TECHNOLOGY FOR OBTAINING AND USING AS POSITIVE CONTROL SAMPLES FOR PCR DIAGNOSTICS OF DANGEROUS VIRAL INFECTIONS

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Abstract: The goal of the work is to develop a technology for obtaining positive control samples based on recombinant retroviral particles, as well as its use in creating reagent kits for identifying the RNA of pathogens of dangerous and especially dangerous viral infections using the reverse transcription polymerase chain reaction method.
Keywords: RNA-containing viruses, molecular genetic diagnostics, recombinant retroviral particles, positive control sample.

INTRODUCTION

One of the main methods for molecular diagnostics of viral infections in recent years has been the polymerase chain reaction (PCR). The creation of modern diagnostic test systems for identifying the genomes of RNA-containing viruses based on the reverse transcription polymerase chain reaction (RT-PCR) method requires the presence of a positive control sample (PCS) in the test system.

MATERIALS AND METHODS

All genetic engineering manipulations were carried out using the collection strain Escherichia coli DH5α (supE44 lacU169 (f80 lacZΔM15) hsdR17 recA1 endA1 gyrA96 thi-1 relA1). Transformation of bacterial cells, isolation of plasmid DNA, and electrophoretic analysis of fragments were carried out according to the recommendations given in the manual [1]; restriction, ligation of plasmid DNA and fragments - in accordance with the conditions recommended by the manufacturer Thermo Scientific (USA).

RESULTS AND DISCUSSION

The “packaging” cell line GP+env-AM12, which produces retroviral virions with an amphotropic host spectrum, as well as the NIH 3T3 cell line, created on the basis of mouse fibroblasts and used to determine the biological titer of the virus, were obtained from the Russian collection of vertebrate cell cultures (rcck p) (St. Petersburg, Russian Federation). Cell lines were cultured in DMEM (Dulbecco's Modified Eagle's Medium) (Sigma, USA) supplemented with 10% fetal calf serum (Sigma, USA), 2 mM L-glutamine (Sigma, USA), 100 U/ml penicillin (Sigma , USA), 100 µg/ml streptomycin (Sigma, USA) at a temperature of 37 °C and 5% co2 content.

When developing diagnostic tests for detecting RNA-containing viruses, the method of obtaining positive control samples is of particular importance. These samples must contain an RNA copy of the target gene, preferably protected by a protein coat, and undergo all the main stages of sample preparation: RNA isolation, reverse transcription and polymerase chain reaction. In our opinion, the most suitable way to obtain control samples is cellular biosynthesis by a “packaging” line of recombinant retroviral particles, into the genome of which a diagnostic target gene is integrated. The specific nucleotide sequence in such a particle is packaged in a protein shell, which protects it from the action of rnases, and is presented in a diploid form in the form of RNA. The idea of using retroviral particles as carriers of genetic information, due to the natural ability of retroviruses to integrate into the genome of the host cell in the form of a provirus and be transcribed in its composition, using the cellular enzymatic system, is the basis for the creation of a high-tech method for obtaining RNA. This technology has been tested in the development of diagnostic test systems for identifying the genomes of pathogens of the most relevant natural focal infections for the Republic of Belarus: hemorrhagic fever with renal syndrome (HFRS), lymphocytic choriomeningitis (LCM) and tick-borne encephalitis (TBE), as well as dangerous hemorrhagic lassa fever, endemic to African countries.

The technological process of biosynthesis of recombinant retroviral particles consists of several stages. The first stage is associated with the construction of a plasmid vector that combines the retroviral and bacterial parts and contains cassettes of regulatory elements necessary for its successful replication in pro- and eukaryotic cell systems, as well as the target gene of the infectious agent.

The bacterial part of the construct includes the pBR322 plasmid and allows for all genetic engineering manipulations for cloning and selection of recombinant plasmids in the bacterial strain E. coli DH5α. The advantage of the plasmid is the fact that its replication occurs independently of the cell cycle. This makes it possible to obtain a large number of copies of the plasmid, which is important for subsequent stages of cloning. The vector includes an ampicillin resistance gene, thanks to which the selection of bacterial clones is possible when grown on a medium with an antibiotic [2].

Two additional functionally significant nucleotide sequences were cloned into the retroviral cassette of the vector: one of them encodes green fluorescent protein (egfp), the other encodes aminoglycoside 3'-phosphotransferase (neo). The transcription of each of these proteins occurs under the control of its own promoter: the green fluorescent protein is transcribed from the cMV promoter (promoter of early genes of cytomegalovirus), the neo gene is transcribed from the 5' promoter of the Moloney murine leukemia virus. Each of the sequences carries its own functional load. The expressed protein eGFP allows one to estimate the titer of retroviral particles using a cytofluorimeter, and aminoglycoside 3'-phosphotransferase allows one to select recombinant cell clones on a medium with an antibiotic. Thus, as a result of genetic engineering manipulations, a plasmid is constructed containing all the necessary elements for successful replication in both bacterial and eukaryotic systems, into the polylinker of which a diagnostically significant target gene is additionally cloned

CONCLUSION

The technology for obtaining positive controls based on recombinant molecules makes it possible to significantly improve the molecular genetic diagnosis of RNA-containing viruses and to adequately assess the effectiveness of the reaction both at the stage of developing a diagnostic test and during its commercial use. This approach to obtaining positive control samples is of particular importance in cases where we are talking about dangerous, especially dangerous and emerging viral infections, as well as pathogenic RNA-containing viruses for which adequate cultivation methods have not been found. At the same time, the proposed technology, in the event of an extreme situation and the need for urgent diagnosis, allows the use of artificially synthesized DNA fragments of viral genomes, without having them available, and obtaining their RNA copies.

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