Perspective
Volume 12:9, 2022

Journal of Bioengineering & Biomedical Science

ISSN: 2155-9538 **Open Access**

Rapid and Effective BAC Recombination Engineering Using the Gain and Loss Screening System

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Introduction

A technique for altering a BAC through homologous recombination is known as recombineering. However, it was difficult to obtain a correct clone in a short amount of time due to the lack of a properly developed screening method for selecting the appropriate clone. We devised a novel screening strategy—a gain and loss screening system—to overcome these obstacles and quickly identify recombined clones. Simple antibiotic inoculation of cells into LB medium revealed the positive clones visually within 24 hours. DNA sequencing proved that this screening method was accurate, revealing that all positive clones had recombinant sequences. In addition, we were able to complete the entire procedure, including the first recombineering, flip-out, and second recombineering, in just 13 days thanks to our novel approach [1].

Mammalian cell culture is one of the most common methods utilized in biopharmaceutical protein manufacturing, which has long been a hot topic in biotechnology. About 100 therapeutic proteins are currently produced by mammalian systems, and this number is expected to rise quickly as new therapeutic antibodies are discovered. Consequently, significant efforts have been made in recent decades to boost protein production in mammalian cell lines. Plasmid-based vectors are the most commonly used tools for protein production. They include promoters, which are in charge of getting a Gene of Interest (GOI) to come out. However, GOI expression in plasmid-based vectors is significantly influenced by the surrounding chromatin at the integration site. After the vector is integrated into an area of "silent chromatin," also known as positional chromatin effects, the expression tends to mutate over time. Consequently, numerous strategies have been developed to avoid the local effects of chromatin. One of the most common methods is to employ a Bacterial Artificial Chromosome (BAC) that can accommodate all of a mammal's loci. BAC can accommodate a complete gene with all cis-acting regulatory elements arranged naturally [2].

Description

The integration site's surrounding chromatin has no effect on BAC, allowing it to accurately provide the desired expression pattern. However, normal cloning procedures, such as restriction enzyme digestion or ligation, are unable to modify the BAC because of its enormous size. Regardless of the DNA's size, BAC recombineering only permits the precise, specific, and accurate exchange of genetic information between two DNA molecules. BAC recombineering takes a lot of time and effort because there are so many false positive background colonies during the screening step. Consequently, less experienced researchers considering BAC as expression vectors for protein production encountered significant difficulties with recombineering [3].

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Received: 24 August, 2022, Manuscript No. jbbs-23-87853; **Editor Assigned:** 26 August, 2022, PreQC No. P-87853; **Reviewed:** 08 September, 2022, QC No. Q-87853; **Revised:** 15 September, 2022, Manuscript No. R-87853; **Published:** 22 September 2022, DOI: 10.37421/2155-9538.22.12.323

The revolutionary gain and loss screening system that we offer here can significantly improve accuracy while reducing time spent on the job. Biopharmaceutical protein expression and other large-vector applications could be made easier and more accessible to scientists with less experience using this innovative technology. A quick and effective screening mechanism is needed for the BAC recombineering technique [4].

The significance of the screening mechanism was brought to light by an approach that utilized extended digestion of the targeting vector to reduce the number of false positive clones. However, it still takes too long to locate a genuine recombinant clone due to the large number of false positive clones. Using PCR amplification of the targeting vector with HR-containing PCR primers was another way to avoid false positive clones from non-cleaved vectors. This method was also hindered by false-positive clones that were obtained from PCR templates or by unavoidable PCR errors that occurred during the PCR amplification of the targeting vector. In the current investigation, we discovered a novel method that visible positive clones within 24 hours of simple cell inoculation. This method focuses on determining whether antibiotic-treated cells can survive in the medium. By combining survival and non-survival in an antibiotic-containing culture, a method was developed to determine whether a clone contains a genuine recombined BAC or comes from a false positive background. The accuracy of this screening was demonstrated by the fact that all positive clones' DNA sequencing results contained recombinant sequences. In the field of BAC recombineering, our study is the first to demonstrate that a novel gain and loss screening strategy can achieve 100% accuracy [5].

Conclusion

The recombination process was aided by the 100% accurate gain and loss screening system, which ensured that each recombination operation was successful simultaneously. Utilizing homologous recombination systems, recombineering is a genetic and molecular biology technique for efficiently recombining linear DNA molecules flanked by long HR. The significance of long HR (approximately 50 bp) is emphasized by the fact that the targeting cassette with long HR (200–500 bp) is more effective for recombineering than the targeting cassette with short HR. In preparation for use in BAC recombineering stages, restriction digestion was used to linearize and purify targeting vectors with long HR. Long-term DNA digestion is necessary for complete digestion; however, incomplete digestion may occur if DNA methylation inhibits enzyme activity.

References

1. Lin, Xinyi, Borui Xu, Hong Zhu and Yongfeng Mei et al. "Requirement and development of hydrogel micromotors towards biomedical applications." *Res* 2020 (2020).
2. Cheng, Tao, Yi-Zhou Zhang,Wen-Yong Lai and Wei Huang et al. "Conductive hydrogel-based electrodes and electrolytes for stretchable and self-healable supercapacitors." *Adv Funct Mater* 31 (2021): 2101303.
3. Jing, Xin, Hao-Yang Mi, Xiang-Fang Peng and Lih-Sheng Turng. "Biocompatible, self-healing, highly stretchable polyacrylic acid/reduced graphene oxide nanocomposite hydrogel sensors via mussel-inspired chemistry." *Carbon* 136 (2018): 63-72.
4. Vázquez-González, Margarita and Itamar Willner. "Stimuli-responsive biomolecule-based hydrogels and their applications." *Angew Chem Int Ed* 59 (2020): 15342-15377.

5. Yao, Bowen, Haiyan Wang, Miao Zhang, Chun Li and Gaoquan Shi et al. "Ultrahigh-conductivity polymer hydrogels with arbitrary structures." *Adv Mater* 29 (2017): 1700974.

How to cite this article: Joy, Bethany. "Rapid and Effective BAC Recombination Engineering Using the Gain and Loss Screening System." J Bioengineer & Biomedical Sci 12 (2022): 323.