Introduction
What would happen if proteins were very long? It’s so interesting, isn’t it? We expected that long-chain protein would be useful. We attempted to create this protein and to express outstanding functions. In general, because mRNA is linear, the protein translated from it is a constant size.

Linear mRNA
Thus, we circularized mRNA in E. coli by using the self-splicing mechanism of group I intron and removed the stop codon. As a result, it allows this circular mRNA to conduct the infinite translation of proteins.

Circular mRNA
In this method, long repeating proteins are created. The proteins will contribute to synthesizing fibers or collecting metal ions efficiently.

Methodology
Experiments
1. Confirm existence of Circular mRNA by reverse transcription of this jointing part and analyze the sequence.
2. Confirm repeating translation by SDS-PAGE.
3. Perform the Western blotting using peroxidase RFP antibody.
4. Reverse-transcribe the specific four fragments of DNA(A–D) and calculate the efficiency of mRNA circularization by the MPN-PCR.
5. Dye protein with the CBB and make the calibration curve between the strength of bands and the concentration of monomer RFP. Determine the quantity of the proteins.

Results and Discussion
Existence of Circular mRNA
The sequence(①) corresponded to the circularized sequence as we had expected. This means that mRNA was circularized. And also, the sequence indicates that the reading frame cannot slip down if a ribosome rotates several laps.

Synthesis of long-chain proteins
The proteins over 250 kDa were detected. This means that long-chain protein was synthesized by the circular mRNA that does not have stop codon.

Derived from RFP
The proteins over 250 kDa were bound with the antibody. It means that the long-chain protein derives from the RFP.

Circularize efficiency
The below figures show each section(A–D) and results of MPN-PCR.

Quantitative determination of protein
Concentration of the monomer RFP was 0.57(mg/mL), and the polymer RFP was 0.41(mg/mL). Thus, their ratio of existence was as follows.

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\frac{[\text{monomer RFP}]}{[\text{polymer RFP}]} = 42\%
\]

Future work
Not only the RFP, but we also succeeded in synthesizing long-chain metallothionein(2014 Nagahama BioBrick), which combines with metals. Therefore, more metals can be collected efficiently.

Caution of coding insert
The coding must be mutated so that the stop codons don’t exist on the reading frame.

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References
- R. PERIMAN, and M. ANJEL, in (1998), RNAs, 6, 1547-1554
- S. UMEKAGE, et al. (2012), Innovation in Biotechnology, 1, 75-80

Conclusions
- Existence of Circular mRNA
- Synthesis of long-chain proteins
- Derived from RFP
- High ability to synthesize protein

Figures
① Subcloning
② Results
③ Methodology
④ Discussion
⑤ Conclusion