Perspective
Volume 12:3, 2022

Journal of Bioengineering & Biomedical Science

ISSN: 2155-9538 Open Access

Gain and Loss Screening System for Rapid and Efficient BAC Recombineering

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Introduction

Recombineering is a method for homologous recombination to change a Bacterial Artificial Chromosome (BAC). Nonetheless, because a screening approach to identify the suitable clone had not been properly developed, obtaining a correct clone within a limited time frame was problematic. To overcome these challenges, we created a new screening method (a gain and loss screening system) that allows us to quickly identify recombineered clones. Within 24 hours, simple inoculation of cells into LB medium with the proper antibiotics revealed the positive clones visually. The accuracy of this screening procedure was confirmed by DNA sequencing, which revealed that all positive clones had recombinant sequences. Furthermore, using our innovative method, we were able to finish the full procedure in just 13 days, including the first recombineering, flip-out, and second recombineering [1].

Biopharmaceutical protein manufacturing has long been a hot topic in biotechnology, with mammalian cell culture being one of the most common methods. Currently, mammalian systems create about 100 therapeutic proteins, and this number is projected to rise rapidly as new therapeutic antibodies are identified. As a result, in recent decades, major efforts have been made to enhance protein output in mammalian cell lines. The most extensively used tools for protein production are plasmid-based vectors. They include promoters, which are responsible for inducing the production of a Gene of Interest (GOI). However, the surrounding chromatin at the integration site has a significant impact on GOI expression in plasmid-based vectors. The expression tends to mute over time after the vector is integrated into a "silent chromatin" area (i.e., positional chromatin effects). As a result, numerous techniques have been developed to avoid chromatin's local effects. The use of a Bacterial Artificial Chromosome (BAC) that can accommodate entire mammalian loci is one of the most extensively used approaches. A whole gene with all cis-acting regulatory elements in their natural arrangement can be accommodated by BAC [2].

Description

As a result, the surrounding chromatin at the integration site has no effect on BAC, allowing it to correctly provide the intended expression pattern. However, due to the huge size of the BAC, normal cloning processes cannot be used to modify it (e.g., restriction enzyme digestion or ligation). BAC recombineering only allows for the accurate, particular, and true exchange of genetic information between two DNA molecules, regardless of the size of the DNA. Due to a large number of false positive background colonies during the screening step, BAC recombineering is labor-intensive and time-consuming. As a result, BAC recombineering posed a significant hurdle to less experienced researchers considering BAC as expression vectors for protein production [3].

The gain and loss screening system, which we offer here, is a revolutionary screening approach that can deliver significantly increased accuracy and reduced working time. This novel technology could be a potent new tool for facilitating biopharmaceutical protein expression and other large-vector applications, as well as making such approaches more accessible to scientists with less experience. The BAC recombineering technique requires a quick and efficient screening mechanism [4].

An approach to limit the amount of false positive clones by performing extended digestion of the targeting vector highlighted the necessity of the screening mechanism. However, due to the large number of false positive clones, finding a true recombinant clone still takes too long. Another technique for avoiding false positive clones from non-cleaved targeting vectors was to use PCR amplification of the targeting vector with HR-containing PCR primers. False positive clones obtained from PCR templates or inevitable PCR mistakes during PCR amplification of the targeting vector also hampered this method. We discovered a novel technique in which simple inoculation of cells visibly indicated positive clones within 24 hours in the current investigation. This technique is focused on whether cells with specific antibiotics can survive in the medium. A technique was developed to detect if a clone contains a real recombined BAC or comes from a false positive background by combining survival and non-survival in an antibiotic-containing culture. DNA sequencing results showed that all positive clones had recombinant sequences, proving the accuracy of this screening. Our research is the first to show that a new gain and loss screening strategy can achieve 100% accuracy, opening up a new vista in the field of BAC recombineering [5].

Conclusion

The 100% accurate gain and loss screening system aided the recombination process by ensuring that each recombination operation was successful at the same time. Recombineering is a genetic and molecular biology technology that uses homologous recombination systems to efficiently recombine linear DNA molecules flanked by lengthy HR. The fact that the targeting cassette with long HR (200–500 bp) is more efficient for recombineering than the targeting cassette with short HR emphasizes the importance of long HR (approximately 50 bp). Restriction digestion was used to linearize and purify targeting vectors with lengthy HR in preparation for usage in BAC recombineering stages. Complete digestion requires long-term DNA digestion, however if enzyme activity is hindered by DNA methylation, incomplete digestion can result.

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Received: 09 March, 2022, Manuscript No. Jbbs-22-61762; **Editor Assigned:** 11 March, 2022, PreQC No. P-61762; **Reviewed:** 25 March, 2022, QC No. Q-61762; **Revised:** 31 March, 2022, Manuscript No. R-61762; **Published:** 07 April, 2022, DOI: 10.37421/2155-9538.22.12. 293

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How to cite this article: Joy, Bethany. "Gain and Loss Screening System for Rapid and Efficient BAC Recombineering." J Bioengineer & Biomedical Sci 12 (2022): 293.