- **Deliverable D8.2:** Reassortant viruses between conventional (e.g. BTV-1 or BTV-4) and novel/atypical strains (e.g. to explore the genetic basis for horizontal transmission (Partners 1, 2, 4, 5). **(M18) (report Dec 2018)**

We have chosen BTV-1 reference strain as the backbone to generate mono-reassortants of BTV-26 for assessing genes facilitating horizontal transmission from infected to non-infected animals, particularly mice. IFNAR knock out mice are susceptible to infection by BTV-1 wild type strain, developing clinical signs which end by death of the mice with 5-7 days post inoculation. Such an observation hinders a contact of a longer duration between infected and non-infected animals. As a collaboration between ANSES and Nottingham, we therefore developed an attenuated BTV-1 by reverse genetics (BTV-1RG), which shows less drastic clinical sings and viremia of up to $10^4$ pfu of BTV-1RG lasting for over 7 days, while animals surviving past end of viremia and the minor clinical signs.

To generate our BTV-1, cDNA from individual full length genome segments were PCR amplified with a forward primer tailed with a T7 promoter to facilitate T7 transcription and a reverse primer tailed with a restriction enzyme, such as BsmBI, which upon cleavage will generate an authentic termini of each genome segment. RNAs were generated using the Mmessage Mmachine Ultra transcription kit and lipofected in the desired combination to rescue either BTV-1RG or mono-reassortants of BTV-26.

The generation of the attenuated BTV1-RG was crucial for establishing a backbone that will serve to swap individual BTV-1 genome segments with their homologues of BTV-26. Using this backbone, we effectively generated mono-resassortants of BTV-26. All mono-reassortants were tested once in mice and did not cause death.