OPEN ACCESS Freely available online

ISSN: 2471-2663
Clinical & Medical Biochemistry

Opinion Article

A Ligation and Restriction Enzyme Independent (LREI) Cloning Technique for Cloning Influenza Gene Segments

Sushant Bialy*

Department of Biochemistry, The Pirbright Institute, Pirbright, United Kingdom

DESCRIPTION

Reverse Genetics (RG) is the process of generation of live virus *in vitro* using synthetic or PCR-amplified genes. This technique allows the generation of mutant influenza viruses of any genotype or phenotype. The RG technique was first used for rabies virus in 1994, and *in vitro* generation of various DNA and RNA viruses, including segmented and unsegmented RNA viruses. RG Technology has also revolutionized the field of influenza and progressing influenza research through genetically engineered recombinant influenza viruses. Reverse genetics as a tool is useful for studying influenza host range, transmission patterns of viral genome replication, pathogenicity, and virulence. This technique is also being implemented to develop influenza vaccines or recombinant influenza viruses that contain reporter genes for studying virus egress and dissemination.

Despite the utility of RG systems, the cloning step remains a limiting factor for the *de-novo* generation of viruses. Gene cloning is a crucial step in RG technology and has gained popularity in terms of usage but the technique involves restriction digest followed by ligation, which sometimes becomes difficult to perform. The primary reasons are presence of internal restriction enzyme sites (e.g. for BsmBI, BsaI, AarI or BbsI) in the different gene segments of field isolates of influenza virus. Further, the degradation of dNTPs in the ligation buffer or inefficient ligase enzyme also results in failure during ligation. RG plasmids containing large inserts (>2000 bp) of influenza virus were also found to be unstable after transformation into *E. coli*, possibly due to their toxicity to the bacterial host. This leads to the incorporation of bacterial sequences into the target insert. Therefore, an alternative cloning strategy is sought. Ideally, it would bypass restriction-ligation steps, increase the efficiency of recombinant plasmid formation, and reduce the chance of genetic recombination within the insert. The development of a Ligation and Restriction Enzyme Independent (LREI) cloning procedure for cloning influenza gene segments into a standard reverse genetic plasmid pHW2000 increases the chances of recombinant plasmid formation, if subsequent growth of bacteria at lower temperatures, will reduce the problem of genetic recombination. The LREI cloning procedure represents an alternative strategy for cloning influenza gene segments that are internally restricted to enzymes used in reverse genetics. Furthermore, the problem of bacterial genetic instability found in influenza gene fragments can be alleviated by culturing the recombinant bacteria at lower temperatures. This technique can be applied to clone any influenza gene segments using common primers, which will help to generate influenza viruses rapidly and facilitate influenza research and vaccine development. The LREI cloning strategy allows the integration of any gene into any vector position, as long as the same mega-primer strategy is followed. The LREI cloning method is faster than conventional cloning methods due to bypassing the restriction digestion of the inserted amplicon and plasmid and the ligation procedure.

The LREI cloning technique is based on the exponential amplification of the mega-primer and the target vector, resulting in a greater number of positive colonies after transformation compared to conventional cloning strategies. This technique is specific and highly efficient in generating cloned plasmids, which are difficult to clone. LREI Cloning increases the chance of forming a recombinant clone, and recombinant bacteria growing at lower temperatures alleviate the problem of genetic recombination, albeit at the cost of plasmid yield. In summary, this technique can be applied to clone all influenza gene segments using universal primers, which will help to rapidly generate influenza virus and make the study of influenza virus biology easier.

Correspondence to: Sushant Bialy, Department of Biochemistry, The Pirbright Institute, Pirbright, United Kingdom, E-mail: sushant.bialy@pirbright.ac.uk

Received: 01-Apr-2022, Manuscript No. CMBO-22-16602; **Editor assigned:** 06-Apr-2022, PreQC No. CMBO-22-16602 (PQ); **Reviewed:** 27-Apr-2022, QC No CMBO-22-16602; **Revised:** 04-May-2022, Manuscript No. CMBO-22-16602 (R); **Published:** 11-May-2022, DOI: 10.35841/2471-2663.22.8.124.

Citation: Bialy S (2022) A Ligation and Restriction Enzyme Independent (LREI) Cloning Technique for Cloning Influenza Gene Segments. Clin Med Bio Chem. 8:124.