Reverse Genetics for Double-Stranded RNA Viruses

H. Attoui[1*] F. Mohd Jaafar[1]
S. Maan[1] P.P.C. Mertens[1]

Since the recognition by Sabin of the specific nature of reoviruses in 1959 and the characterisation of their genome as double-stranded ribonucleic acid (dsRNA) by Gomatos in 1960, there have been many attempts to rescue viruses by transfecting cells with viral dsRNA. These attempts were largely unsuccessful. In 1990, it was proposed by Roner and Joklik that messenger (m) RNA transcribed from orthoreovirus cores was infectious when that mRNA was transfected into cells together with rabbit reticulocyte lysates that were pre-incubated with denatured viral dsRNAs. However, attempts to reproduce these 'rescue' experiments failed in the hands of other scientists. A breakthrough came in 2007 when Kobayashi and Dermody established a plasmid-based reverse genetics system for the orthoreoviruses, which represents the first reliable, synthetic based, reverse genetic system for a dsRNA virus. The system made it possible to study the role of specified amino acids in the outer capsid protein by generating 'designer' mutants. A second and potentially even more significant breakthrough came in the same year when Boyce and Roy showed that mRNA transcribed from the bluetongue virus core was infectious, allowing rescue of the virus in BSR cells, a clone of BHK-21 cells. This same group extended their work to the generation of synthetic transcripts from complementary (c) DNA copies of each of the 10 genome segments cloned into plasmids, driven by the T7 polymerase. We report refinement of a T7 base transcription approach, which uses simple linear polymerase chain reaction (PCR) amplicons of the individual virus genome segments from any reovirus, for transcription of full length, fully capped mRNA transcripts. These transcripts were used to transfect BSR cells and permitted rescue of the corresponding viruses. There was no requirement for a second transfection of either capped or uncapped messages. The efficiency of the system was significantly improved, using modulators of the cells' innate immunity; particularly, 2-aminopurine considerably enhanced the rescue and shortened the time for appearance of lysis plaques. This system was successfully used for 12 segmented coltiviruses, the 12 segmented seadornaviruses and 10 segmented orbiviruses. The authors suggest that this simplified rescue-strategy should be applicable to any dsRNA virus, particularly the members of the 15 recognized genera of the family *Reoviridae*. The system is currently being tested for mono-partite dsRNA viral genomes.

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1. Arbovirus Molecular Research Group, Vector-borne Disease Programme, Institute for Animal Health, Pirbright Laboratory, Woking, Surrey, GU24 0NF, United Kingdom.

* Corresponding author
Tel.: +44 14 83 23 11 94; Fax: +44 14 83 23 24 48
E-mail: houssam.attoui@bbsrc.ac.uk