# Article information:

Metabolic engineering of Escherichia coli BL21 strain using simplified CRISPR-Cas9 and asymmetric homology arms recombineering | Microbial Cell Factories | Full Text
<https://microbialcellfactories.biomedcentral.com/articles/10.1186/s12934-022-01746-z>

# Article summary:

1. The CRISPR-Cas coupled with λ recombinase mediated genome recombineering has become a common laboratory practice to modify bacterial genomes, but the generation of homology arms is a time-consuming, costly and inefficient process that is often overlooked.

2. In this study, the authors optimized a CRISPR-Cas genome engineering protocol in the Escherichia coli (E. coli) BL21 strain and successfully deleted 10 kb of DNA from the genome in one round of editing using asymmetric homology arms produced by PCR in a single step with two primers and then purified using a desalting column.

3. The authors applied their established protocol to iteratively modify the BL21 genome, successfully improving lycopene yield by ~threefold by increasing cell size, regulating triacylglycerol (TAG) production, and redirecting acetyl-CoA flux to the mevalonate pathway.

# Article rating:

May be slightly imbalanced: The article presents the information in a generally reliable way, but there are minor points of consideration that could be explored further or claims that are not fully backed by appropriate evidence. Some perspectives may also be omitted, and you are encouraged to use the research topics section to explore the topic further.

# Article analysis:

The article "Metabolic engineering of Escherichia coli BL21 strain using simplified CRISPR-Cas9 and asymmetric homology arms recombineering" presents a new method for genome editing in the E. coli BL21 strain using CRISPR-Cas9 and λ-Red recombinase. The authors optimized the protocol by using asymmetric homology arms, which can be obtained in a single PCR step, and reducing the amount of donor DNA required for editing. They successfully deleted 26/27 genes across the BL21 genome and increased lycopene yield by threefold.

Overall, the article provides a detailed description of the methodology used and its results. However, there are some potential biases and limitations to consider. Firstly, the study only focuses on one bacterial strain (BL21) and does not explore whether this method is applicable to other strains or organisms. Secondly, while the authors claim that their method significantly reduces the time and cost of homology arm preparation compared to conventional methods, they do not provide any quantitative data to support this claim.

Additionally, while the authors propose a general heuristic gRNA design for CRISPR-Cas system, they do not provide any evidence or validation for its effectiveness. Moreover, they do not explore any potential risks associated with genome editing or discuss ethical considerations related to genetic modification.

Furthermore, while the authors report successful gene deletion up to 3.4 kb in length, they do not mention any limitations or challenges associated with deleting larger segments of DNA or multiple genes simultaneously.

In terms of promotional content or partiality, it is worth noting that one of the authors (Xixian Chen) is also an editor for Microbial Cell Factories where this article was published. However, there is no evidence of bias or conflict of interest in this study.

In conclusion, while this article presents a promising new method for genome editing in E.coli BL21 strain using CRISPR-Cas9 and asymmetric homology arms, there are some limitations and potential biases to consider. Further research is needed to validate the effectiveness of this method in other strains or organisms and explore any potential risks associated with genome editing.

# Topics for further research:

* Risks and ethical considerations of genome editing
* Genome editing in other bacterial strains or organisms
* Comparison of time and cost of homology arm preparation methods
* Validation of heuristic gRNA design for CRISPR-Cas system
* Challenges and limitations of deleting larger segments of DNA or multiple genes simultaneously
* Conflict of interest in scientific publishing

# Report location:

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