Title : RNA interference in gene cloning/expression

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Abstract

DNA cloning is a simple, straightforward process. It has been put into practice as a standard lab procedure for cloning the gene of interest for specific protein expression and other molecular or cellular studies. The present study is the first of its kind to hypothesize the formation of interfering recombinant plasmid during a DNA cloning reaction that is believed to interfere with the protein or gene expression. This might lead to a false conclusion in a cellular study and also may lower the protein expression during the production stage.

Keywords: DNA Cloning, RNAi, Protein Expression

Introduction

Earlier it was a notion that all biological species maintained a characteristic purine vs. pyrimidine ratio, indicating that it is unlikely for any foreign DNA from an unrelated species to become functional in a different host. Breaking this convention, Cohen *et al.* (1973) reported the construction of a new biologically functional recombinant plasmid species by *in vitro* joining of fragments of two separate plasmids and introduced the resulting recombinant DNA molecules into bacteria. The recombinant plasmids were shown to possess genetic properties and nucleotide base sequences from both the parent DNA molecules (Cohen et al, 1973). A few months later, Chang and Cohen further reported that genes carried by EcoR1-digested *Staphylococcus* plasmid DNA fragments can be introduced into *E.coli*

antibiotic resistance plasmid pSC101. The new interspecies recombinant plasmid DNA carrying nucleotide sequence information from two different parent DNA molecules were able to replicate as biologically functional unit in a foreign host (Chang et al, 1974). Subsequently, the Boyer's and Cohen laboratories published collaborative experiments demonstrating that EcoR1-digested fragments of amplified frog DNA, coding for 18S and 28S rRNA, can be ligated *in vitro* to the bacterial plasmid pSCl01. They also reported that the recombinant molecular DNA plasmids containing both the eukaryotic and prokaryotic DNA, when introduced into the *E.coli* showed stable replication and transcription of 18S and 28S rRNA (Morrow et al, 1974). These technical breakthroughs has laid the foundation for recombinant DNA work and opened a new branch of science "Genetic engineering or recombinant DNA technology or Molecular Cloning" which have had a dramatic impact on all aspects of molecular biology/Cell biology worldwide. Many experimental biologists learn these fundamental skills in their training and today this ability to manipulate DNA is considered as a cornerstone of modern biology. The DNA cloning technique has produced significant insights into the working of genes and cells in health and diseases. This has brought about changes in the nature of biotechnology and biopharmaceutical industries worldwide (Cohen SN, 2013).Today in most of molecular biology lab, it is a standard lab practice to clone a gene of interest for protein expression and other molecular study.

The steps in DNA cloning involves gene amplification by polymerase chain reaction (PCR) and subsequent introduction of two different restriction enzymes sites at the two ends (Scharf et al,1986). The amplified PCR products are isolated through the gel purification method and later subjected to digestion using two different restriction enzymes. The plasmid digestion is carried out using the same two restriction

enzymes. Subsequently, both the digested template DNA and plasmid are ligated using T4 DNA ligase, and the recombinants are transformed into the bacteria. Later the appropriate clones are screened either by using PCR or restriction digestion of recombinant plasmid. Even though these steps look simple, one can face problems in cloning the gene of interest, which can be frustrating and time-consuming (Matsumura I, 2014). Based on the intended use of recombinant protein, the strategy is applied to express the recombinant protein in different hosts (for e.g., bacteria, plants, yeast, insect, mammalian cell line, etc.) (Figure 1). Following the introduction of the recombinant plasmid into the cell, the strategy is expanded to screen the individual cell population with the highest production. The cell line that shows appropriate growth rate and gives higher yield is chosen for the production of recombinant protein (Wurm, F. M. 2004). Here a question arises, why all colonial populations do not exhibit the same level of expression ? Through this prespective, I wish to highlights a different situation with the same cloning steps, in which, if the plasmid is not completely digested might give rise to interfering recombinant plasmid (IRP) that would further suppress the expression of the gene of interest. If there is a complete digestion of the template DNA and incomplete digestion of plasmid with only one enzyme, digested template DNA would not ligate with the plasmid. This condition may lead to a third situation that might lead to the incorporation of the template DNA into the plasmid in a reverse tandem order (Figure 2). The expression of these new recombinants (IRP) will give rise to the expression of both sense and antisense RNA hybrids. This would later result in the production of siRNA that can specifically silence the expression of their target (Fire et al, 1998). Screening of these IRP recombinants, either by restriction digestion or PCR, might produce the same results that may lead to a false conclusion that cloning has occurred in a

normal way. This can also explain the reason why Napoli and Jorgensen when over-expressed chalcone synthase gene (a key enzyme in flavonoid biosynthesis responsible for the deep violet coloration in petunias) in the petunia flowers unexpectedly resulted in the formation of white petunia flowers (Napoli et al, 1990).

For the production of therapeutic proteins, the mammalian cells are preferred over other hosts, and 60–70% of the recombinant protein pharmaceuticals are derived from the mammalian cell. In the last few decades, more than 100-fold yield improvements over titer has been achieved in the mammalian cell bioreactors by improvement in media composition and process control. This has also been possible due to the introduction of novel vector system and host cell engineering (Wurm, FM 2004). Further improvement in the process is possible with screening out of any interfering recombinant plasmid at the initial stages. At present, many of the genes cloned in different vectors are already available through different commercial suppliers. Hence, qualitative checking needs to be done to detect whether the supply contains an interfering recombinant vector or not. The suppliers need to provide a certification of their products ensuring it to be free of any interfering recombinant vector.

Hopefully suggestions within current study will make molecular cloning as a more powerful toolbox for exploring, manipulating, and harnessing DNA that will further lead to the expression of safer therapeutic proteins at lower cost and solve the existing irreproducibility problem in the field of life science worldwide.

Competing financial interests

The author declares no competing financial interests.

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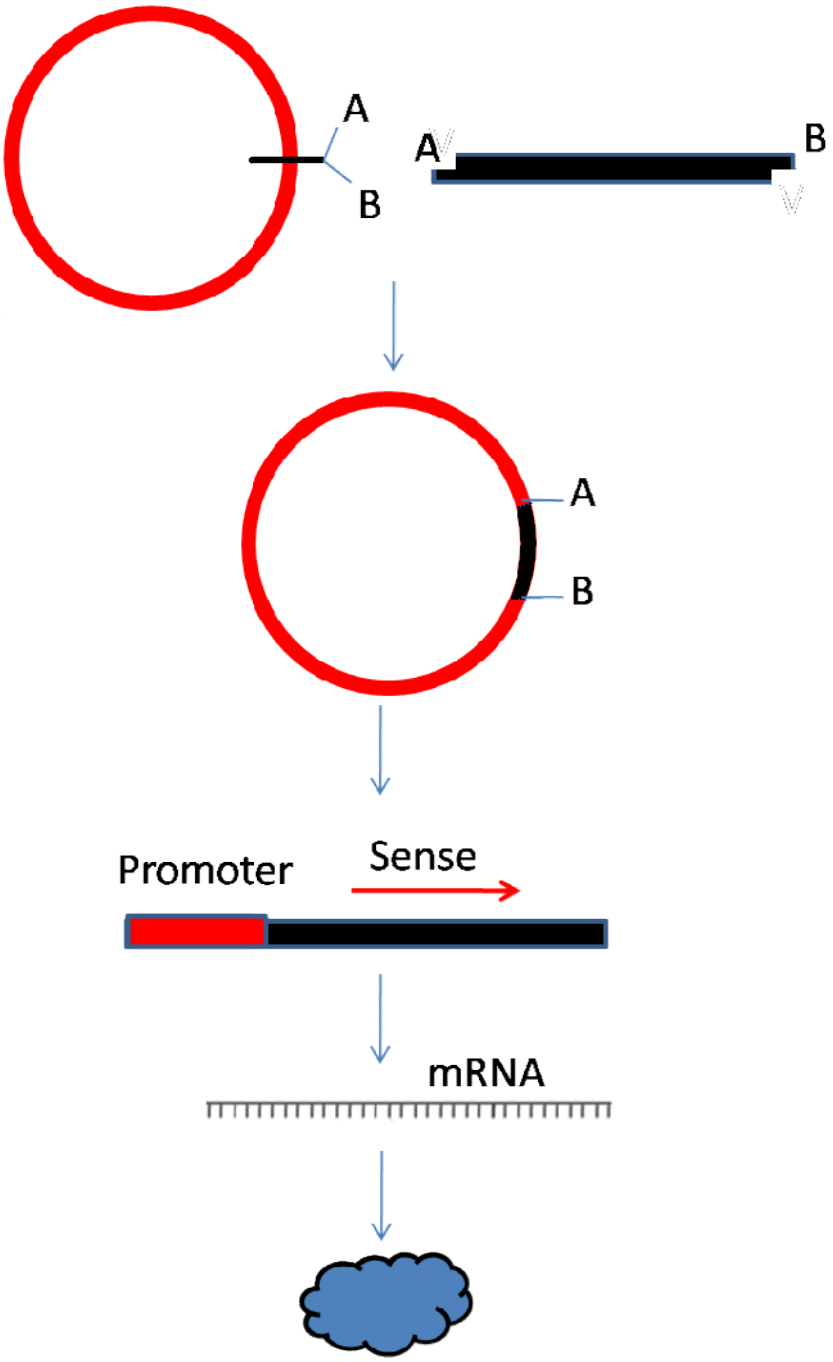

Fig.1 : Basic steps of gene cloning.

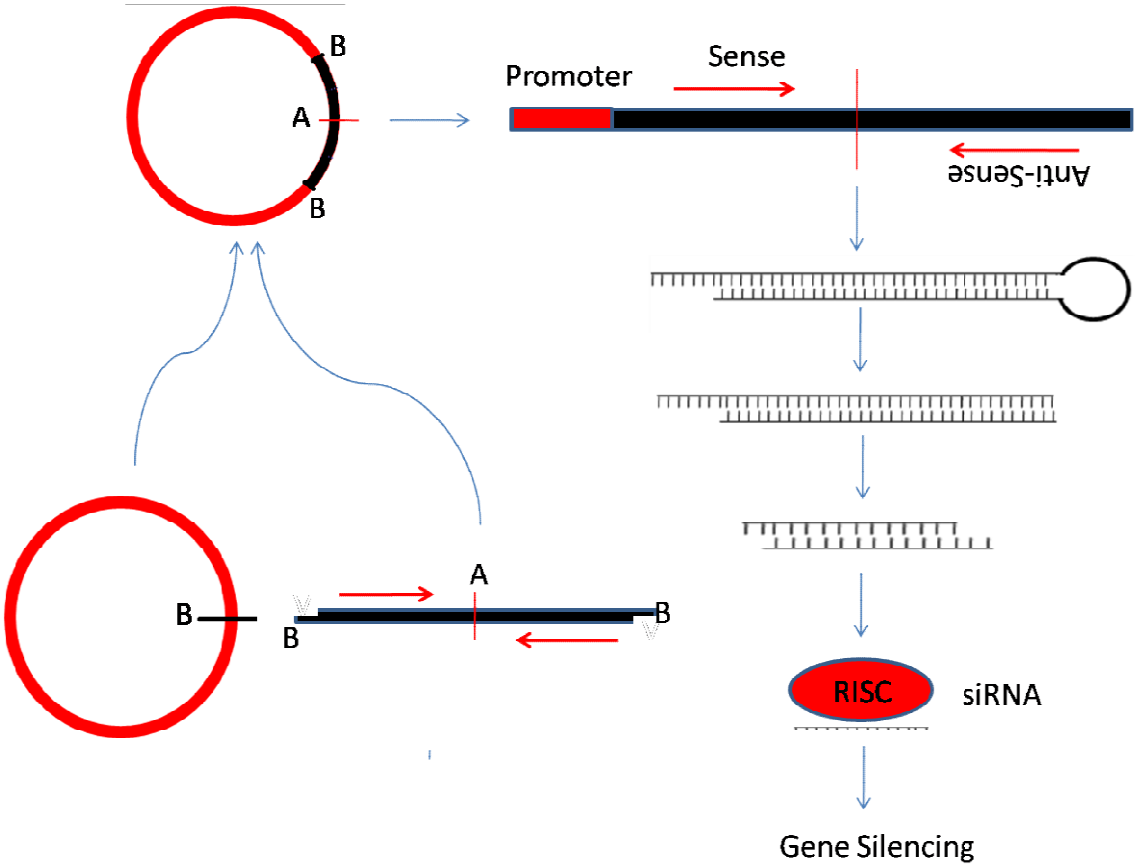

Fig.2 : Generation of IRP (Interfering recombinant plasmid) during a cloning step, which later can give rise to siRNA