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Recombinant hRSV expressing mCherry or luciferase without fitness alteration

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The human respiratory syncytial virus (hRSV) is an ubiquitous pathogen that causes severe respiratory tract infection worldwide in young infants as well as in elderly or immunosuppressed adults. To date, there are no vaccine nor specific antiviral drugs against human RSV excepted a humanized neutralizing monoclonal antibody directed against F protein. Development of specific antiviral drugs has been limited by the lack of knowledge of the RSV replication mechanisms. We set up a reverse genetic system for hRSV based on coexpression of L, P, N and M2-1 proteins and a complete hRSV antigenome of RSV long strain in BSRT7 cells. Different restriction sites were inserted at intergenic regions allowing easy modification of RSV genome.

We successfully rescued a wild type hRSV recombinant virus which was proven to be stable and to grow almost as well as the parental virus. We first introduced an additional coding sequence in the hRSV cDNA and successfully rescued recombinant viruses expressing mCherry or firefly luciferase. These viruses were shown to be stable and exhibit no alteration of fitness in cell culture as compared to the wild type recombinant virus. Expression of mCherry is correlated to infection rate and allows the monitoring of RSV multiplication in cell culture and can be measured in 96 wells cell culture plate using a fluorimeter. This may be useful to screen antiviral molecules or to study seroneutralization. Luciferase expressing RSV is under characterization but may be more sensitive for these applications. Mice were challenged with recombinant hRSV and hRSV-mCherry. Measured pulmonary viral loads were comparable between the wild type virus and the recombinant RSV expressing either Luc or mCherry. Replication of luciferase virus in mice is under evaluation. These recombinant viruses may be useful to detect infected cells *in vivo* and to monitor the efficiency of antiviral strategies in mouse model.