

Nattokinase Overproduction and Establishment of Genetic Engineering Technology in *Bacillus subtilis* var. natto

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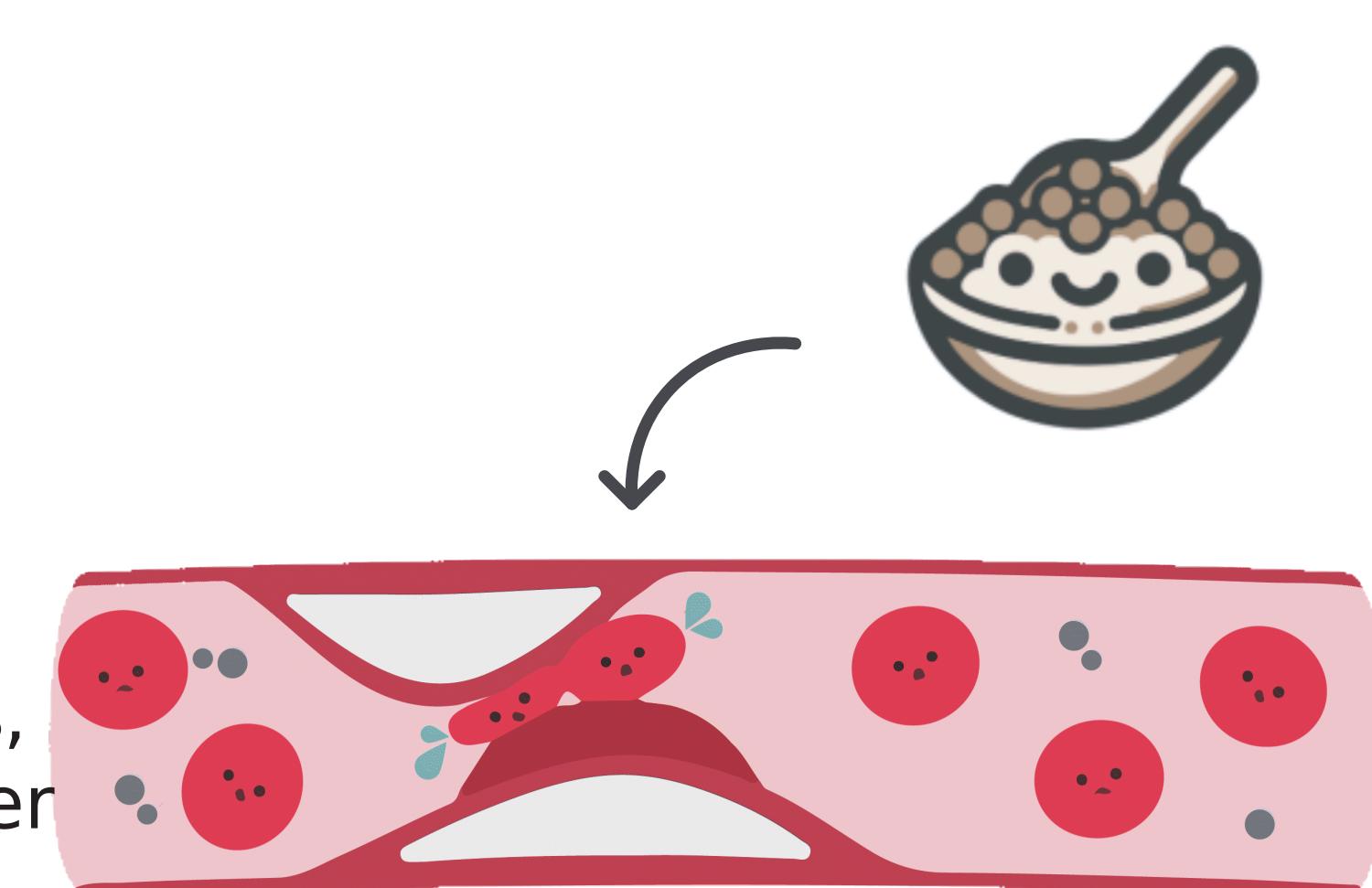
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BACKGROUND

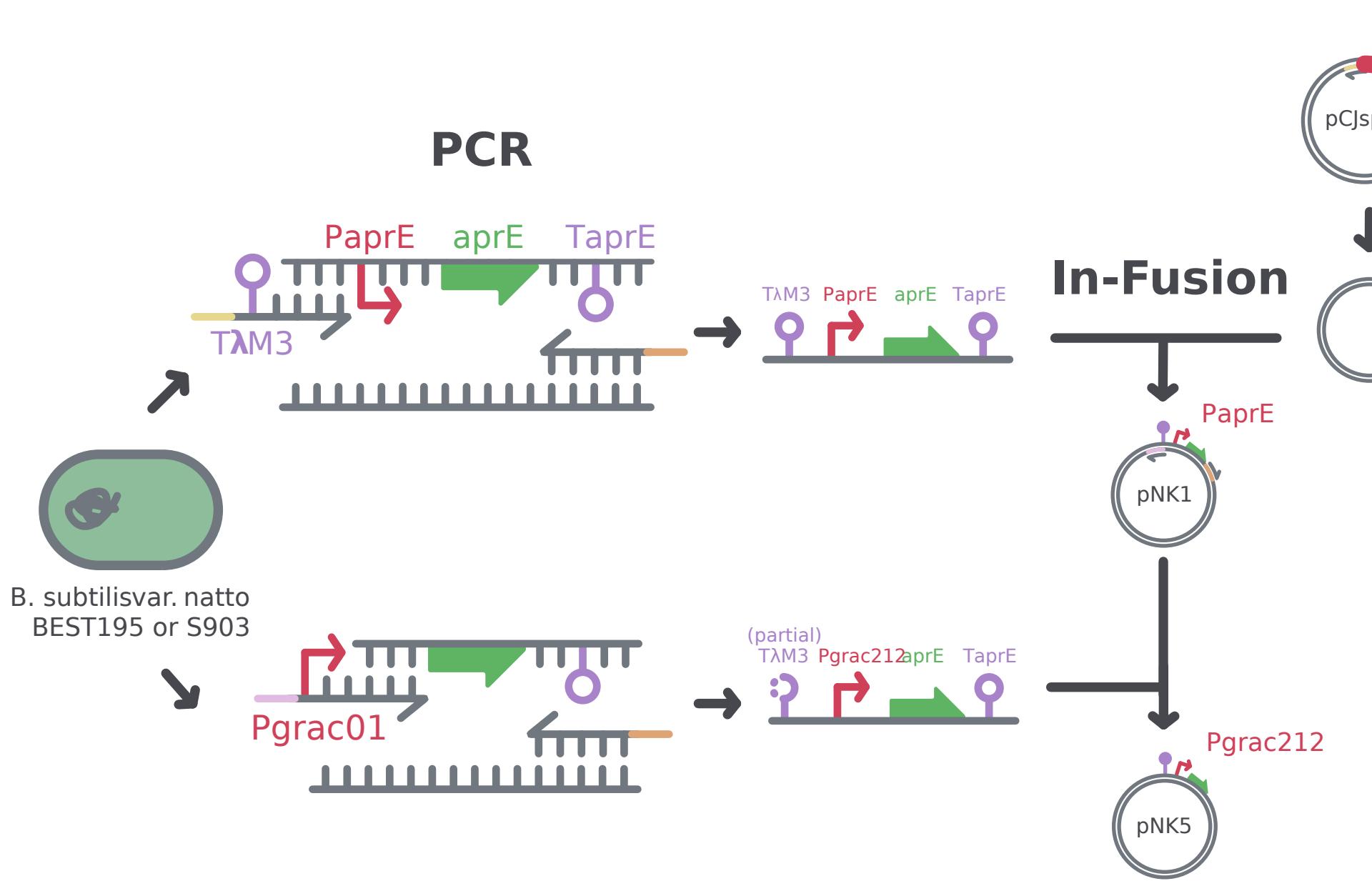
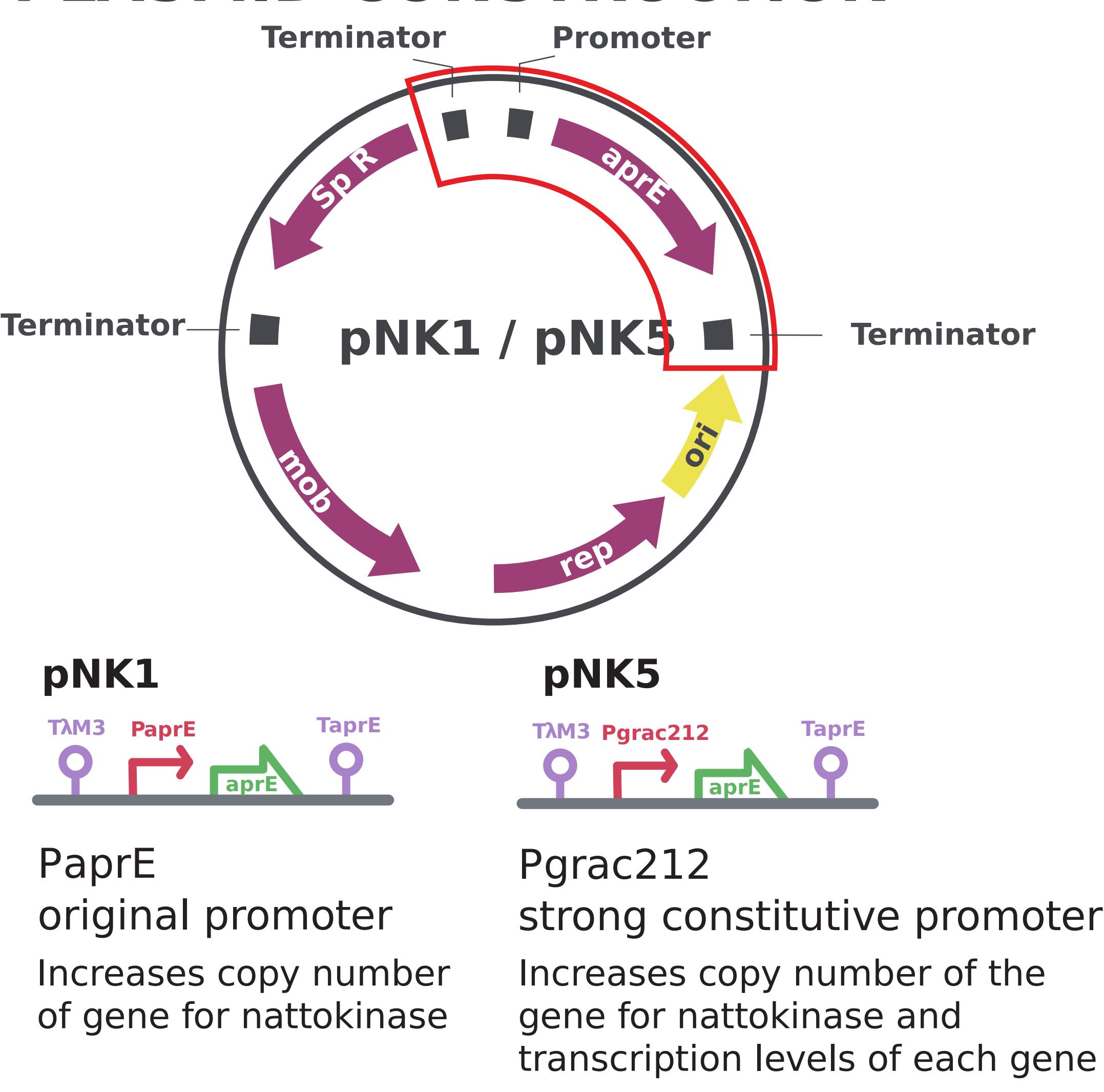
Thrombosis accounts for one in four deaths worldwide. It is a general term for diseases caused by blood clots obstructing blood vessels.

Natto has long been recognized in Japan as a health-promoting food.

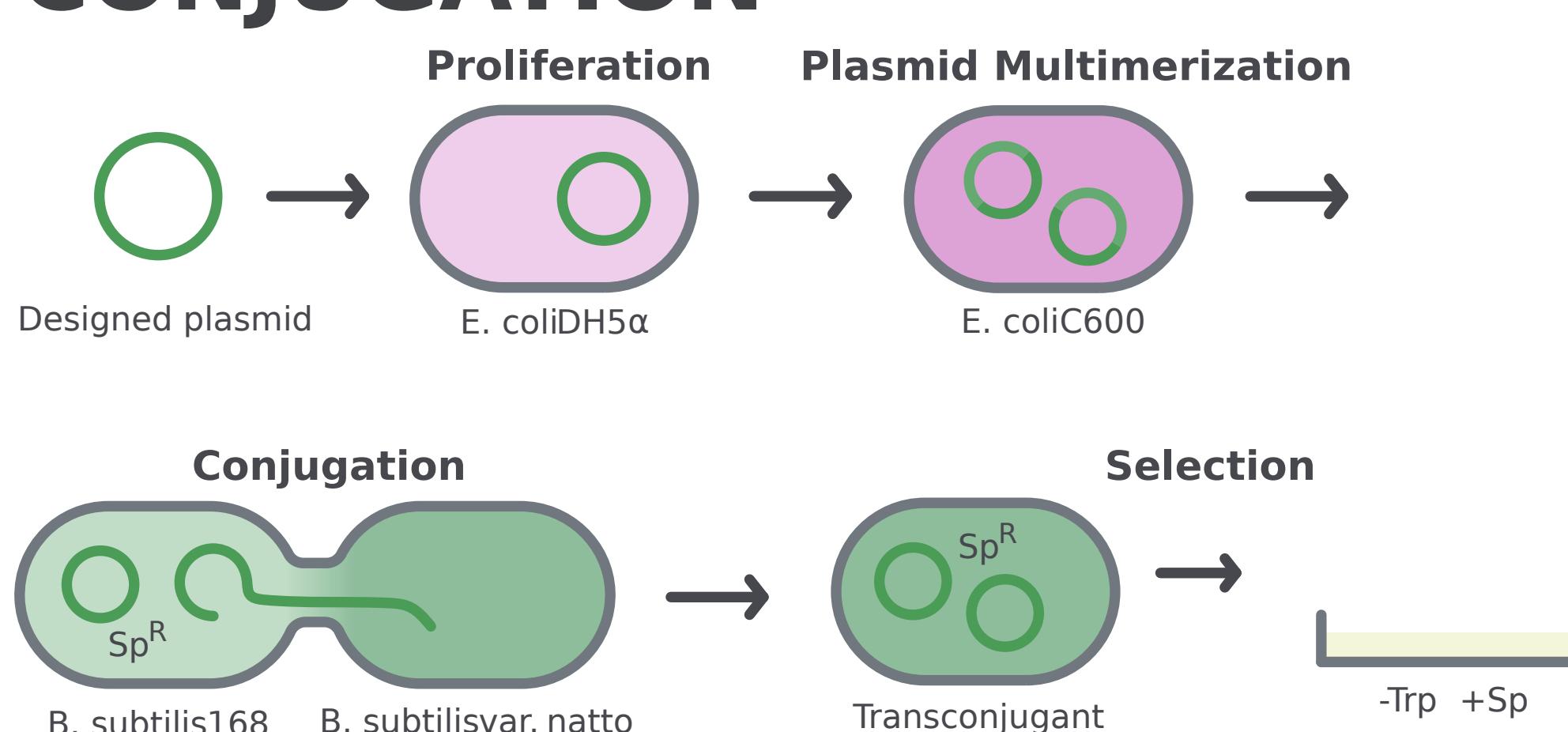
Nattokinase, an enzyme found in natto, is garnering attention as a preventative treatment for thrombosis due to its fibrinolytic effects. To stabilize the production of nattokinase, our project aims to engineer *B. subtilis* var. natto, which is the bacteria strain that naturally produces nattokinase to overproduce nattokinase. In the process, we also aimed to establish the genetic engineering technology for this bacteria as it is a difficult strain to engineer due to its low natural competency.



PLASMID CONSTRUCTION



CONJUGATION



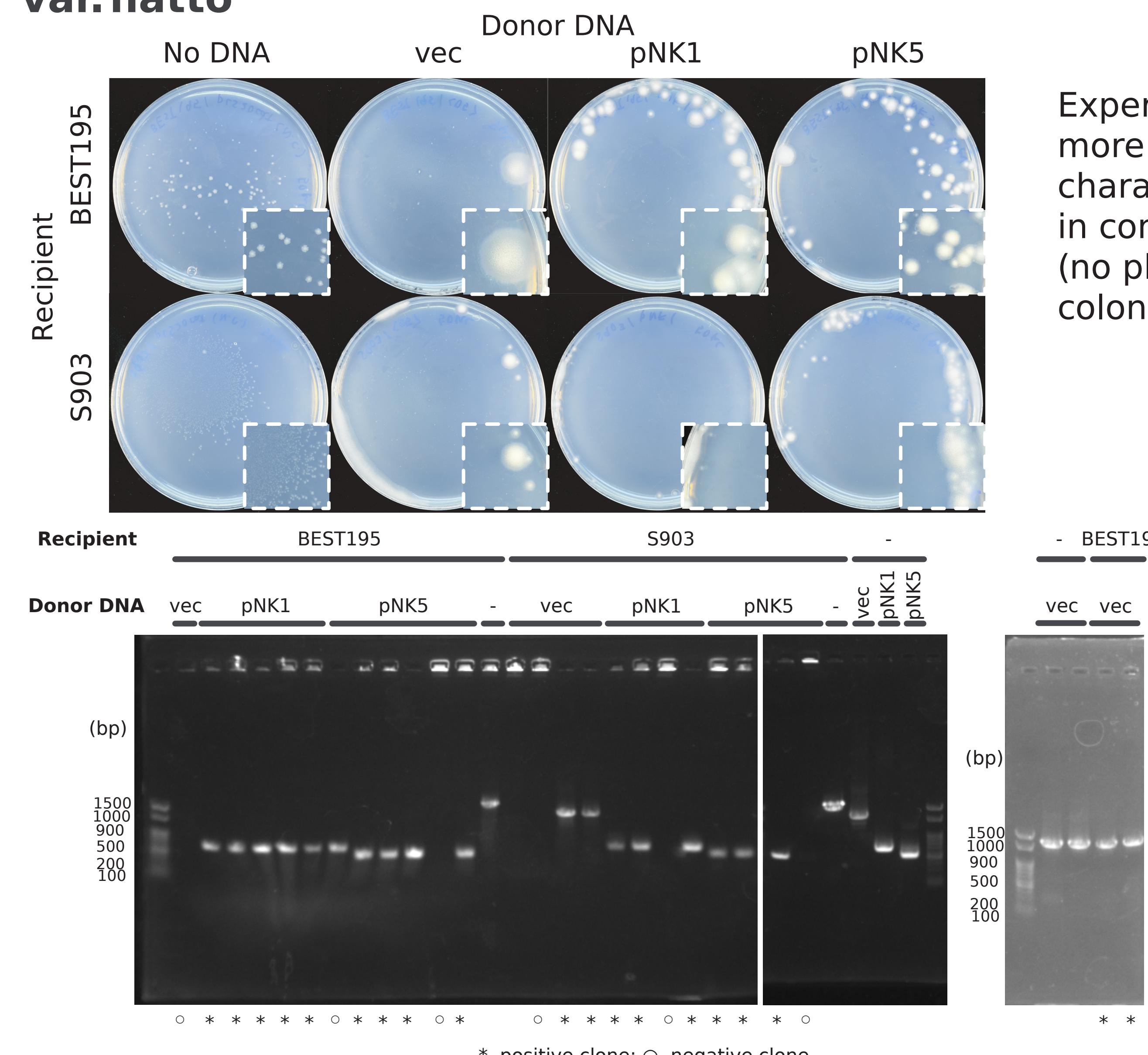
BEST195 - experimental strain

S903 - commercially available strain

S903 was isolated from the product "すごい納豆S-903", produced by Takano Foods, to confirm effectiveness of the plasmids in a commercially available strain.

RESULTS AND DISCUSSION

Conjugational transfer of nattokinase plasmid from *B. subtilis* var. natto



Experimental plates show bigger, more visible and opaque colonies, characteristic of *B. subtilis* var. natto, in contrast to the negative control (no plasmid) plates with smaller colonies.

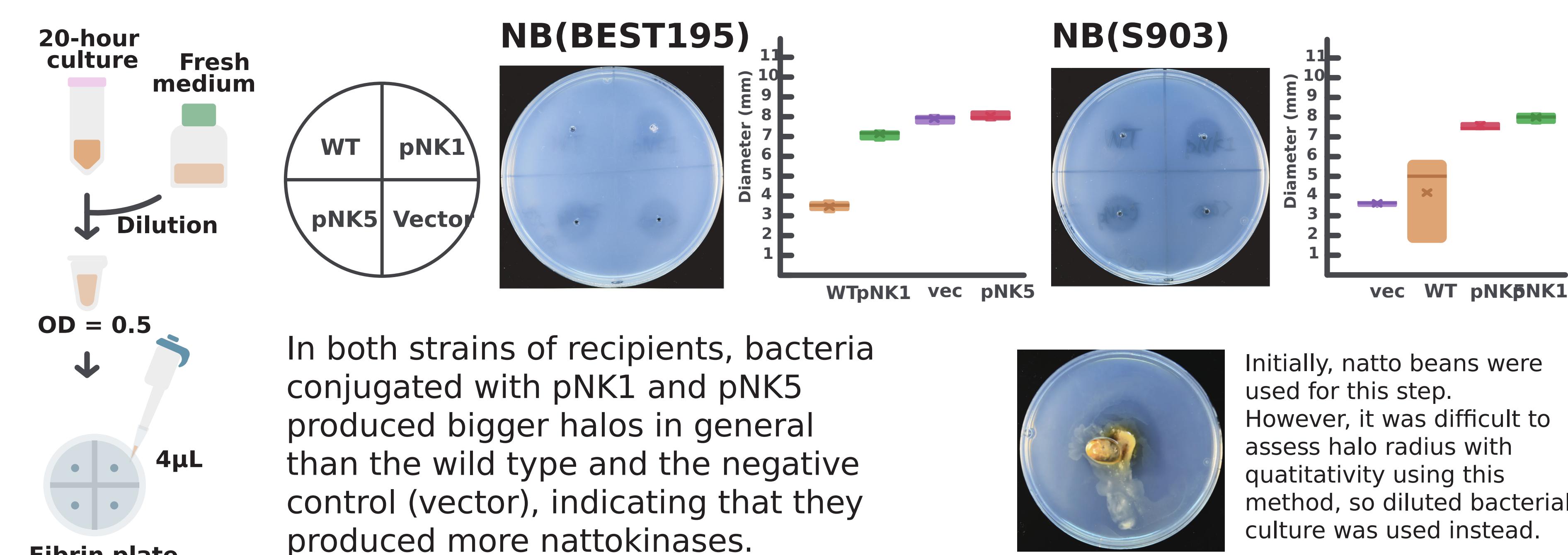
Colony PCR was performed on the experimental colonies. Bands at the expected lengths are observed for at least one colony from each combination of donors and recipients.

Expected lengths:
vec: 1,588bp
pNK1: 535 bp
pNK5: 511 bp

76 % of the experimental clones tested were positive clones.

Fibrin plate assay for nattokinase overproduction

Nattokinase degrades fibrin. Therefore, the fibrin plate assay was used to assess the fibrinolytic activities of nattokinase derived from various strains.



In both strains of recipients, bacteria conjugated with pNK1 and pNK5 produced bigger halos in general than the wild type and the negative control (vector), indicating that they produced more nattokinases.

FUTURE PERSPECTIVES

- Develop a better methodology (self-cloning) using only genes derived from *B. subtilis* var. natto. This will make it easier to implement products using this *B. subtilis* var. natto, so that they can be consumed as is and purification methods would be unnecessary.
- Test with other strains of *B. subtilis* var. natto, other foods fermented with *B. subtilis* var. natto. This will ensure the reproducibility of our results and allow this method to be used more widely.

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