2020 Rare Disease Challenge

BEEO TEAM

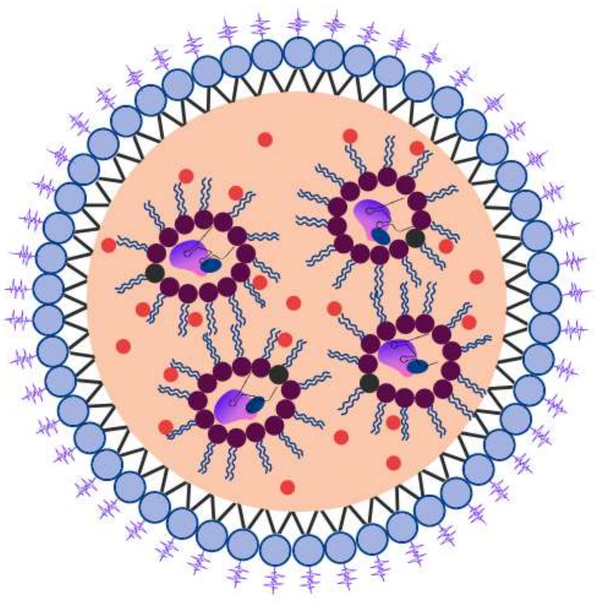
Yaşar Yurtsever[1], Ebrar Güneş[1], M. Feray Çoşar[1], M. Sami Özaydın[1], Ahmet Kara[1]

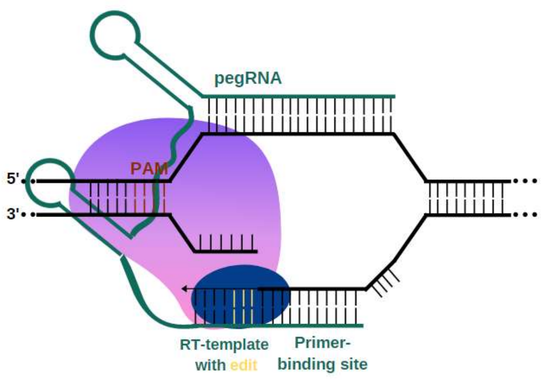
Istanbul Technical University, BSc Molecular Biology and Genetics' 23

ABSTRACT

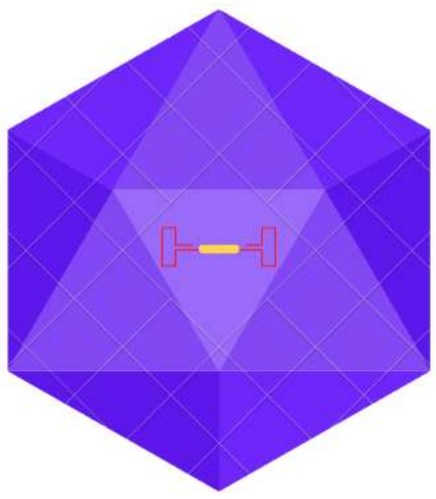
Glycogen storage disease, one of the rare diseases, causes glycogen accumulation in some organs and tissues as a result of the incomplete steps of glycogenolysis-glycolysis reactions. There are many subtypes of glycogen storage disease (GSD) and the most common subtype is type 1. GSD type 1 occurs at 1 in every 100,000 people. GSD is divided into type 1, a and b. GSD-1a represents more than 80% of GSD-1 cases and is the most common of the GSD types. R83C and Q347X GSD are the two most common mutations in type 1a. [1] As the BEEO team, we created 2 main projects for these 2 most observed mutations. Among these, "Prime Editing 4.0" that invented by the BEEO team, designed for R83C, and "Prime Editing Standard" for Q347X were created. An alternative project to the "Prime Editing 4.0" project for the R83C mutation has been presented.

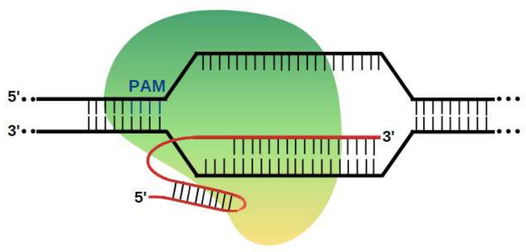
PRIME EDITING 4.0



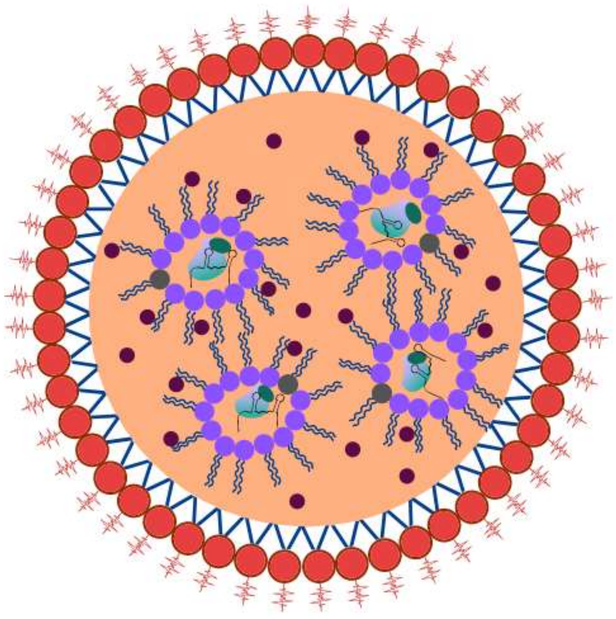


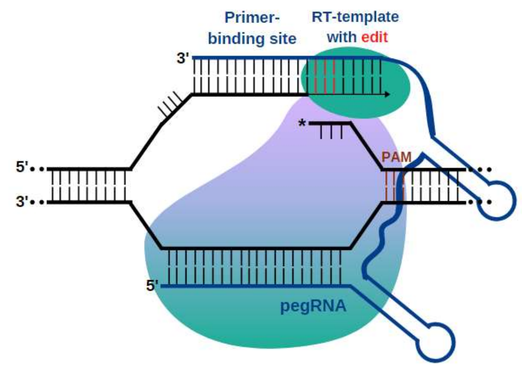
ALTERNATIVE PROJECT OF PRIME EDITING 4.0 CRISPR-CPF1





PRIME EDITING STANDARD





* The all figures were designed by team BEEO.

CONCLUSION

In the new generation Prime Editing 4.0, the mutant LbCpf1 was chosen due to its high targeting success and its suitability for the R83C mutation. [2] LbCpf1 has been mutated from the R1006A and R1218A regions, which regulate cleavage activity, cutting the single chain and become a nickase. [3] The LbCpf1 nickase was created by the BEEO team for the first time. Mutant M-MLV Reverse Transcriptase (RT) with enhanced activities was used. [4] PegRNA has been designed according to 5'-TTTV-3' pam sequence. PegRNA, LbCpf1 and M-MLV RT formed the ribonucleoprotein complex. Cationic lipids were chosen as the transfer method for reasons such as specificity and low immune response compared to viruses. [5] As an alternative to this project, the CRISPR / Cpf1 system was designed and used for AAV2 / 8 transmission. In our "Prime Editing Std" project, Prime Editing 2 (PE2) system was used to treat point mutation Q347X. Since 95% targeting success was observed in PegRNA (primeedit.nygenome.org), the system was not changed. An innovative transfer method with cationic lipid has been chosen. [6]

REFERENCES

1. Chou, J. Y., & Mansfield, B. C. (2008). Mutations in the glucose-6-phosphatase-alpha (G6PC) gene that cause type Ia glycogen storage disease. *Human mutation*, *29*(7), 921–930. https://doi.org/10.1002/humu.20772
2. Mali S. (2013). Delivery systems for gene therapy. *Indian journal of human genetics, 19(1)*, 3–8. https://doi.org/10.4103/0971-6866.112870
3. Stella, S., Alcón, P., & Montoya, G. (2017). Structure of the Cpf1 endonuclease R-loop complex after target DNA cleavage. *Nature, 546*(7659), 559-563. doi:10.1038/nature22398
4. Anzalone, A. V. et al. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature*, *576*(7785), 149–157. https://doi.org/10.1038/s41586-019-1711-4
5. Tang, X., Lowder, L., Zhang, T. et al. A CRISPR–Cpf1 system for efficient genome editing and transcriptional repression in plants. *Nature Plants 3*, 17018 (2017). https://doi.org/10.1038/nplants.2017.18
6. Wei, T. et al. (2020). Systemic nanoparticle delivery of CRISPR-Cas9 ribonucleoproteins for effective tissue specific genome editing. *Nature communications*, *11*(1), 3232. https://doi.org/10.1038/s41467-020-17029-3

SOCIAL IMPACT

- An information poster was made and shared on social platforms.

- 3 blog posts have been written on popular platforms.

- Awareness surveys, informative and accurate content for the disease were created on our Twitter page. Awareness efforts of the team and patient families were supported.

- We wrote a review book for patients' families to understand the scientific contents they read more easily. A compilation book in Turkish, plain and containing up-to-date information about the disease was provided free of charge for families.

- The #nadiredair series was made on YouTube by taking the questions of the patients' families. 6 live broadcasts were performed in this series. (Collaboration with 2 teams.)