Development of a Novel High Throughput Directional Cloning Platform

Ron Hernan and Leanne Snyder
Sigma-Aldrich Biotechnology, St. Louis, MO

Abstract

The cloning and expression of a large number of genes has been an enormous challenge for gene function studies in the post-genomic era. One of the major challenges has been the lack of a universal restriction enzyme system for generating the vectors most required for directed cloning. Consequently, cloning strategies have been limited to the use of several minimal combinations of restriction enzymes, and are often associated with plasmid instability and spontaneous deletions. To overcome these problems, Bacterial Double-Hind III (BHI) and CMH are incorporated during PCR amplification with restriction enzyme sites engineered into primers. Digestion of the amplified product with restriction enzymes results in repair generated ligated ends which can be ligated into appropriate mammalian expression vectors. We have cloned nearly 20 unique E. coli ORFs using this technique previously. In this study, we report the results of a larger scale study examining the efficiency, performance, and quality of the BHI/CMH cloning system relative to comparable systems. We were able to achieve maximum incorporation without significant effects on PCR, allowing for controlled digestion with Exonuclease III to produce a 5' overhang. Amplified products are ligated into a plasmid using a tac promoter to replicate the BHI/CMH site, while the CMH site is symmetrically blocked by ampicillin and thymine. The need for a directional cloning platform that is sequence independent and can be used in an automated format was met with the development of the novel cloning system we describe herein.

Introduction

Functional analysis of genes reading from bacterial or mammalian sources requires the amplification and directional cloning of target sequences in the expression vectors. Repetitive and time-consuming strategies have been traditionally employed for these purposes. In particular, bacterial ORFs have been cloned and expressed using the system of Francis et al. (1991) which involves PCR amplification followed by digestion with EcoRI and Hind III. While this system has been extremely useful, it requires the design of primers containing specific restriction enzyme sites. Therefore, the probability of finding restriction enzyme sites at the ends of ORFs is less than 1/2048. The need for a directional cloning platform that is sequence independent and can be used in a high throughput format was met with the development of the novel cloning system we describe herein. This method uses Exonuclease III, a double-stranded specific exonuclease that catalyzes the digestion of double-stranded DNA. The probability of finding restriction enzyme sites at the ends of ORFs is less than 1/2048.

Results

The system allows one to design primers for amplification without consideration of internal restriction enzyme sites. The system of Francis et al. (1991) involves PCR amplification followed by digestion with EcoRI and Hind III. While this system has been extremely useful, it requires the design of primers containing specific restriction enzyme sites. Therefore, the probability of finding restriction enzyme sites at the ends of ORFs is less than 1/2048.

Conclusions

The system allows one to design primers for amplification without consideration of internal restriction enzyme sites. The system of Francis et al. (1991) involves PCR amplification followed by digestion with EcoRI and Hind III. While this system has been extremely useful, it requires the design of primers containing specific restriction enzyme sites. Therefore, the probability of finding restriction enzyme sites at the ends of ORFs is less than 1/2048.

Acknowledgements

We would like to acknowledge Jing Xiao, Shu-Fen Li, Katrina Soder and Stephanie Lauer for their help in this work.

References

Bacterial ORFs


Corresponding Author

Fax: (314) 286-7645

PO BOX 14508

E. coli ORFs

Corresponding Author

Fax: (314) 286-1765