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A Review of Artificial Genetic Constructs and Their Applications as Positive Controls

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Abstract

Advances in nucleic acid based molecular diagnosis techniques, provided the basis for efficient, accurate and rapid detection of biological/pathological agents. However, these techniques suffer from major limitations including lack of the genomic sequence of high-risk pathogens to calibrate techniques as positive controls, false positive results and restrictions on access to existing commercial tests. However with the advent of synthetic biology, it is possible to construct appropriate positive controls. Artificial genetic constructs are the synthetic constructs that contain genetic materials from highly dangerous bacteria or viruses, which can be used as positive controls in molecular techniques such as PCR. In this review, we introduce positive controls and simulator positive controls firstly and discuss about artificial genetic constructs, procedure of design and synthesis secondly. Finally, we discussed about single and chimeric artificial genetic constructs as two main categories of these positive control.

Keywords: Positive Control, Artificial Genetic Constructs, Molecular Techniques, Chimeric

1. Context

A positive control is a group in an experiment that receives a treatment with a known result. It is an essential part of all research designs (1). For example, in an experiment, measuring the amount of an element in a set of extracts, a positive control would contain the purified element, while a negative control would contain no element. In such experiment, the positive controls should indicate a large amount of target element, but no or very low amount of that element is expected to be identified in the negative controls (2).

All microbiological tests require both positive and negative controls. In such an experiment, the positive control contains the purified microorganism.

Routinely, in molecular diagnosis of pathogens, including bacteria, the genome of the targeted pathogen is used as positive control; however, in some cases these pathogens are highly infectious and dangerous to work with. Thus, recently, a number of new studies have suggested the use of simulator as a positive control, instead of pathogen itself. A genetically simulator of the pathogens (bacteria or viruses) is a pathogen from the same class that simulates properties of the target pathogen, but it is safer than the target pathogen. The most important of these simulators are: Bacillus subtilis spores (instead of Bacillus anthracis), Pantoea agglomerans (instead of any bacteria threatening plants) and M13 phage (instead of any threatening or pathogenic virus). Even though, these natural simulators pave the way for pathogen screening, they are not fully efficient as they do not share exactly the same genomic material of the pathogen of interest. To overcome this shortcoming, in recent years, researchers have proposed the idea of using artificial genetic constructs as positive controls (3). Such artificial genetic constructs potentially provide positive controls that overcome the problems such as: (1) lack of the genome of high-risk pathogens to be used as positive controls for assessment of techniques' accuracy, (2) false positive results occurrence, and (3) lack of easy access to available commercial kits for detecting pathogens (4, 5).

In current review, we first introduce positive controls and simulator positive controls; then, we discuss about artificial genetic constructs as well as approaches for their design and synthesis of them. Finally, we provide an overview of two main categories of the simulator positives, including single and chimeric synthetic artificial constructs.

2. Artificial Genetic Construct

Vectors are considered to represent an ideal candidate to be constructed as positive controls. Routinely, these circular polynucleotides genetic carriers are engineered for molecular cloning and transportation of genetic fragment (6-8). Based on their proven ability to successfully transport genetic fragments into the cell of interest, they provide a suitable candidate to be designed as a safe and non-infection positive control for using in research and molecular diagnosis. Therefore, currently they are the main construct to be utilized as potential candidate for artificial genetic construct. In next section we provide an overview of steps for designing a vector based artificial genetic construct, as positive control.

3. Platform for Design and Synthesis of an Artificial Genetic Construct

3.1. Design of the Construct

The first step for designing an artificial genetic construct is identification of the whole genomic sequence of target pathogens and deciphering the signature sequences. In details, after extracting the genomic sequence of all the bacterial strains or viral serotypes these sequences are aligned together and eventually the common or conserved region or regions between them will be selected and called the signature sequence.

3.2. Specific Changes in the Signature Sequence Before Insertion into the Construct

Before synthesis of the construct, special changes must be made in signature sequence. These changes are necessary for later verification test. For example, after placement of specific change in signature sequence, restriction enzyme sites will be introduced to provide proper vector insertion site. Another example of such changes are addition or removal of a sequence from signature sequence in construct, which makes it possible to distinguish. Moreover, regions for specific purposes can be added to this construct. For example, by placing a restriction enzymes cutting site in the signature sequence, the results of PCR reaction can be confirmed on the basis of the length of detected fragments. As the genome of interest in suspected sample lacks such restriction site, after treatment with specific restriction enzyme, the PCR products from artificial constructs and genome of interest will make two fragments with different sizes on the gel. Besides, by applying these enzymes, prior to PCR reaction, the removal of artificial constructs which may exist due to laboratory contamination in suspected sample, will be ensured. In fact, these actions are also aimed at reducing errors and eliminating false positive results.

3.3. Synthesis of the Construct

At the next step, with the aid of either offline or online available software, construct is designed and then it is chemically synthesized.

3.4. Examine and Verify Whether the Structure is Properly Constructed

In this step we need enzymatic digestion. If the construct has been made accurately, the enzyme digestion will cut the target sequences, which were inserted at the synthesis step. Thus, the PCR product will produce fragments with different length.

These four steps are essential for design and synthesis of the construct.

4. Category of Artificial Genetic Construct

There are two categories of synthetic artificial construct including: single artificial genetic construct and chimeric artificial genetic construct.

4.1. Single Artificial Genetic Construct

In this category, a part of the microorganism genome is selected and the necessary modifications are made on it, and finally the desired sequence is synthesized and cloned. A number of studies have reported administration of this construct as positive control. In 2004, Inoue et.al, reported preparation of a synthetic vector containing the strains of B. anthracis (PAI and PAII), which is usually included in a PCR test of Bacillus anthracis (Figure 1) (9).

In another study, Boonham et.al. produced a synthetic oligonucleotides as a positive control, for evaluating the specificity of real-time PCR assays in the diagnosis of the potato spindle tuber viroid (10). In 2007, Christensen et.al. reported a synthetic DNA molecule, containing a mixture of oligonucleotides (100 to 120 bps). Each one of these oligonucleotides is designed for the detection of one or more disease-causing mutation(s), depending on the proximity of the mutations to one another. This synthetic DNA molecule was reported to provide an appropriate candidate as a standard test control for one or more genes in cystic fibrosis (11). In a study by Krüttgen et al., a blaNDM-1 synthetic gene was reported as a suitable positive control for in-house assays of blaNDM-1 detection (12).

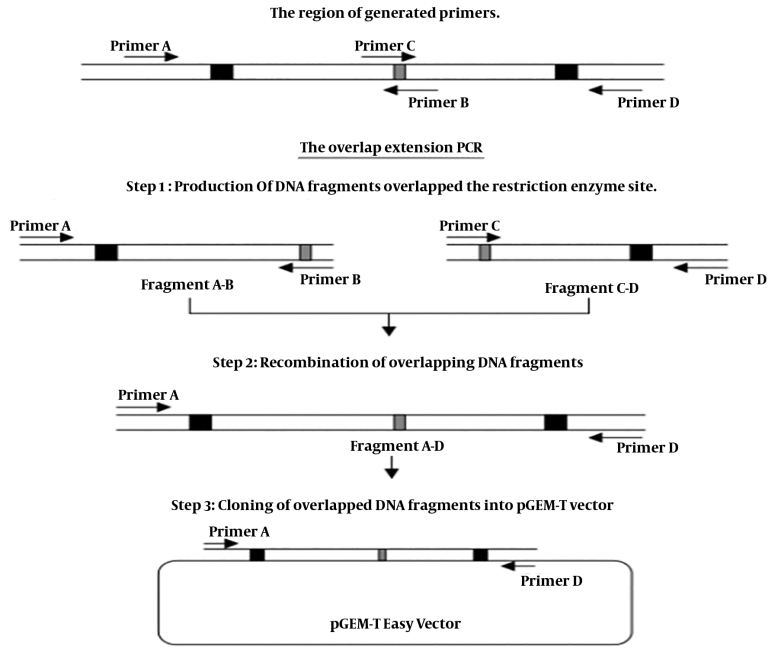

Figure 1. Schematic picture of construction recombinant plasmid DNAs (9)

4.2. Chimeric Artificial Genetic Construct

Currently, a number of studies have reported application of chimeric artificial genetic construct as positive controls. In 2004 Charrel et.al, developed a new artificial positive control by synthesizing three different plasmids entitled Cat.A, Cat.B and Cat.VEF. The first two plasmids contained pathogen agents in CDC Category A and B, respectively; while third plasmid contained sequences from Variola and organisms that cause rash-like illnesses. In order to avoid contamination by plasmid DNA, the native sequences was modified by replacement of 8 nucleotides from exogenic sequence with a 20 nucleotide that contains a Not I site. This modification allows Not I pre-digestion of the sample. Besides, amplified DNA from the control could be distinguished from natural agent via Not I digestion, sequencing as well as by analysis of melting curves (Figure 2) (13).

After that in 2009, Carrera et al., developed a non-virulent simulant as a positive control for several virulent organisms such as Burkholderia mallei, Rickettsia sp., Coxiella burnetii, Brucella sp. and variola (smallpox) virus and etc. (Figure 3) (5).

In 2009, this research team published another study reporting designing & constructing a non-virulent simulant to replace several pathogenic viruses. This safe stimulant was designed to include the nucleic acid signatures of Venezuelan Equine Encephalitis virus, Rift Valley Fever virus, Influenza virus, Machupo virus, in a single construct (4). Another research in this field was is published by Sohni et.al, in 2008. This research team designed an internal amplification control (IAC) for detecting the rpoB gene in Bacillus Anthracis, by using the TaqMan assay. Synthetic IAC oligonucleotides were subcloned in vector pDG1730 (14). Simultaneously, Pitirat Boonsuk et.al, applied the same approach for diagnosis of influenza virus H1, H3, and H5 by multiplex PCR and designed and constructed a total of six plasmids (15). In 2013, Caasi et al., published their work reporting synthesis of a multi-target and safe positive control for routine PCR-based assays (16). This construct contained primer sequences targeting four viruses (Barley yellow dwarf virus, Soilborne wheat mosaic virus, Triticum mosaic virus and Wheat streak mosaic virus), that were pathogenic to wheat. It was applied as internal control, for the plant mitochondrial nad5 gene.

In 2014, Karlsson et al., used two approaches for developing a positive control: In first approach a control sequence was cloned into the pEX-A vector and in second approach control sequence were generated by PCR amplification. The aim of this research was developing an safer assay to be performed in laboratories that has no access to

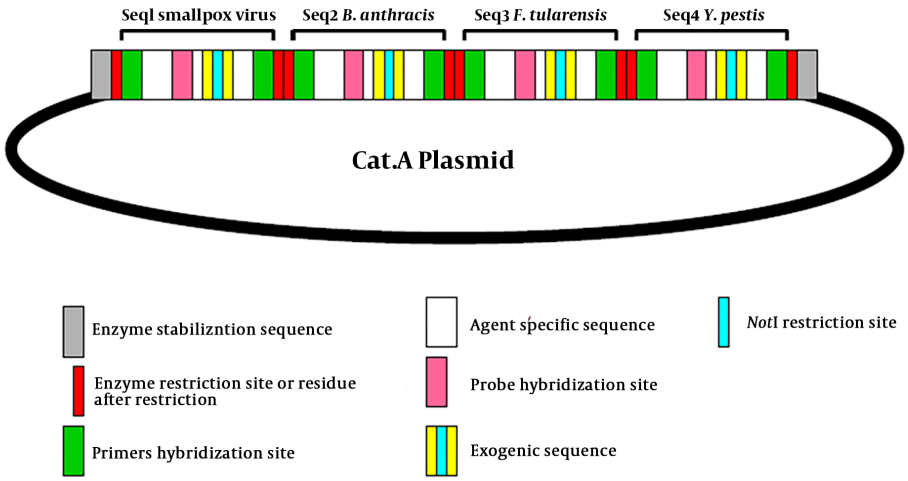

Figure 2. Schematic picture of CatA plasmid. green/white/pink: specific sequences genome of category agents, yellow/blue: exogenic sequences, blue: Not I restriction enzyme site, green: primers for amplified specific genome, pink: probes (13).

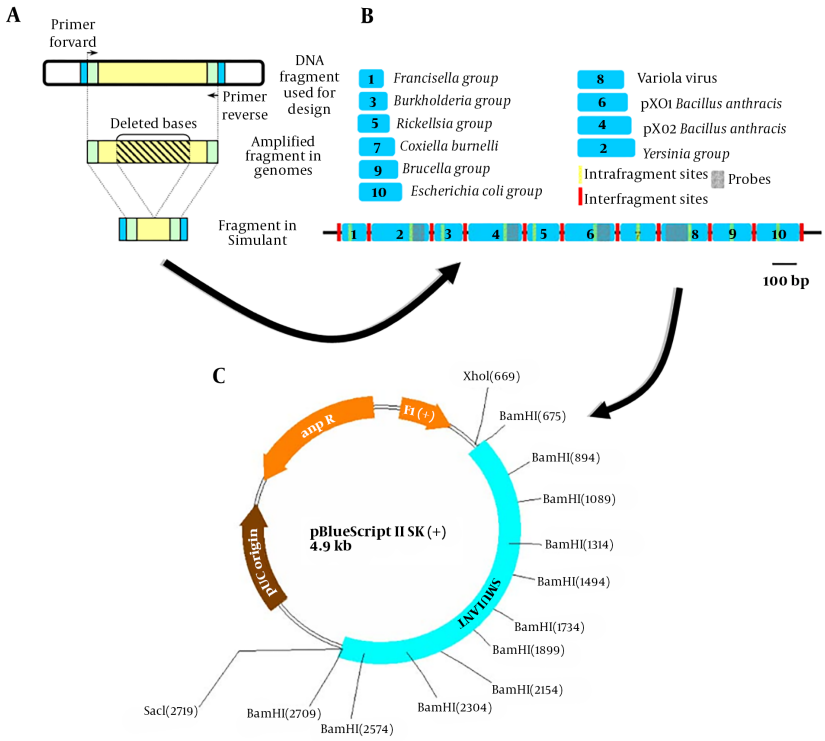

Figure 3. Scheme picture of the multi-target, safe and chimeric artificial positive control. Every pathogens agent group showed schematically by number (5).

genomic controls (17). In another research Arif et al., used a synthesized DNA, containing forward and reverse priming sequences as well as probe complement priming sequences that were ligated into the multiple cloning site (MCS) in the vector pUC57 as an artificial positive control (18). In 2016, Dobhal developed an artificial positive control for Rose rosette virus (RRV) (Figure 4) (19).

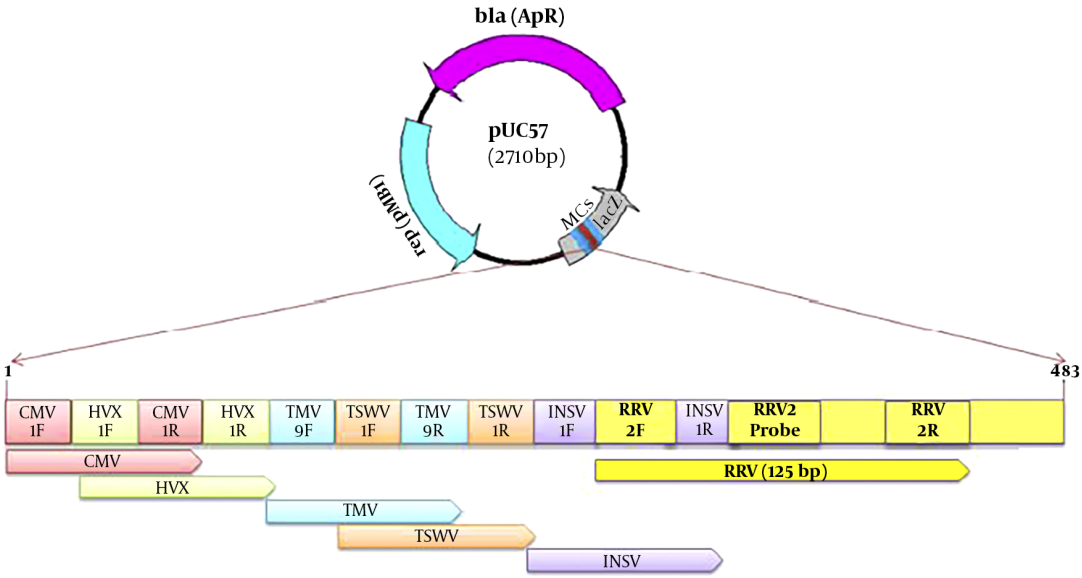

Figure 4. Schematic picture of artificial positive control that shows forward and reverses complement primer sequences (19)

Acknowledgments

Positive controls are essential for PCR reliability and presence of appropriate positive control is very important in clinical diagnosis. The birth of synthetic biology offers new approaches for designing and developing artificial positive control for reducing mistakes and fixing problems caused by the lack of high-risk pathogen genomes to calibrate techniques.

Footnotes

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