virus with the correct balance of antigenic expression of the two proteins and it must be genetically stable.

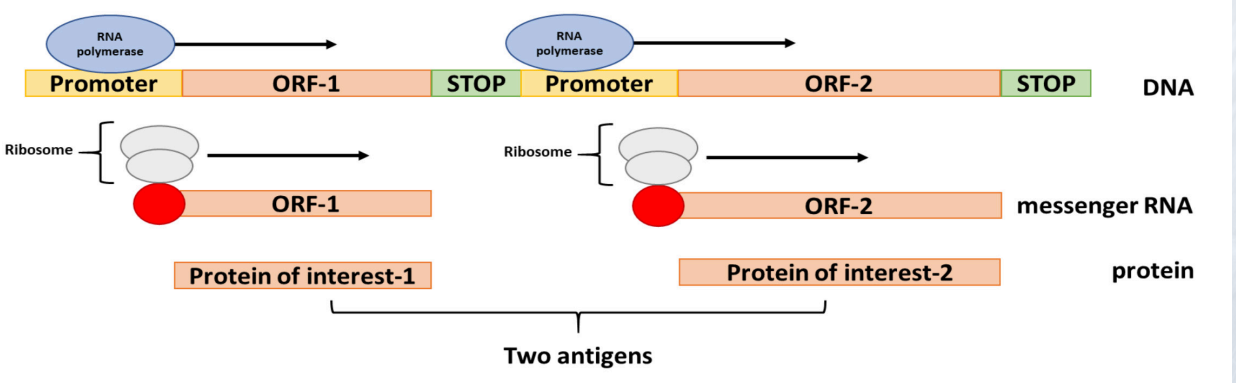
There are many, many combinations of promoter-ORF or IRES-ORF sequences and locations, but most do not result in properly balanced antigen expression or good replication and many constructs are not genetically stable. Many candidates are tested and rejected in the process of developing a stable and efficacious rHVT vaccine.

WHY IS VIRUS STABILITY SO IMPORTANT?

HVT is a herpesvirus. As such, it is never completely eliminated from the chicken after vaccination. It continues to stimulate immunity for the life of the bird. An rHVT vaccine must be able to replicate well in the chicken and the insertion of the antigens must remain stable in the genome of HVT while it remains in the bird to ensure a long duration of immunity.

Whenever we replace or add an ORF to the HVT genome, we risk creating a virus that does not replicate well in the bird, or that may lose stability with replication. Decreased stability could result in reduced duration of immunity.

Figure 4: Two promoter-ORF sequences translated to two different proteins



HOW IS INNOVAX®-ND-IBD MADE?

Innovax®-ND-IBD is constructed from an FC 126 HVT backbone with two promoter-ORF sequences in the US2 region (Figure 4). The Newcastle disease (ND) F sequence was derived from Nobilis® Clone 30 and the infectious bursal disease (IBD) VP2 sequence was derived from the Faragher strain. The expression of the F protein and of the VP2 protein are carefully balanced and are genetically stable:

- Efficacy has been demonstrated against virulent ND (Texas GB per USDA 9CFR and Herts ND strain per EU Pharmacopoeia) with onset of immunity at 4 weeks.
- Efficacy has been demonstrated against classical IBD and Delaware Variant E IBD (per USDA 9 CFR) and vvIBD strain CS 89 (per EU Pharmacopoeia) with onset of immunity at 2 to 3 weeks.
- US challenge studies of vaccinates at 60 weeks of age using Texas GB NDV demonstrated excellent protection. This demonstrates Innovax®-ND-IBD replication and stability in the bird.

HOW ARE OTHER rHVT DUAL CONSTRUCT VACCINES MADE?

Other rHVT dual constructs may be made with two promoter-ORF sequences, similar to Innovax®-ND-IBD, but the promoter sequences and the insertion locations of the promoter-ORF pair may be in different locations in the HVT genome. The size of the promoter-ORF sequence and the location in the HVT genome do not make a construct better or worse.

Another approach to building a dual-construct is to use one promoter-ORF sequence followed by an IRES-ORF sequence (Figure 5). Again, the size of the promoter-ORF sequence and the location in the HVT genome, or the use of the IRES-ORF sequence will not make a construct better or worse …in the end, it is simply a different way of reaching the goal of a stable virus with balanced antigen expression and good genetic stability.

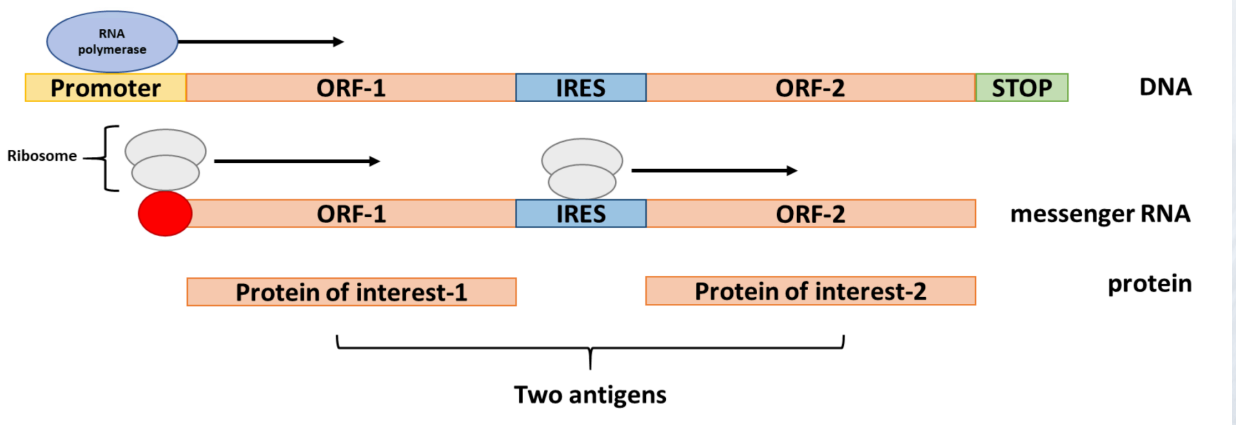
The important tests of all rHVT DNA construct vaccines are:

- Efficacy of the vaccine when challenged with ND and IBD, indicating a balanced expression of the rHVT construct.
- Onset of immunity, indicating good replication of the vaccine virus in the bird.
- Immunity that can last a long time, indicating virus genetic stability in the bird.

Thus, there are many ways to achieve efficacy, rapid onset of immunity and long duration of immunity. But no matter how the rHVT vaccine is constructed, it must achieve these critical parameters.

ONCE A VACCINE IS DEVELOPED, FIELD PERFORMANCE WILL BE THE INDICATOR OF TRUE SUCCESS

Figure 5: One promoter-ORF sequence followed by an IRES-ORF sequence



Data on file.

MERCK Animal Health