



Oregon State
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Biosynthetic Studies of the α -amylase inhibitor Trestatin in
Streptomyces dimorphogenes

Ananiya Demessie^{1,2}, Taifo Mahmud²

¹College of Agricultural Sciences, ²College of Pharmacy
Oregon State University, Corvallis, Oregon

The evolution of sweet tooth



Humans evolved to crave sugar

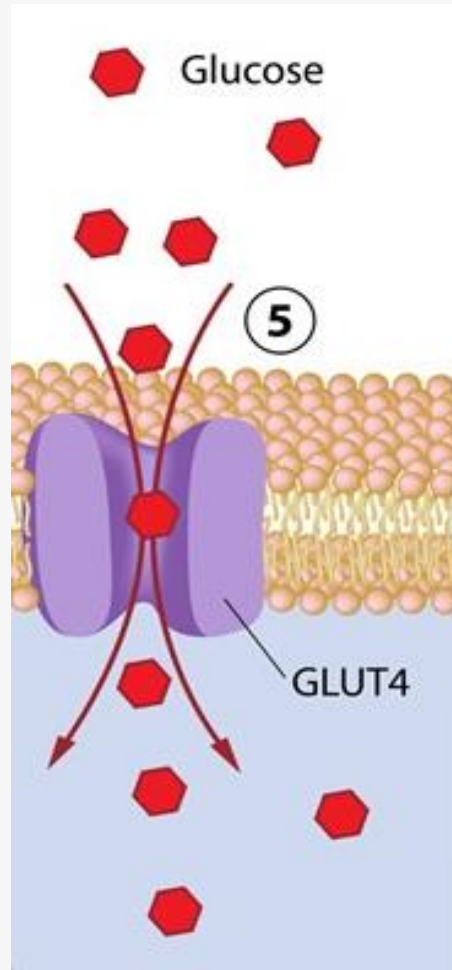
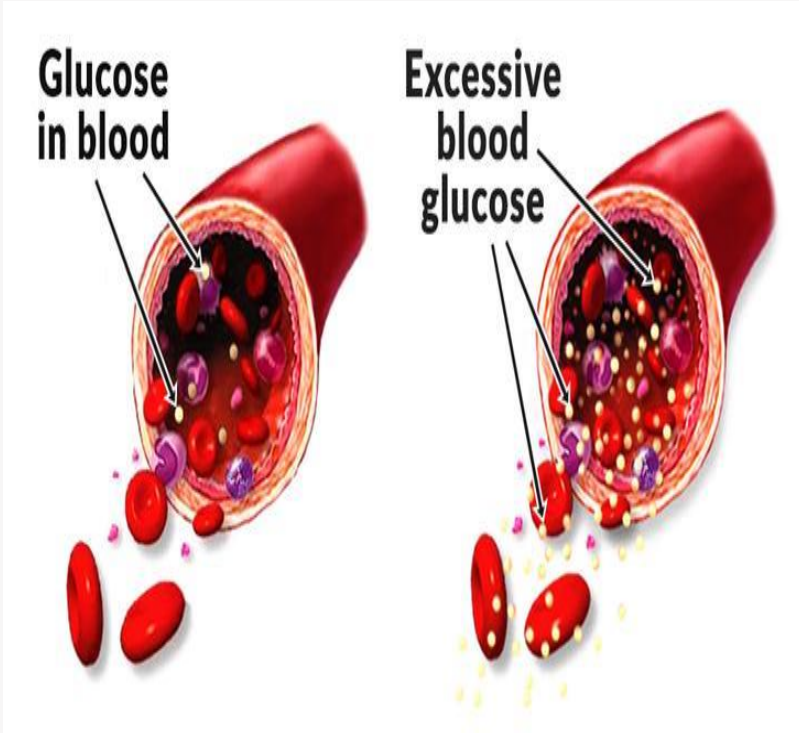
SUGAR ADDICTION: THE PERPETUAL CYCLE



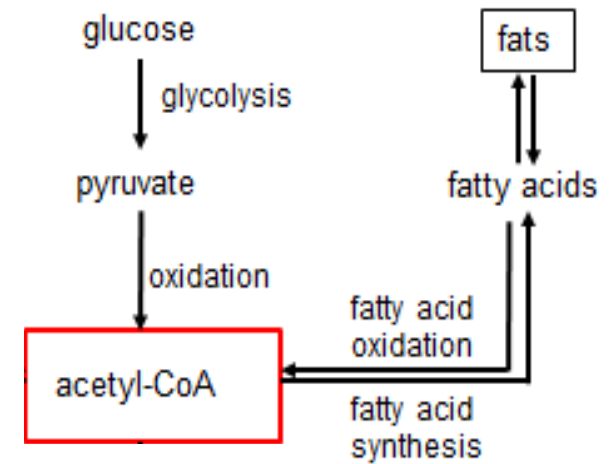
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Excess glucose in blood can lead to diabetes and obesity



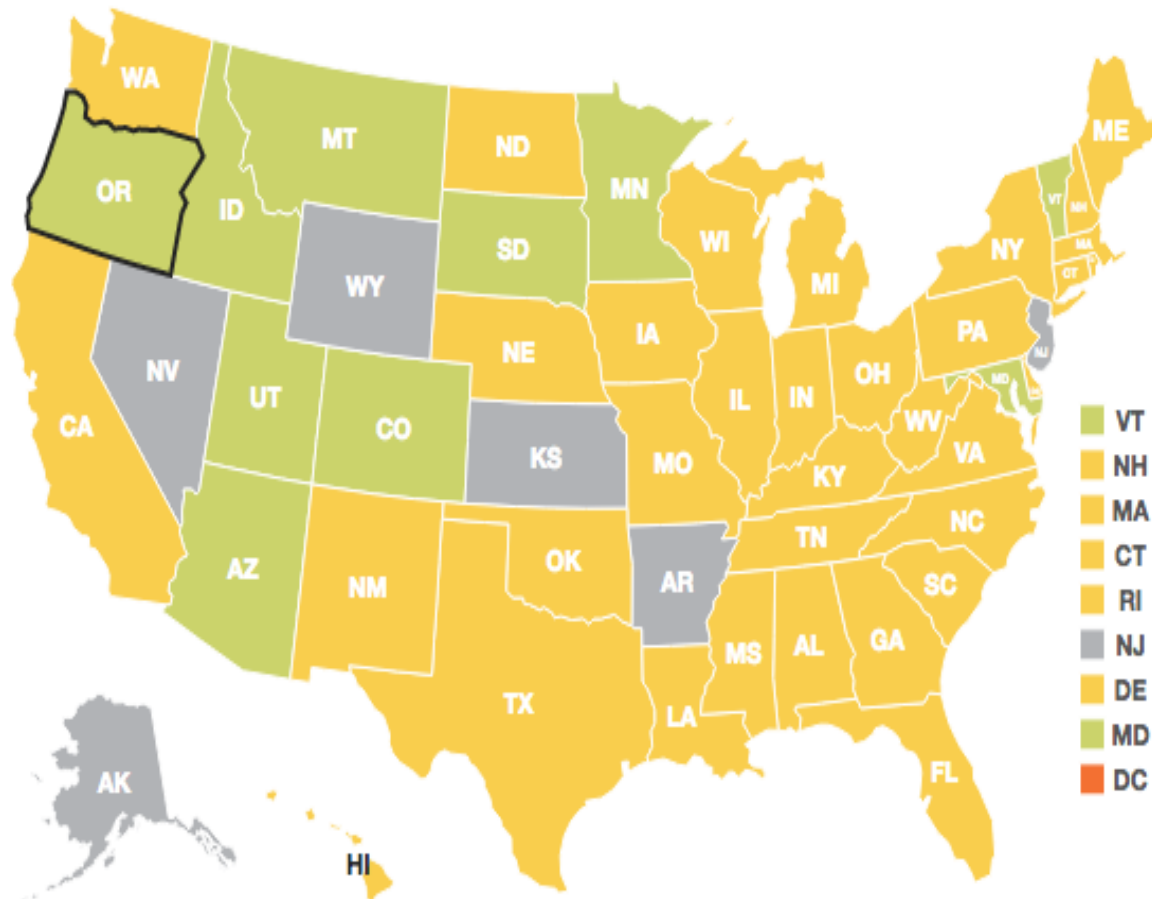
ACETYL CoA – THE CROSSROADS



Adult diabetes rate in the US (1990 vs 2015)

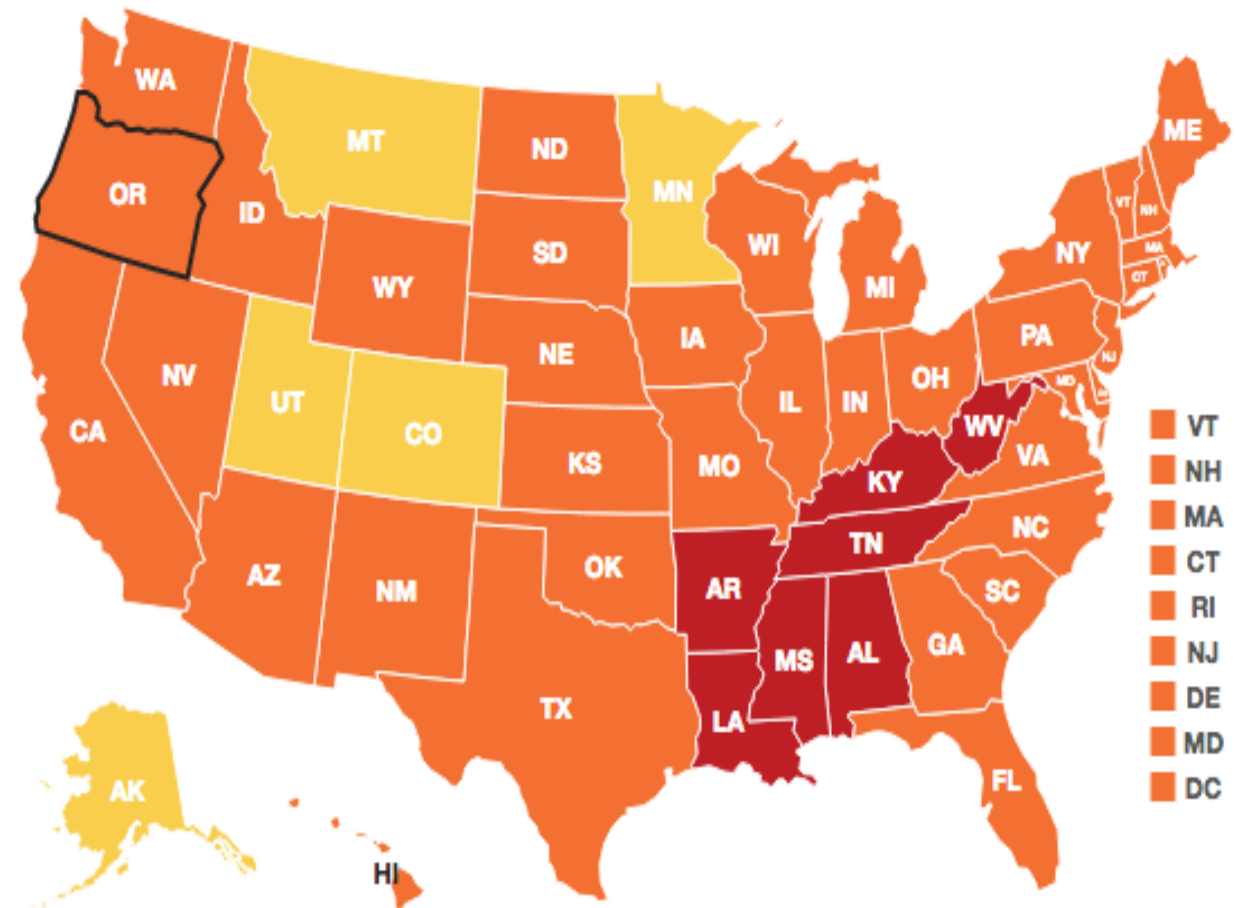
Percent of adults with diabetes

0 - 3.9% 4 - 7.9% 8 - 11.9% 12 - 15.9% 16%+



Percent of adults with diabetes

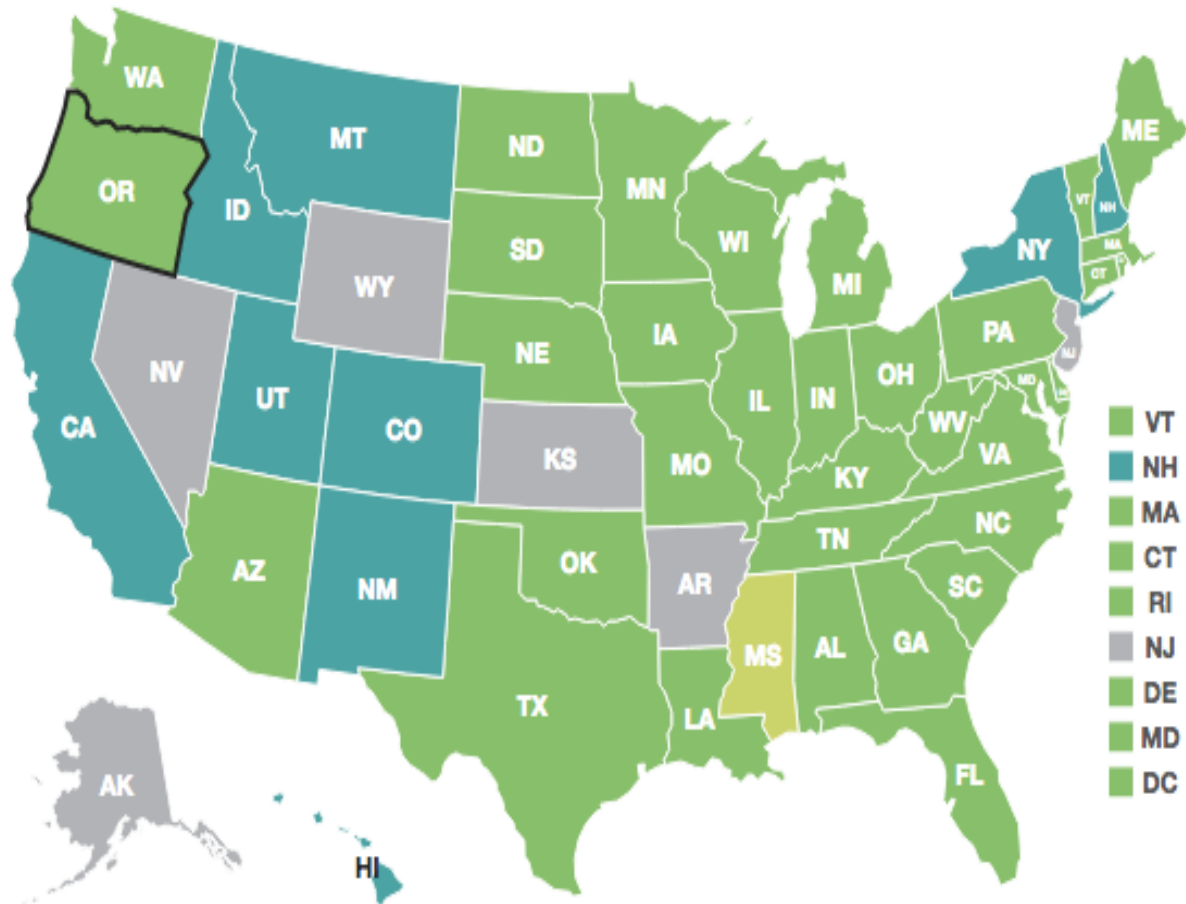
0 - 3.9% 4 - 7.9% 8 - 11.9% 12 - 15.9% 16%+



Adult obesity rate in the US (1990 vs 2015)

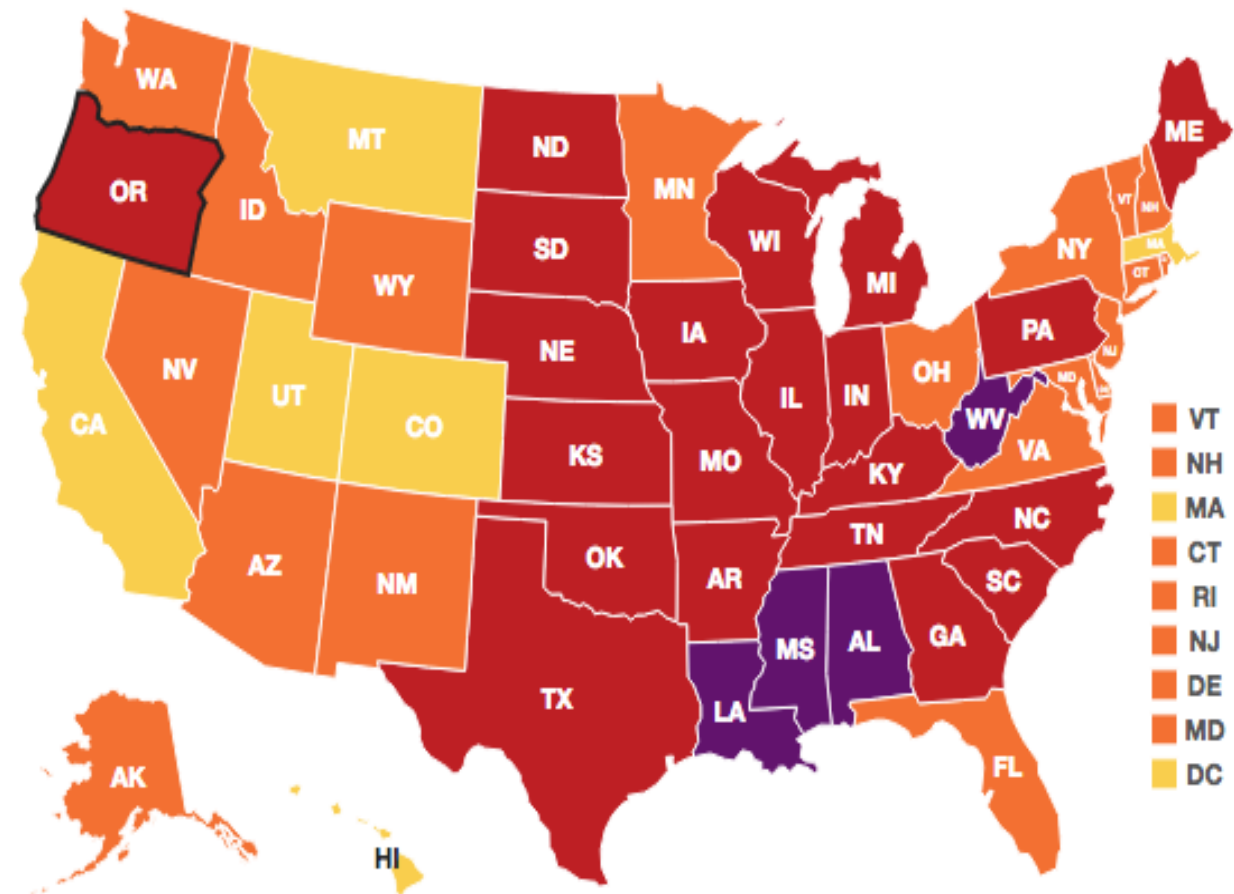
Percent of obese adults (Body Mass Index of 30+)

0 - 9.9% 10 - 14.9% 15 - 19.9% 20 - 24.9% 25 - 29.9% 30 - 34.9% 35%+

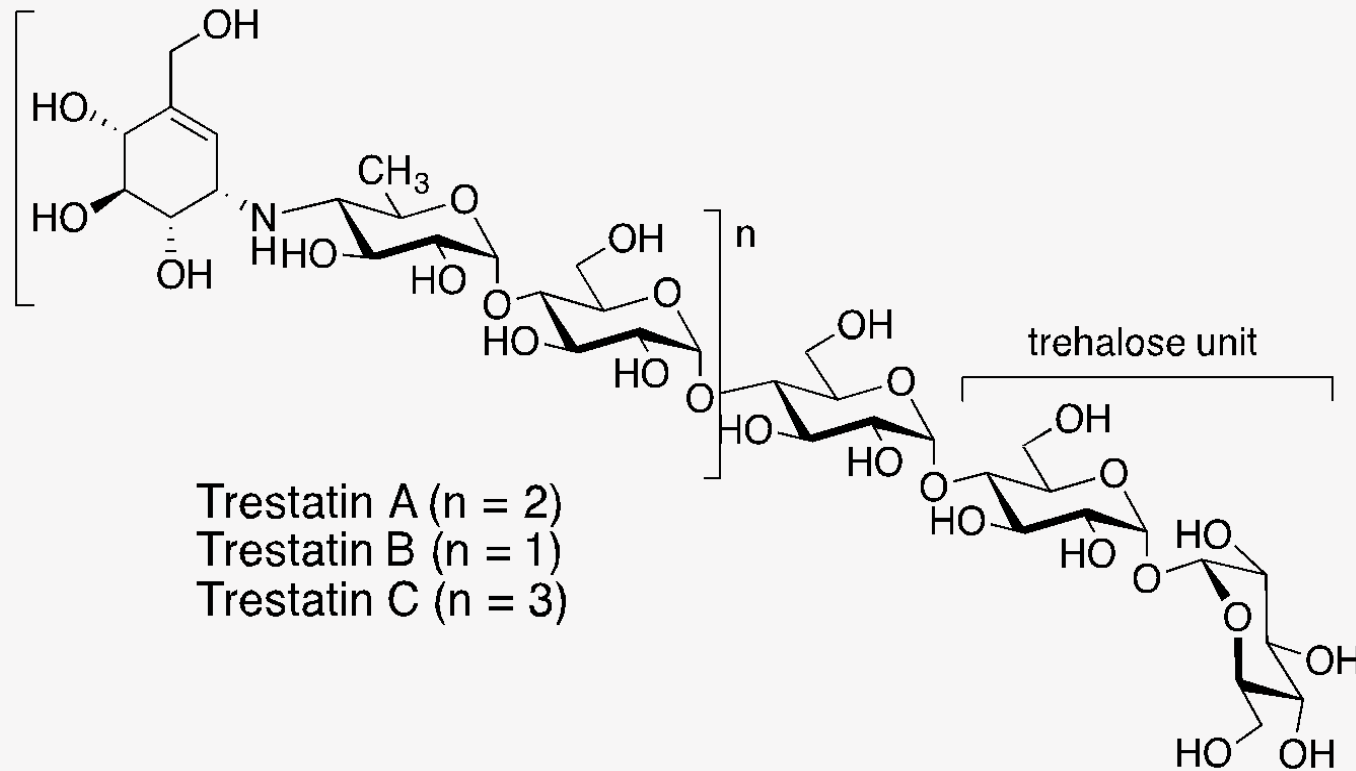


Percent of obese adults (Body Mass Index of 30+)

0 - 9.9% 10 - 14.9% 15 - 19.9% 20 - 24.9% 25 - 29.9% 30 - 34.9% 35%+



Trestatins are produced by *Streptomyces dimorphogenes*

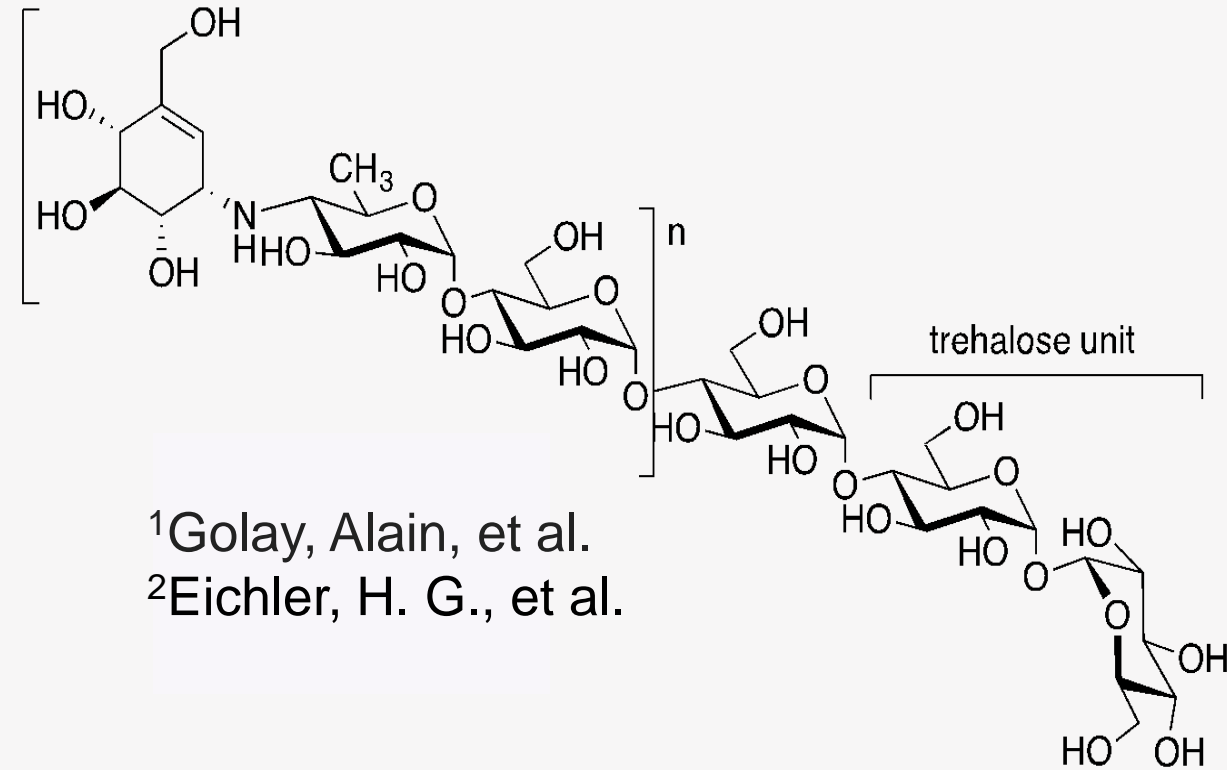
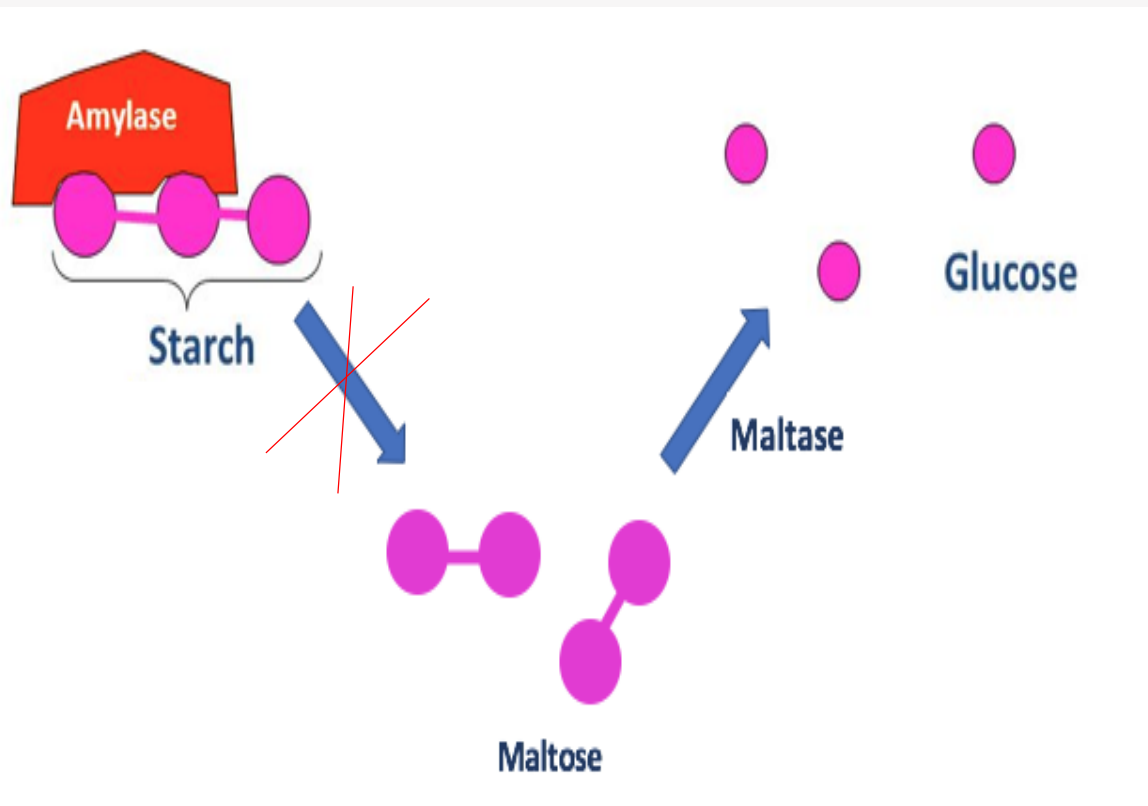


Streptomyces dimorphogenes

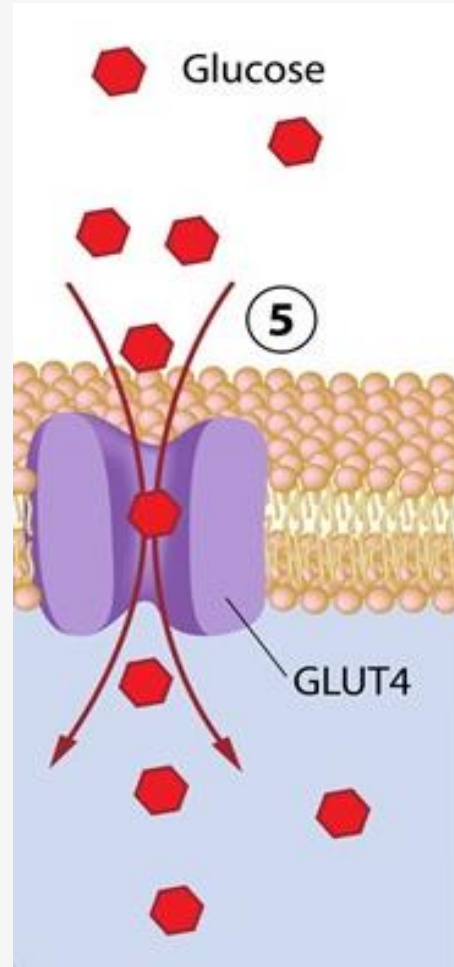
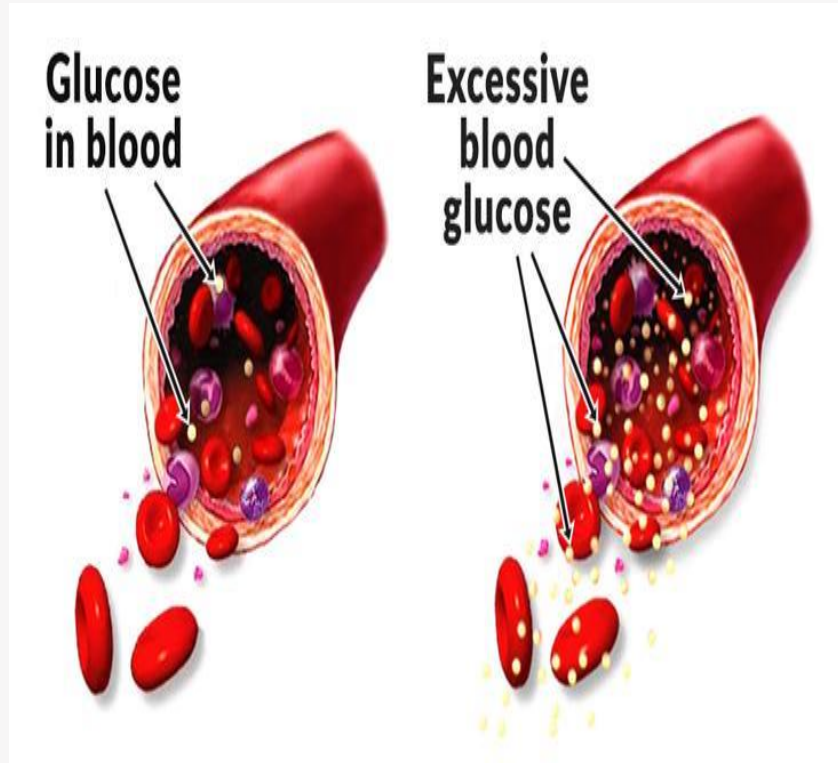
Chemical structures of trestatins

Trestatins as useful therapeutic agents for the treatment of diabetes and obesity

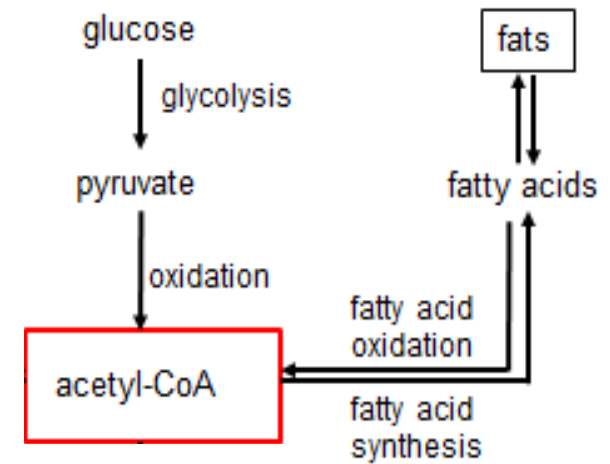
- Potent α -amylase inhibitor^{1,2}



Importance of α -amylase inhibitor



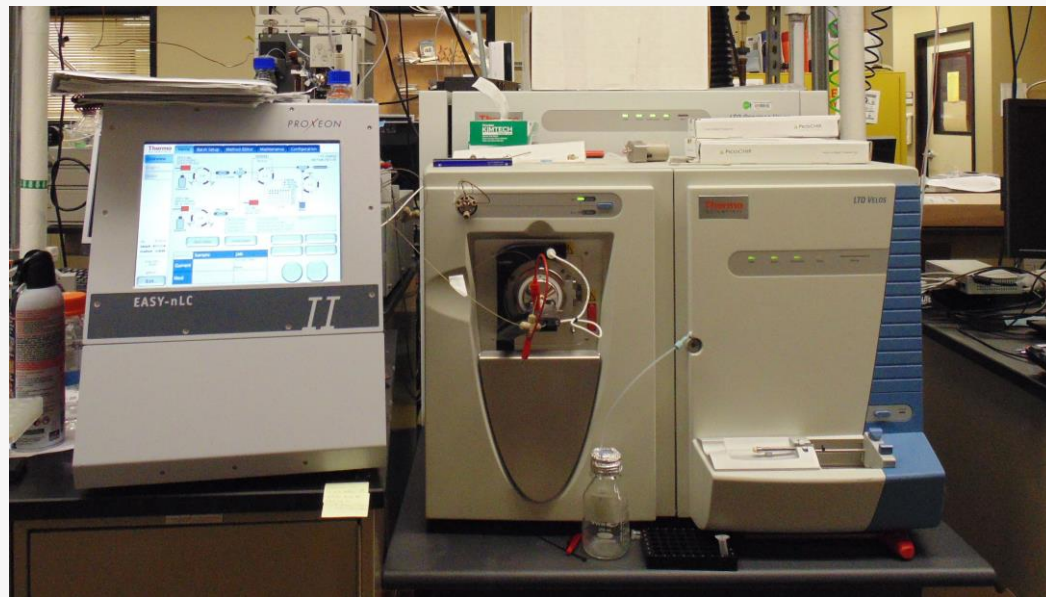
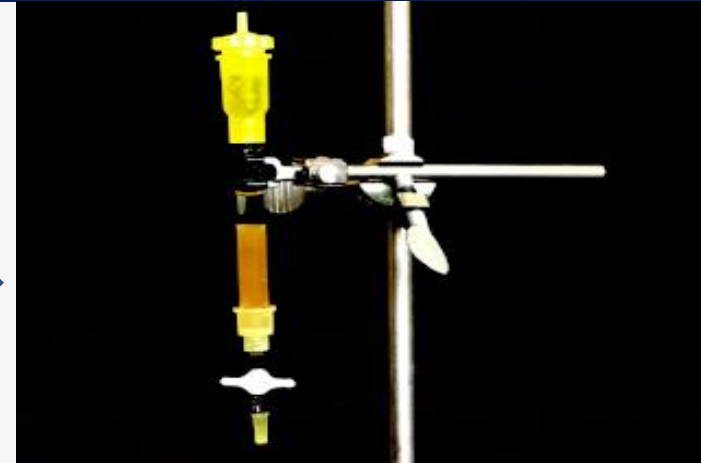
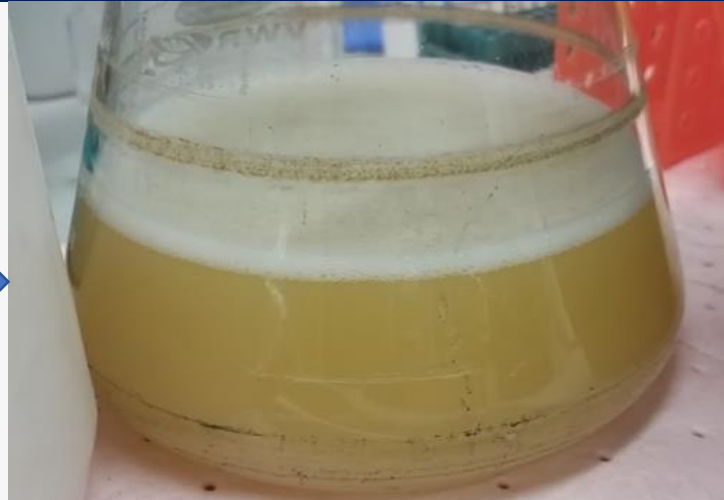
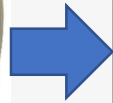
ACETYL CoA – THE CROSSROADS



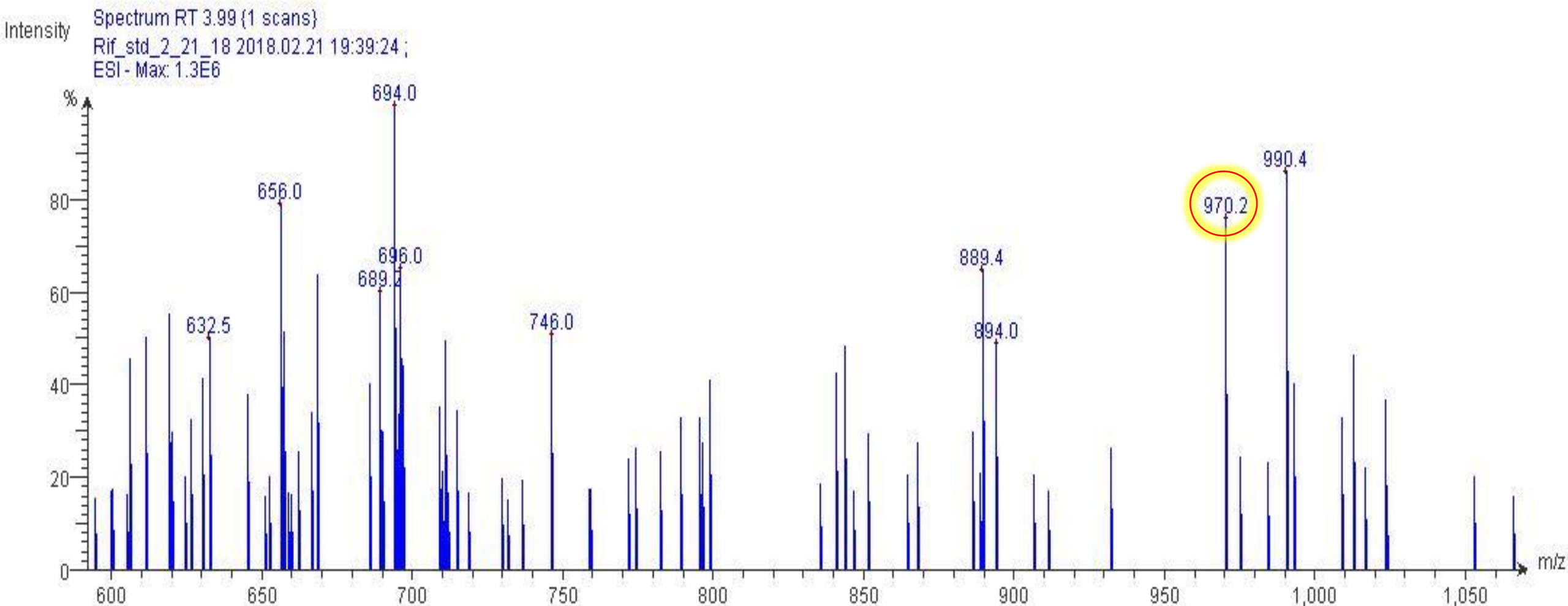
Objective

Investigating the function of individual genes within the trestatin gene cluster by gene inactivation

Checking for the production of trestatin in *Streptomyces dimorphogenes*



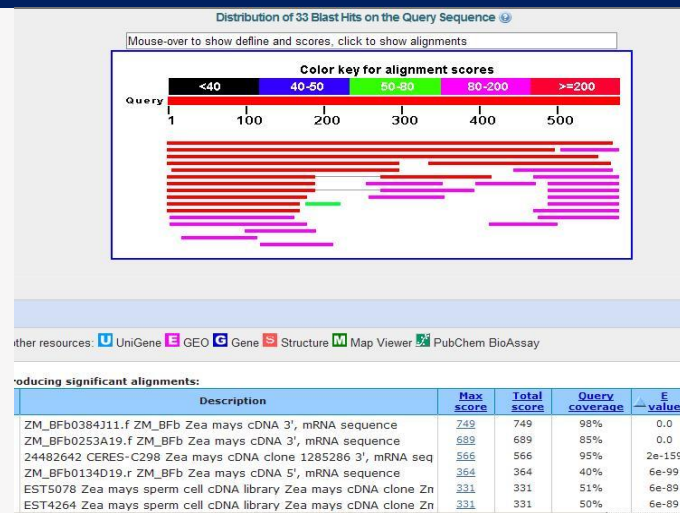
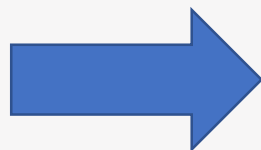
Confirmation of trestatin B production



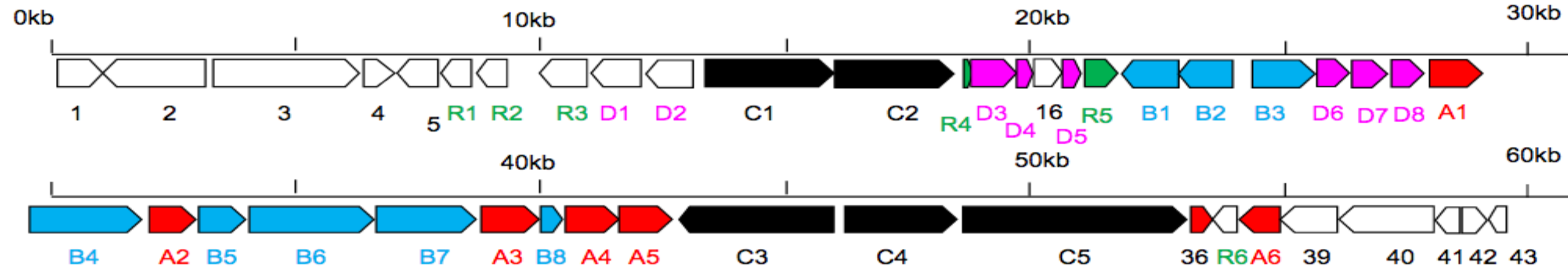
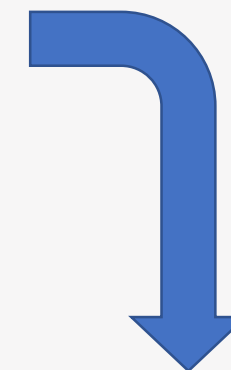
Identification genes within the trestatine biosynthetic gene cluster



Genome sequence data

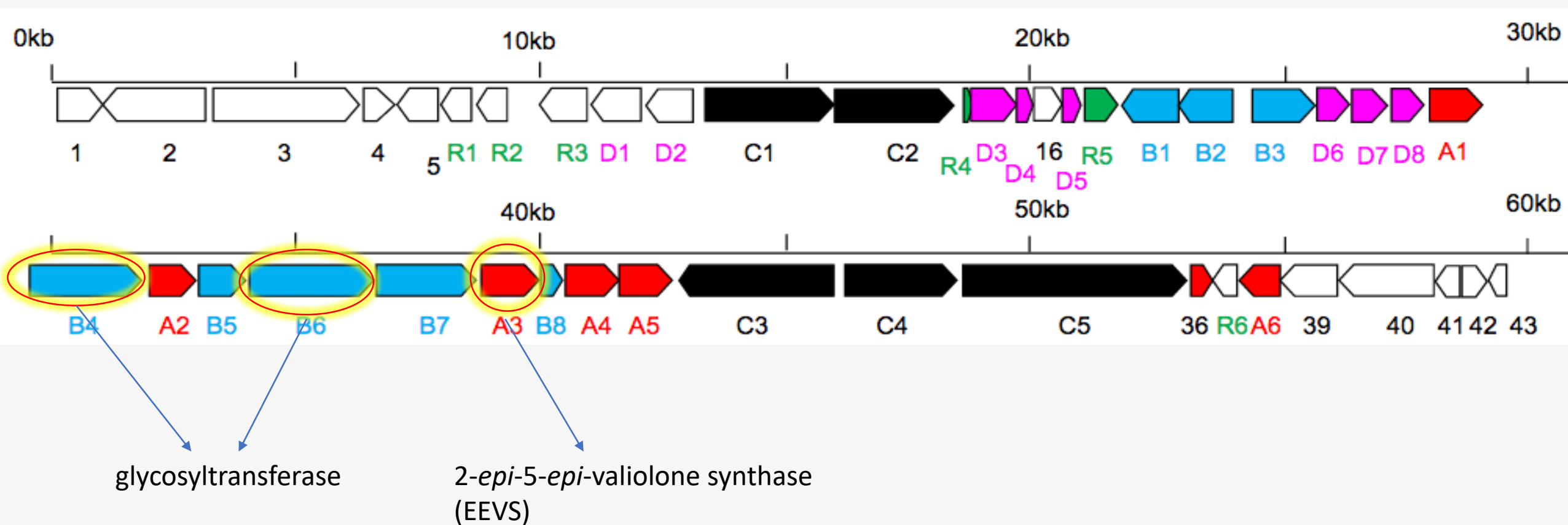


BLAST



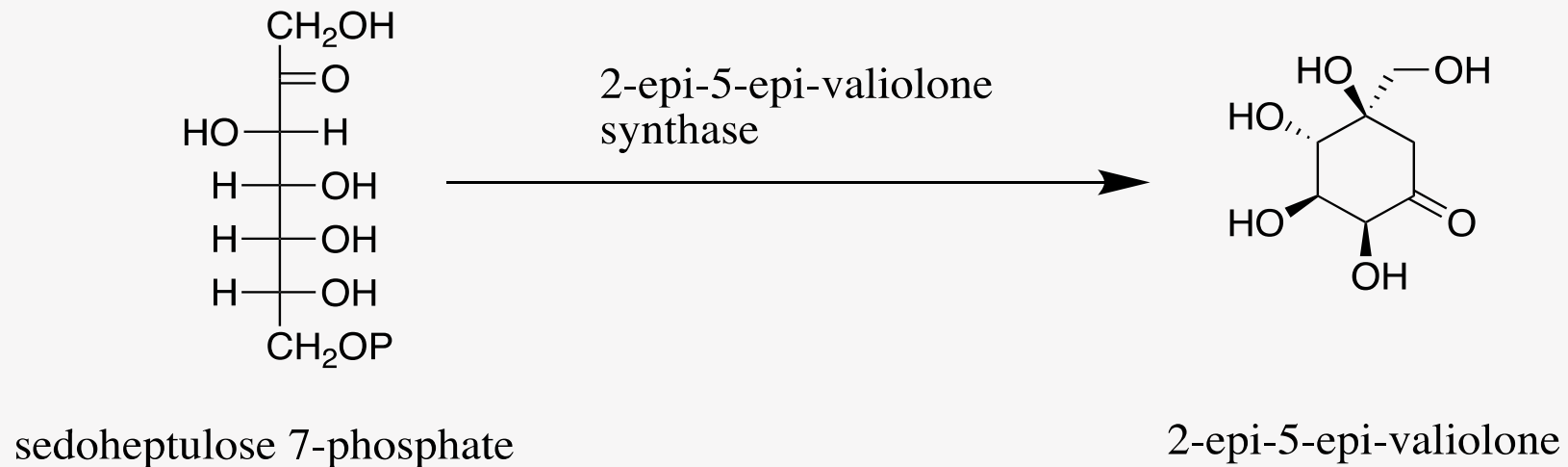
Trestatin biosynthetic gene cluster

Some major genes include trsA3, trsB4 and trsB6

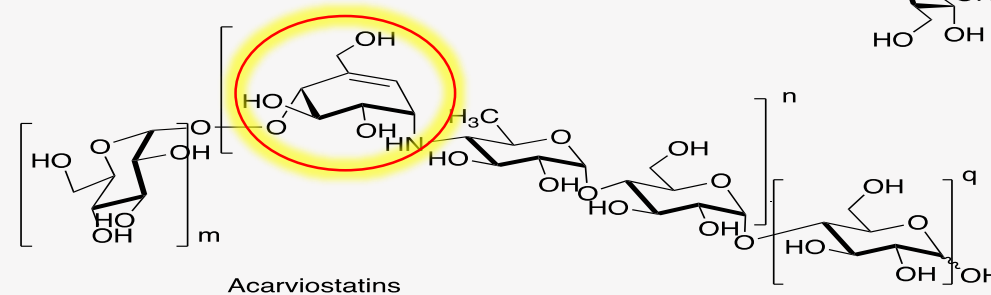
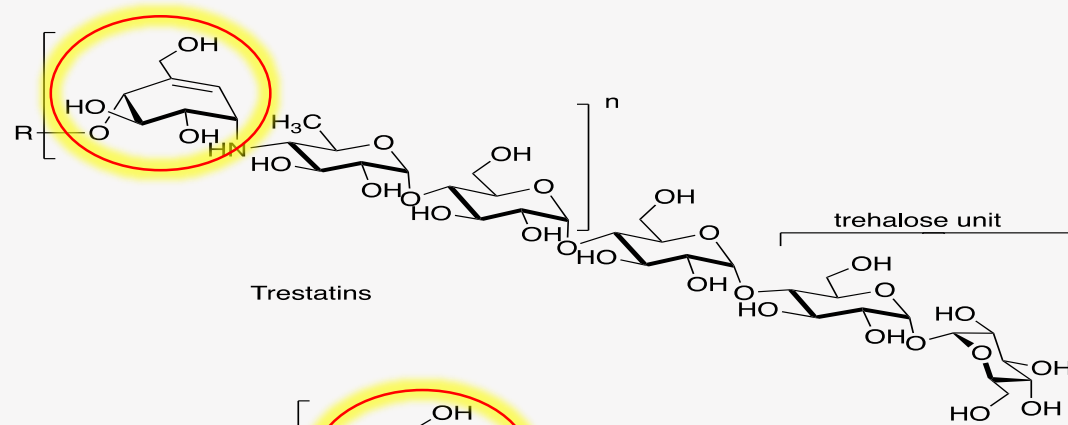
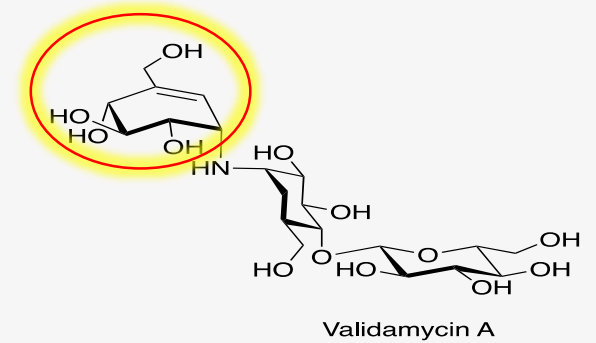
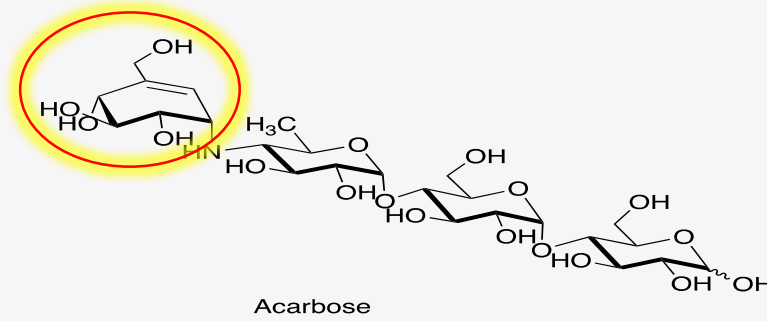
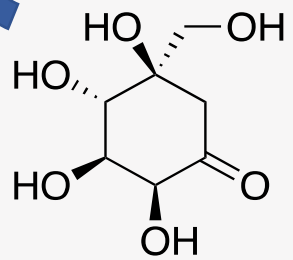
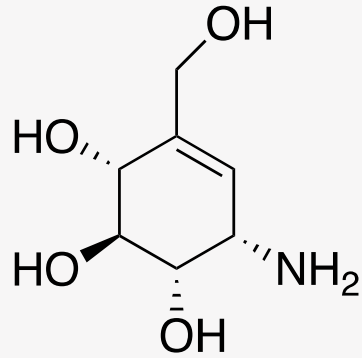


2-*epi*-5-*epi*-valiolone synthase (EEVS)

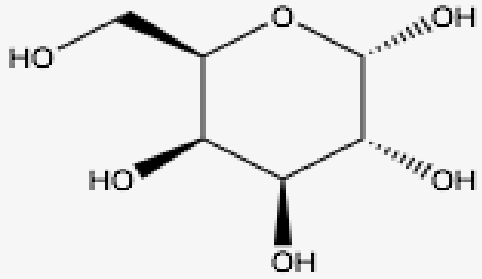
catalyzes the cyclization of sedoheptulose 7-phosphate to 2-*epi*-5-*epi*-valiolone



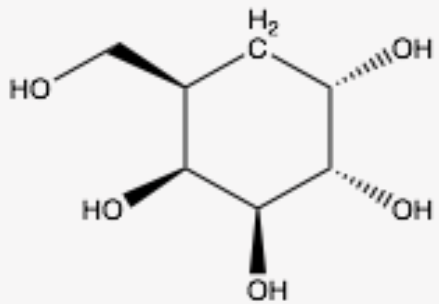
C₇N-aminocyclitol-containing natural products (pseudooligosaccharides)



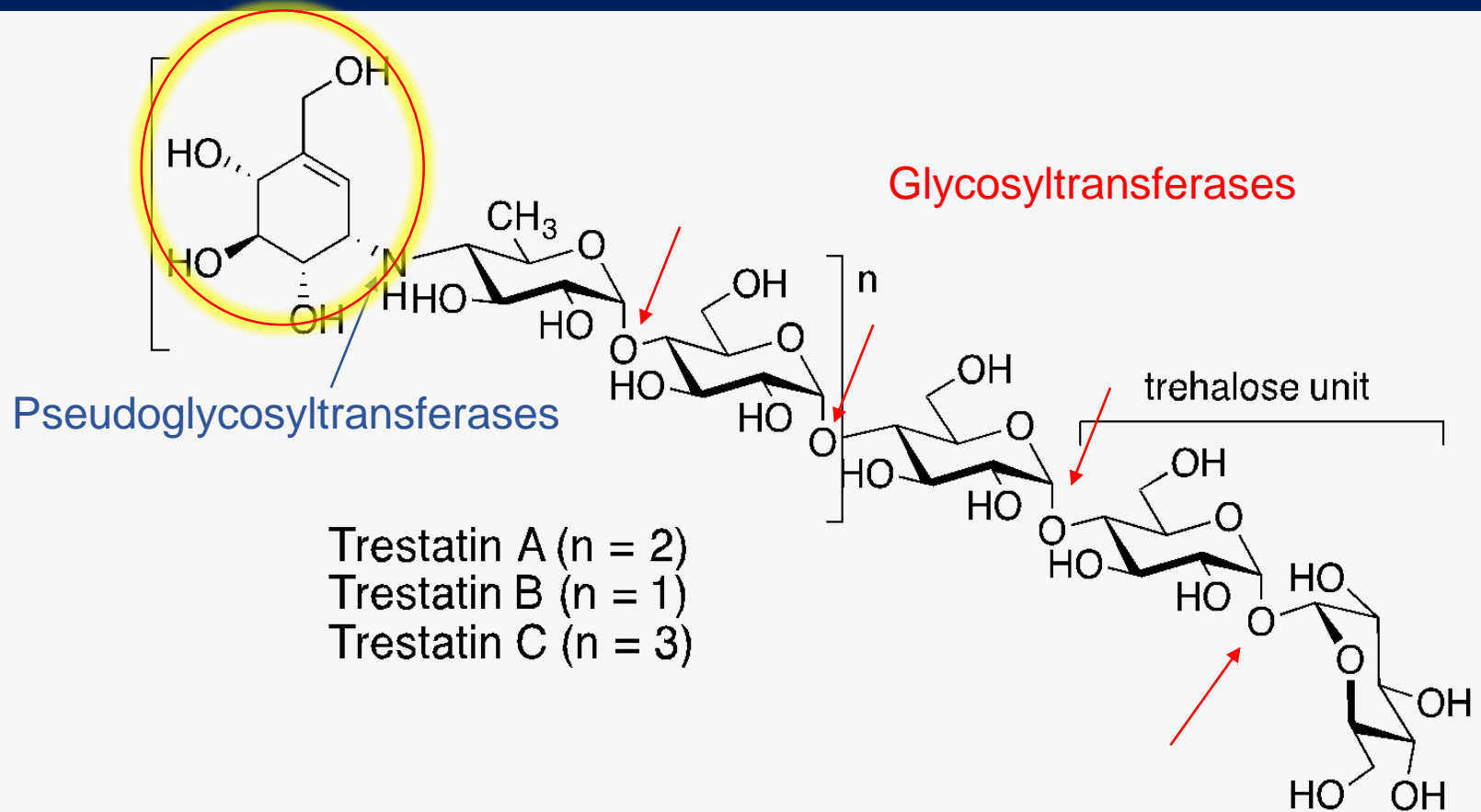
Glycosyltransferases vs Pseudoglycosyltransferases



α -D-galactopyranose



Pseudo- α -D-galactopyranose

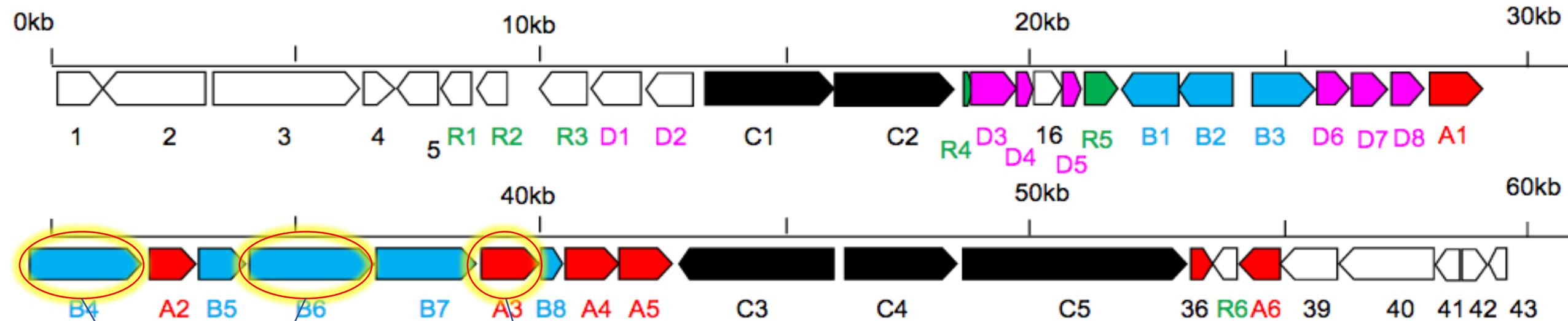


Pseudoglycosyltransferases

Glycosyltransferases

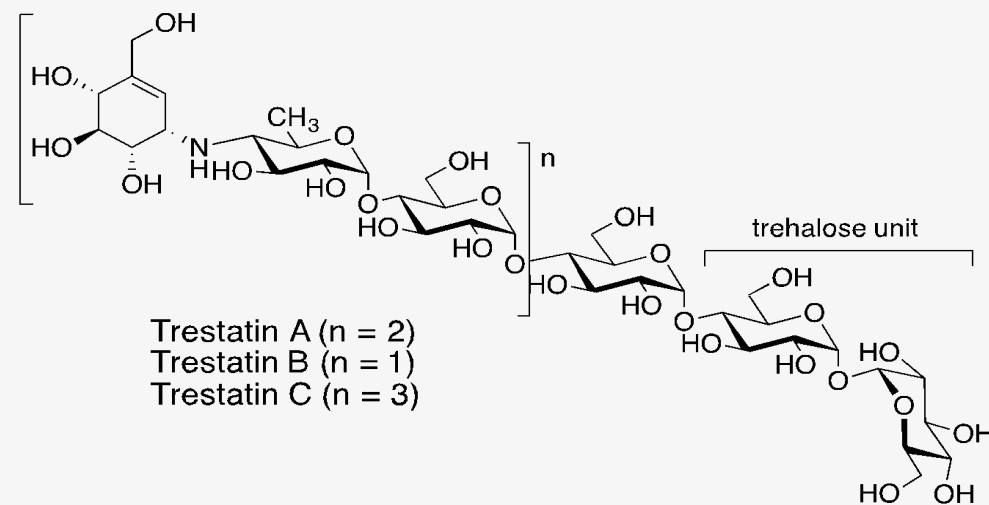
- Trestatin A ($n = 2$)
- Trestatin B ($n = 1$)
- Trestatin C ($n = 3$)

trsB4 or trsB6 might code for pseudoglycosyltransferase

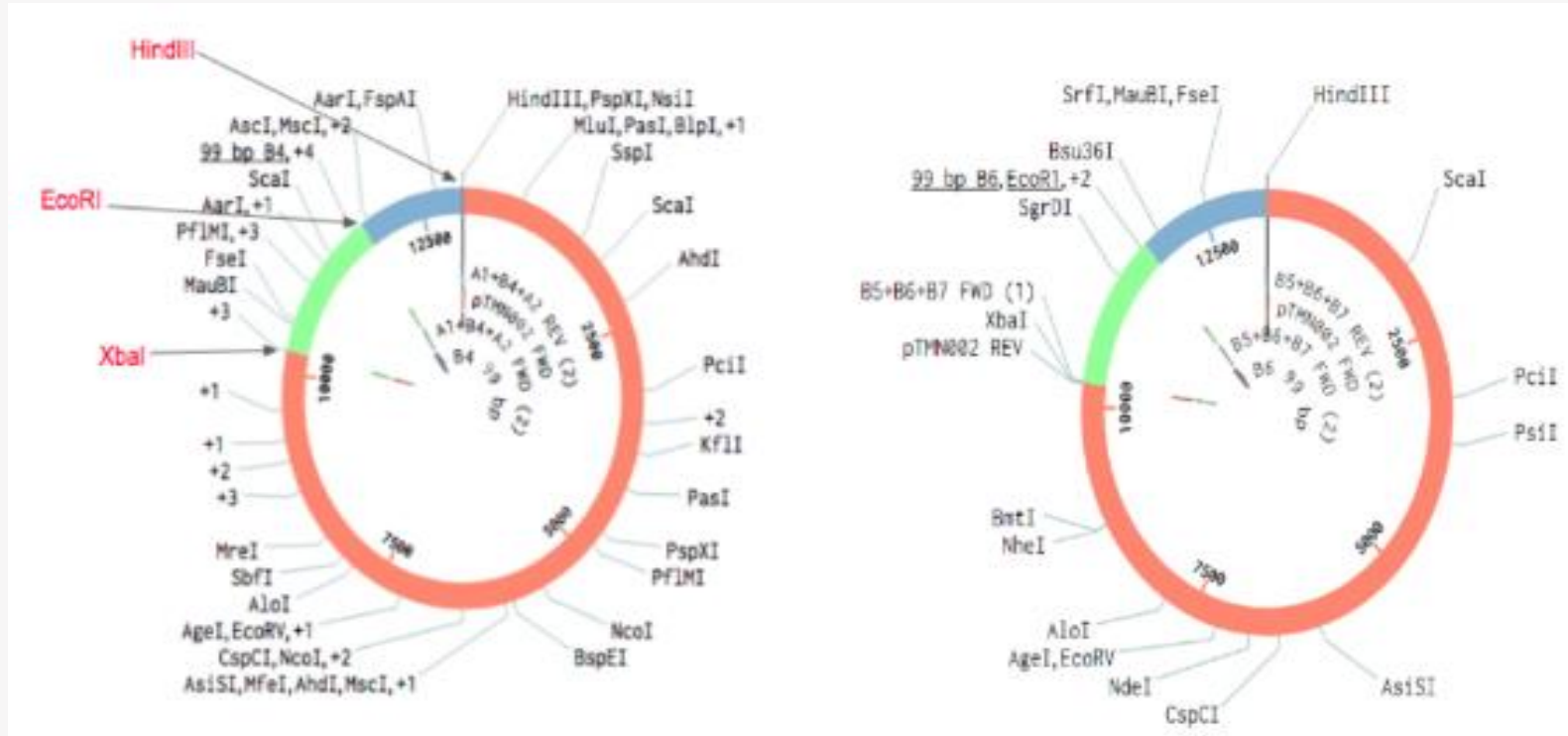


Glycosyltransferases

2-*epi*-5-*epi*-valiolone synthase (EEVS)



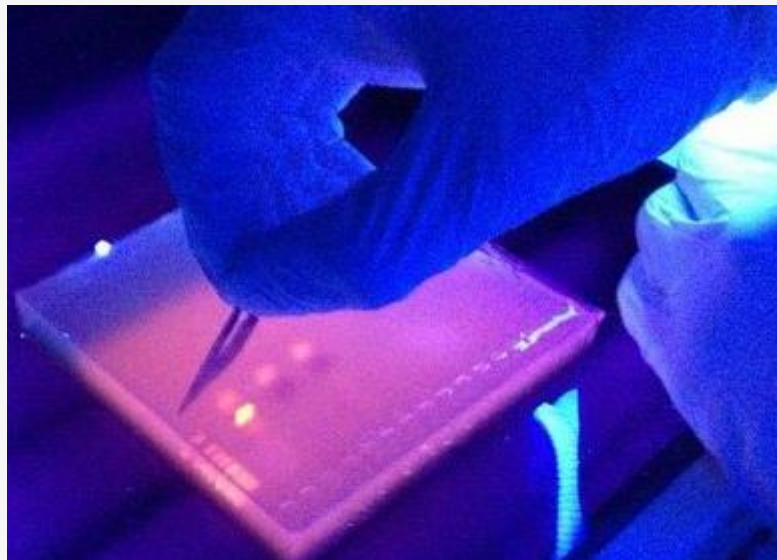
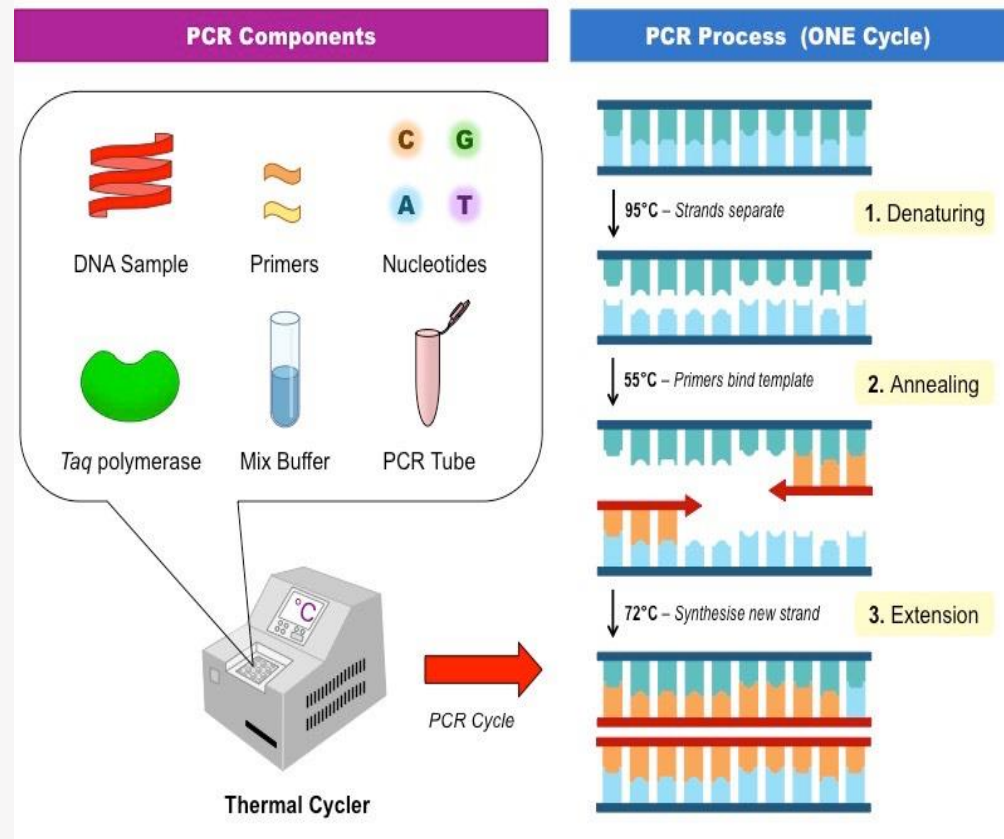
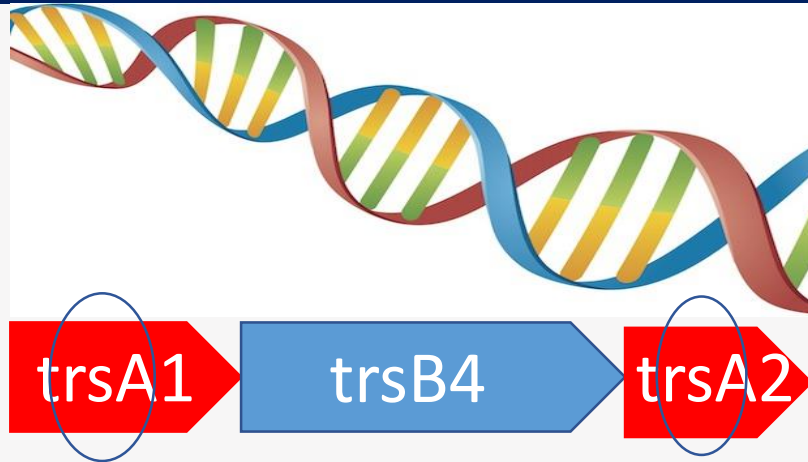
pTMAD003 and pTMAD004



pTMAD003

pTMAD004

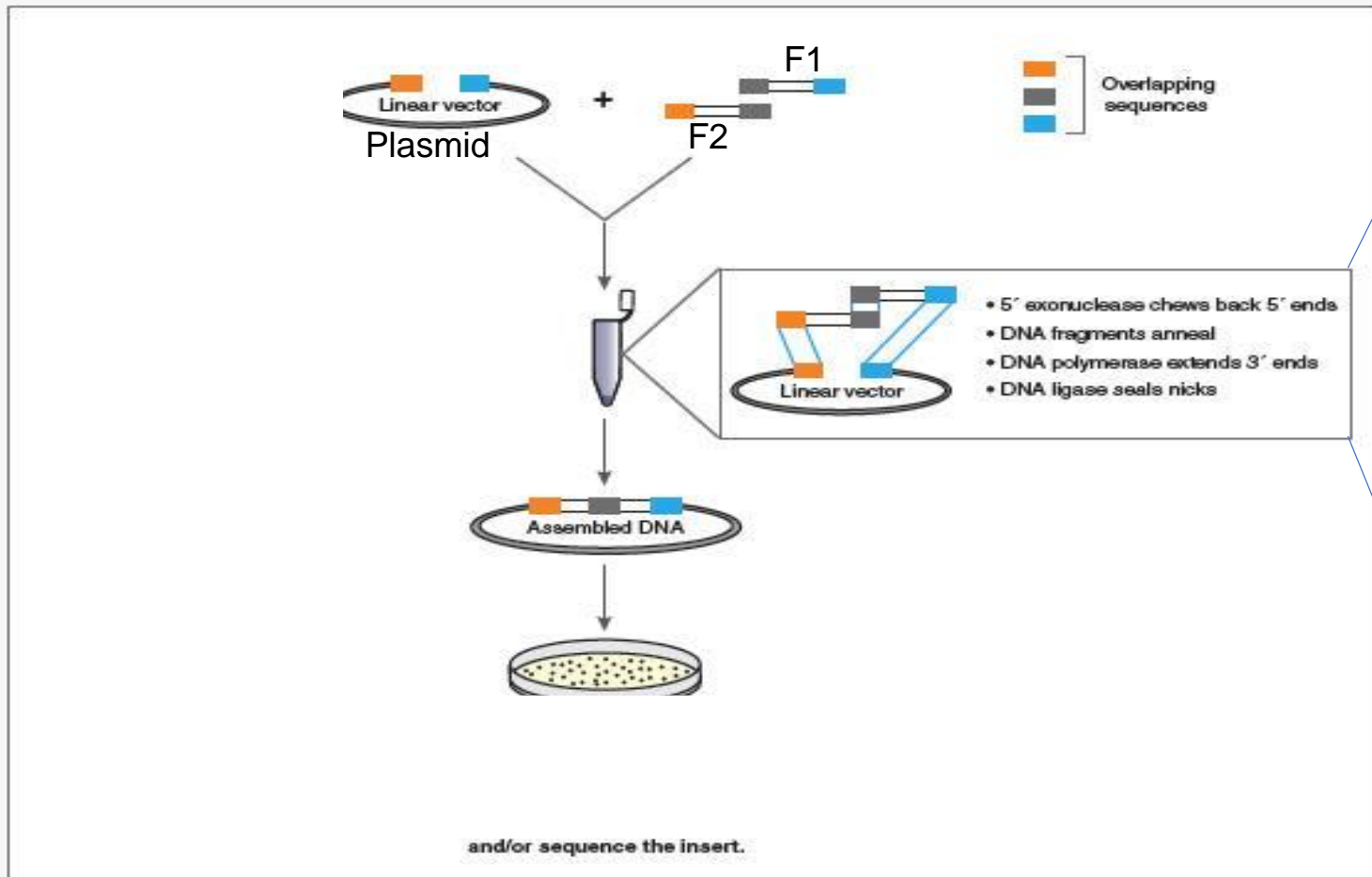
Construction of pTMAD003



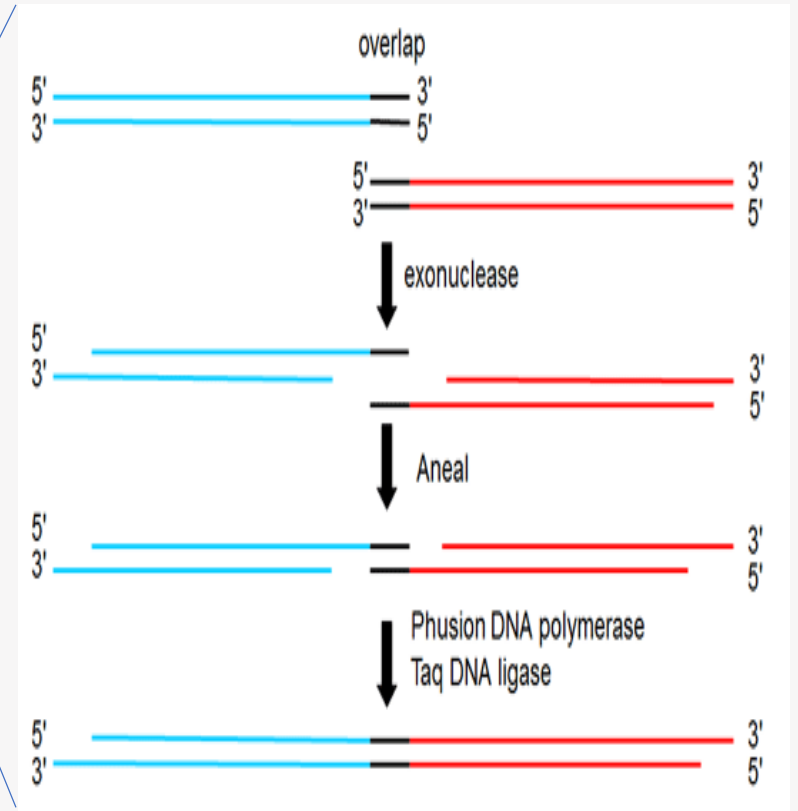
DNA
assembly

Polymerase chain reaction (PCR)

DNA assembly overview

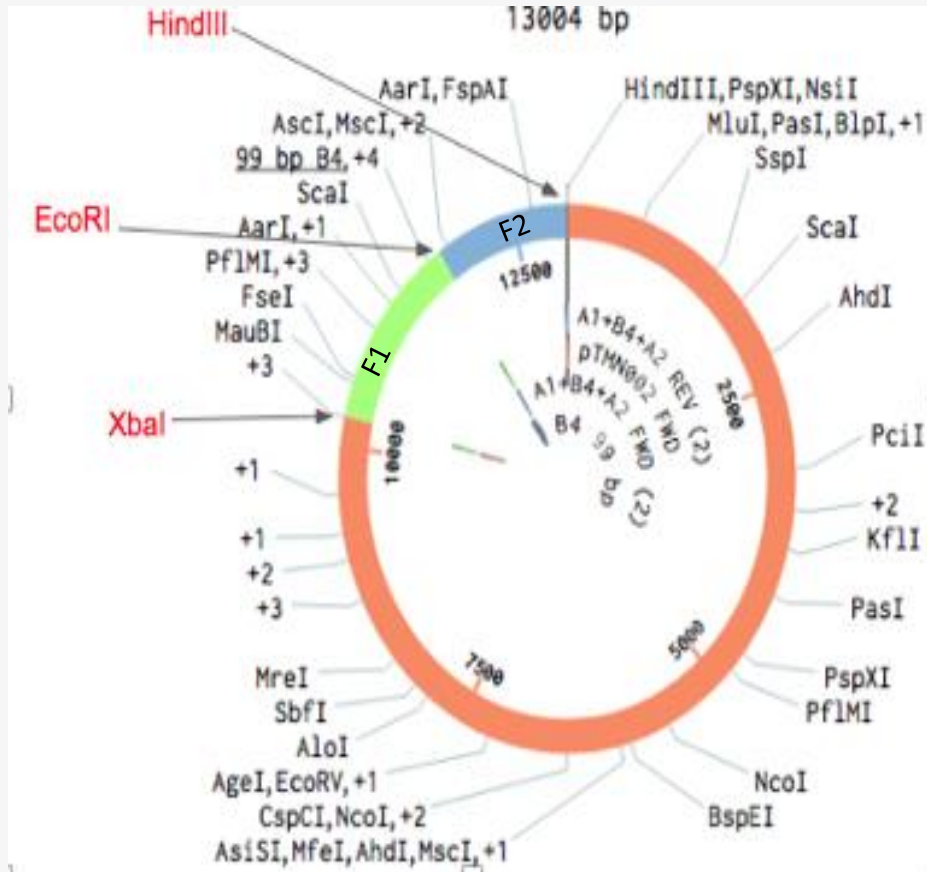


DNA assembly diagram



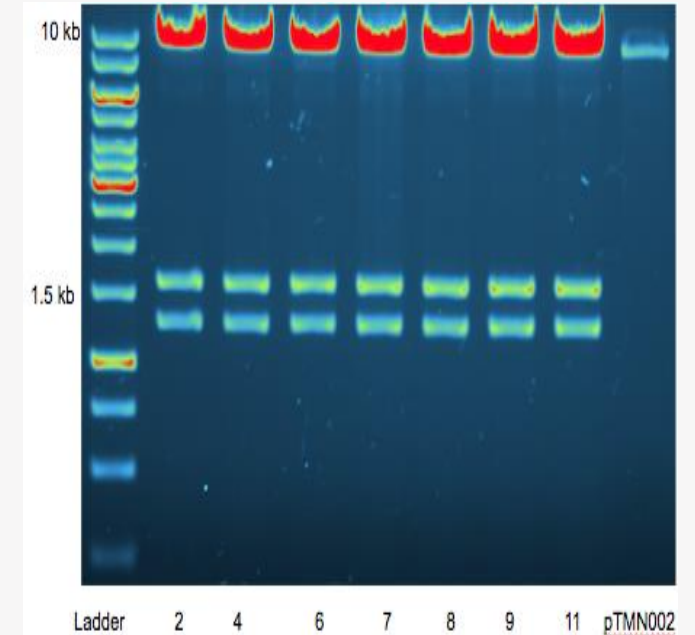
Assembly master mix action

Fragments from trsA1 and trsA2 were successfully cloned into pTMN002 plasmid, giving pTMAD003

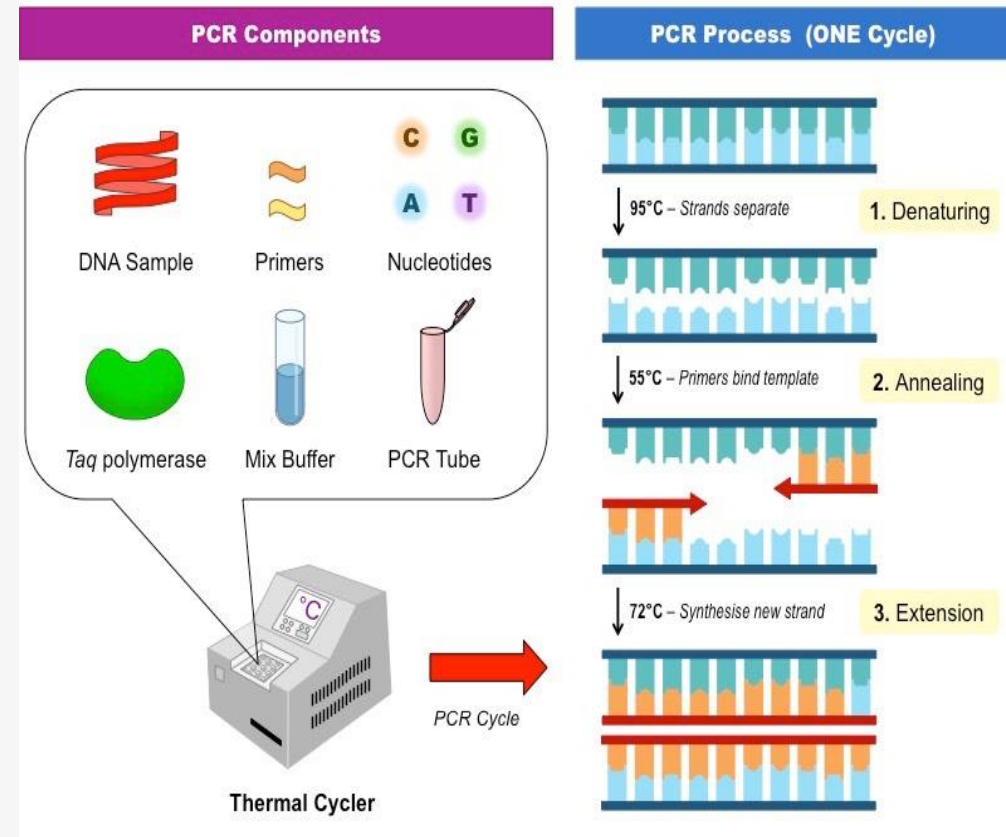
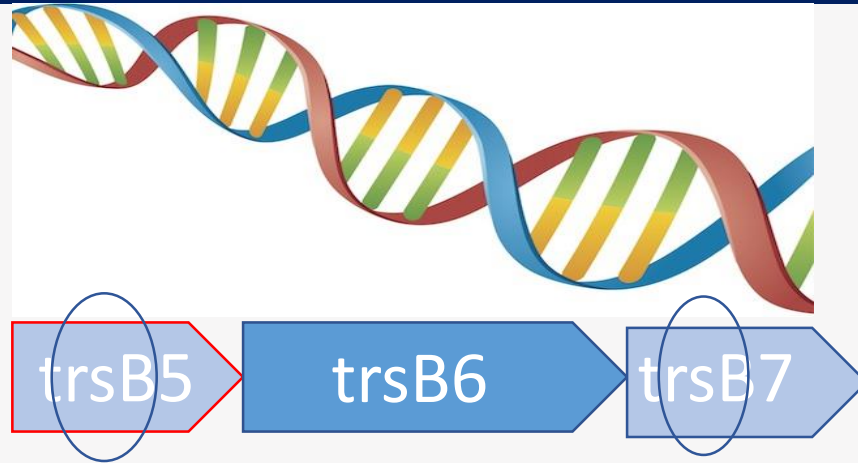


pTMAD003

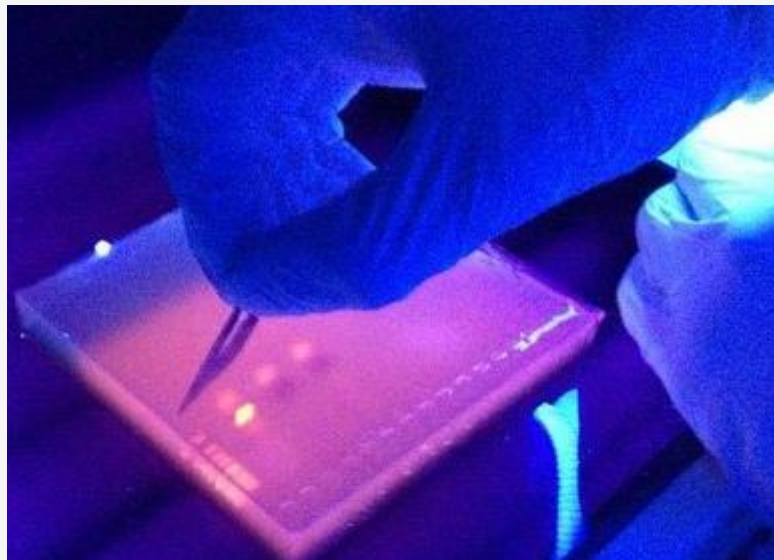
+XbaI, +HindIII
and +EcoRI



Construction of pTMAD004

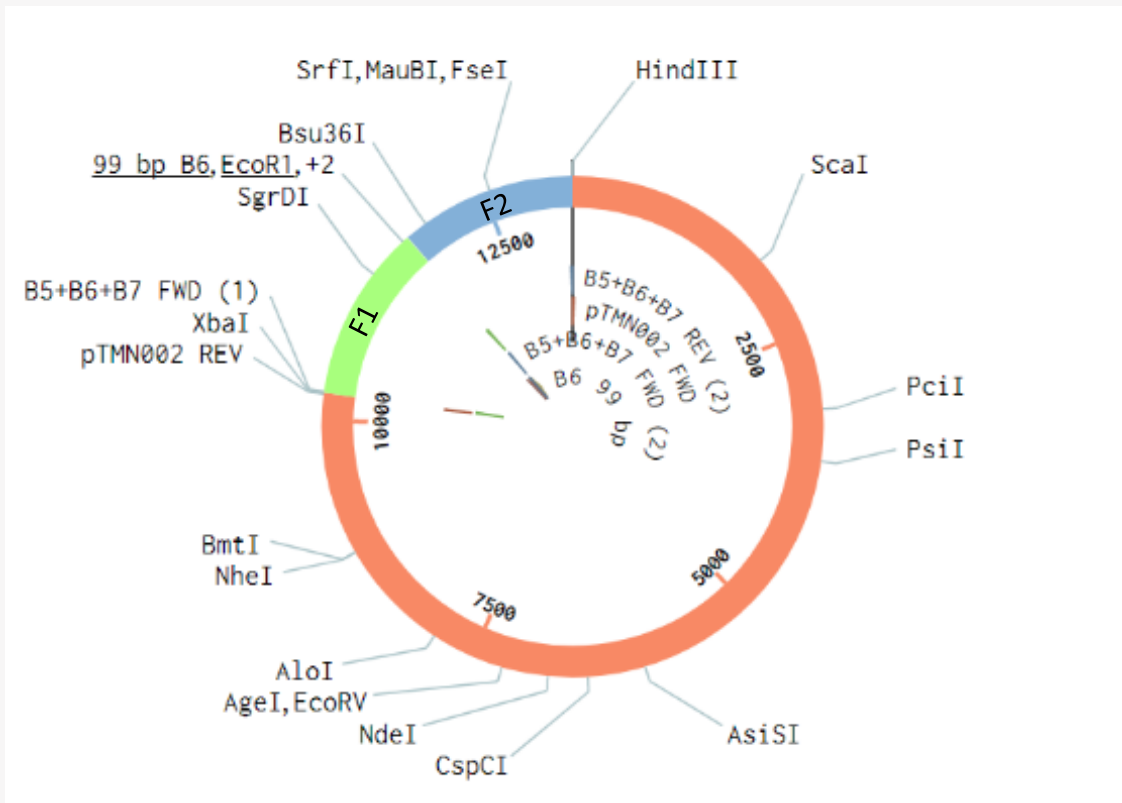


Polymerase chain reaction (PCR)



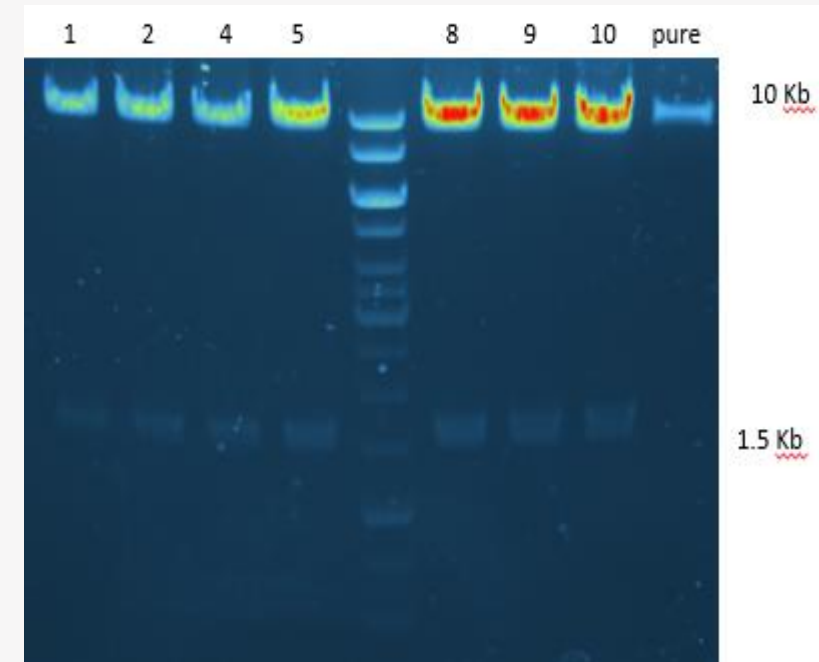
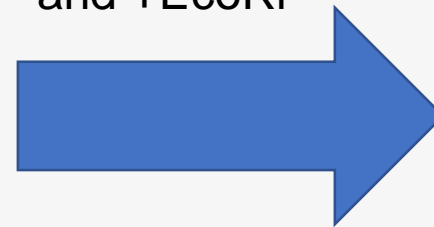
DNA assembly

Fragments from trsB5 and trsB7 were successfully cloned into pTMN002 plasmid, giving pTMAD004

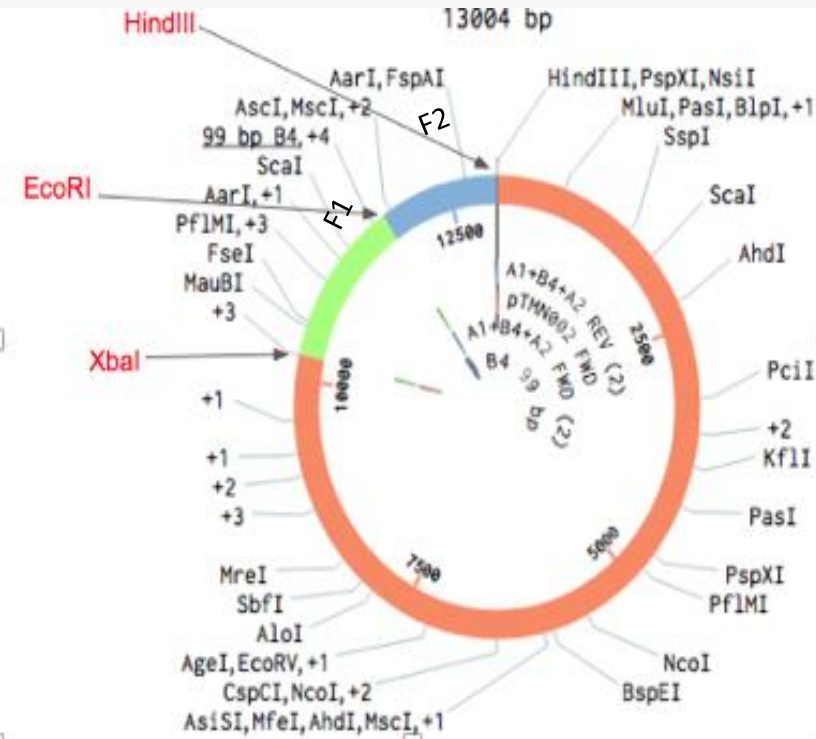


pTMAD004

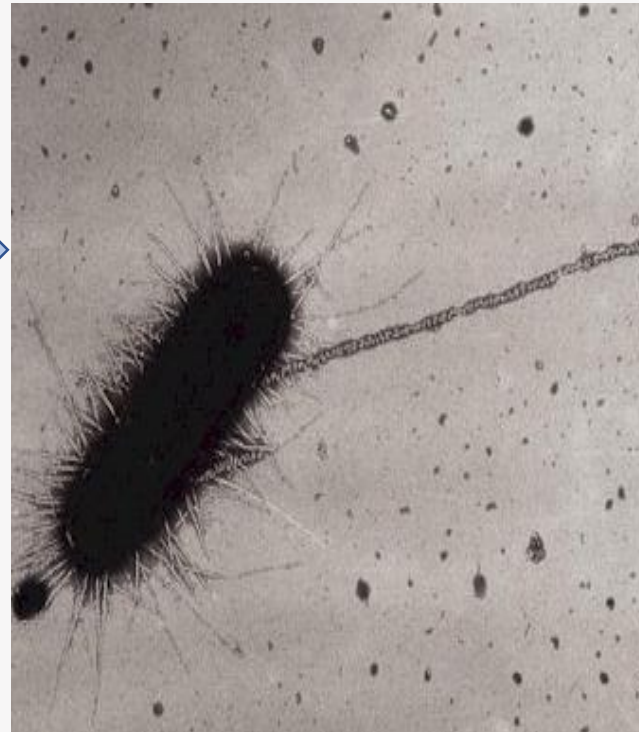
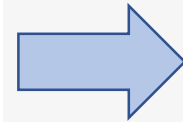
+XbaI, +HindIII
and +EcoRI



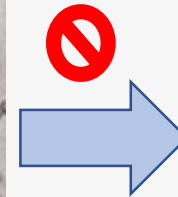
Conjugation between *E. coli* and *Streptomyces dimorphogenes* was not successful



Plasmid pTMAD003

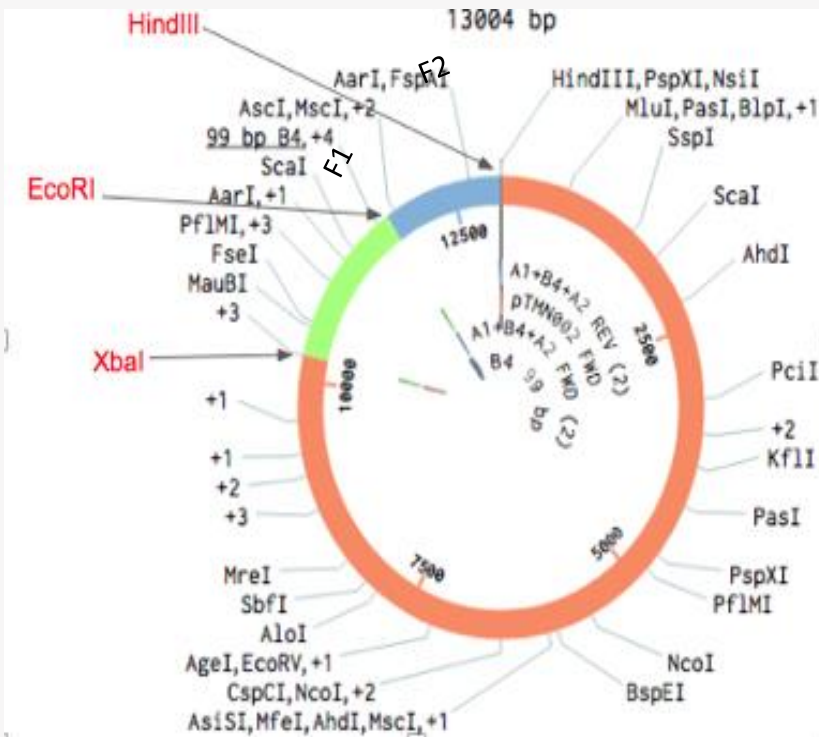


E. coli ET12567/pUZ8002

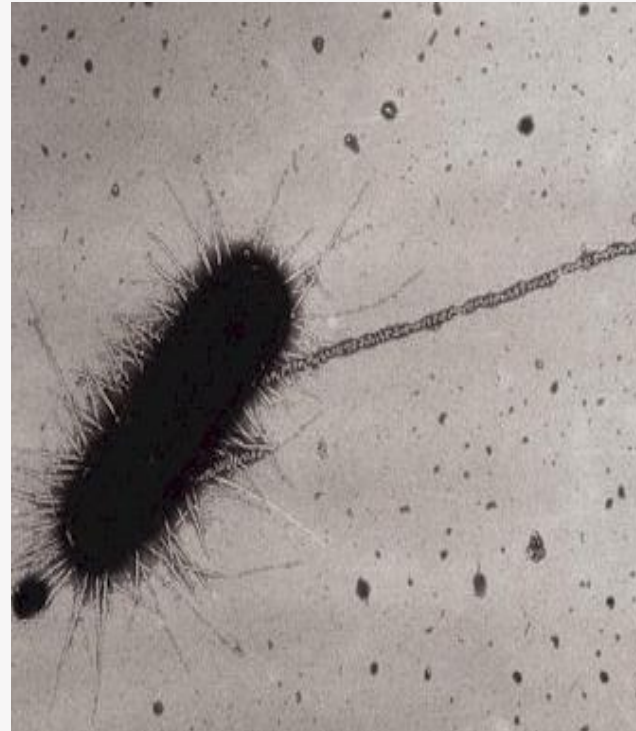
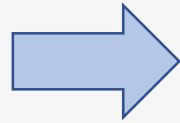


Conjugation between *E. coli* ET12567/pUZ8002/pTMAD003 and *Streptomyces dimorphogenes*

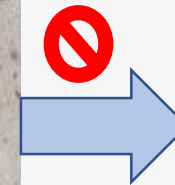
Conjugation between *E. coli* and *Streptomyces dimorphogenes* was not successful



Plasmid pTMAD004

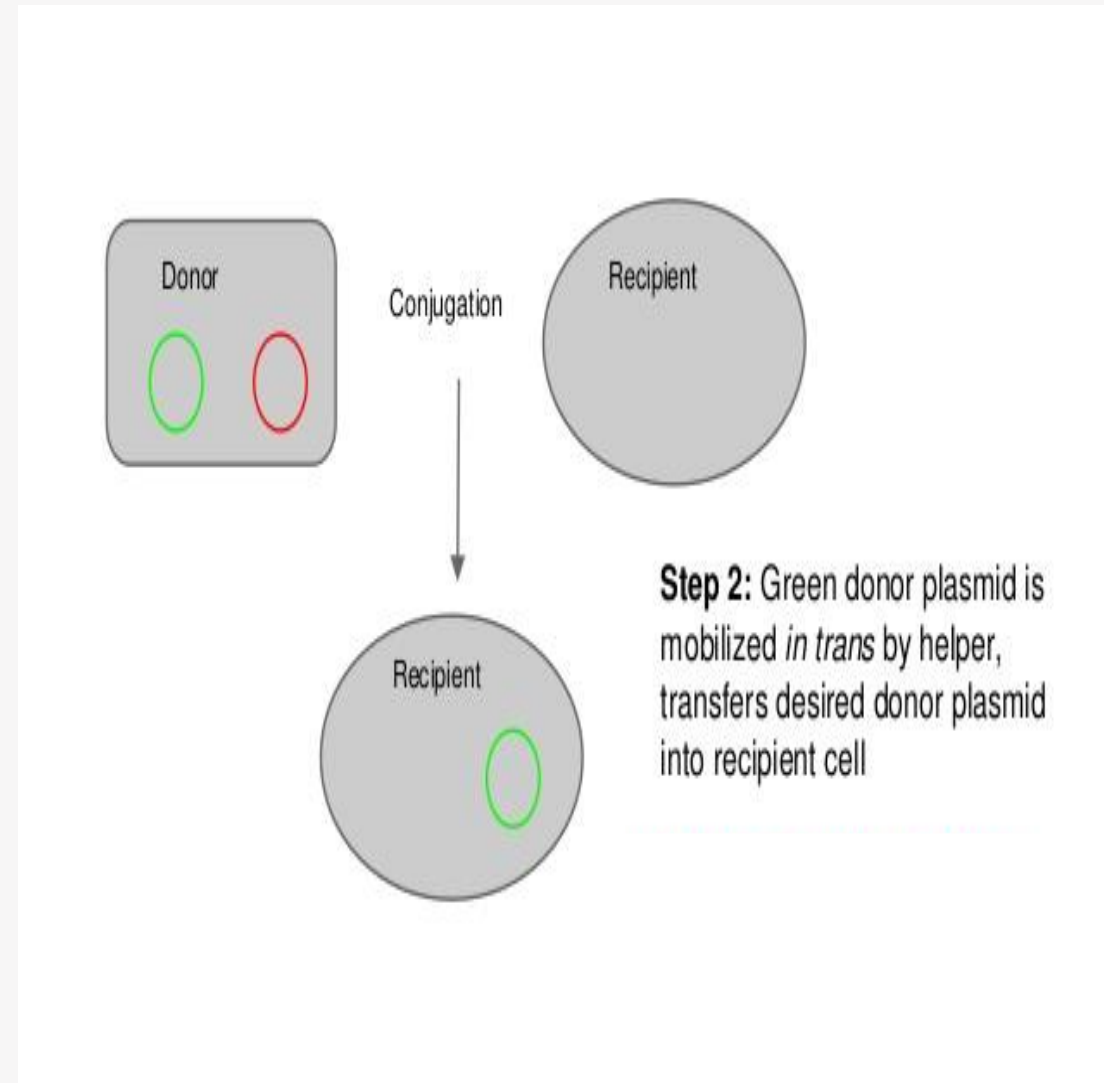
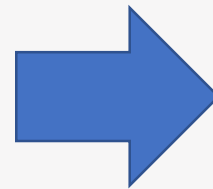
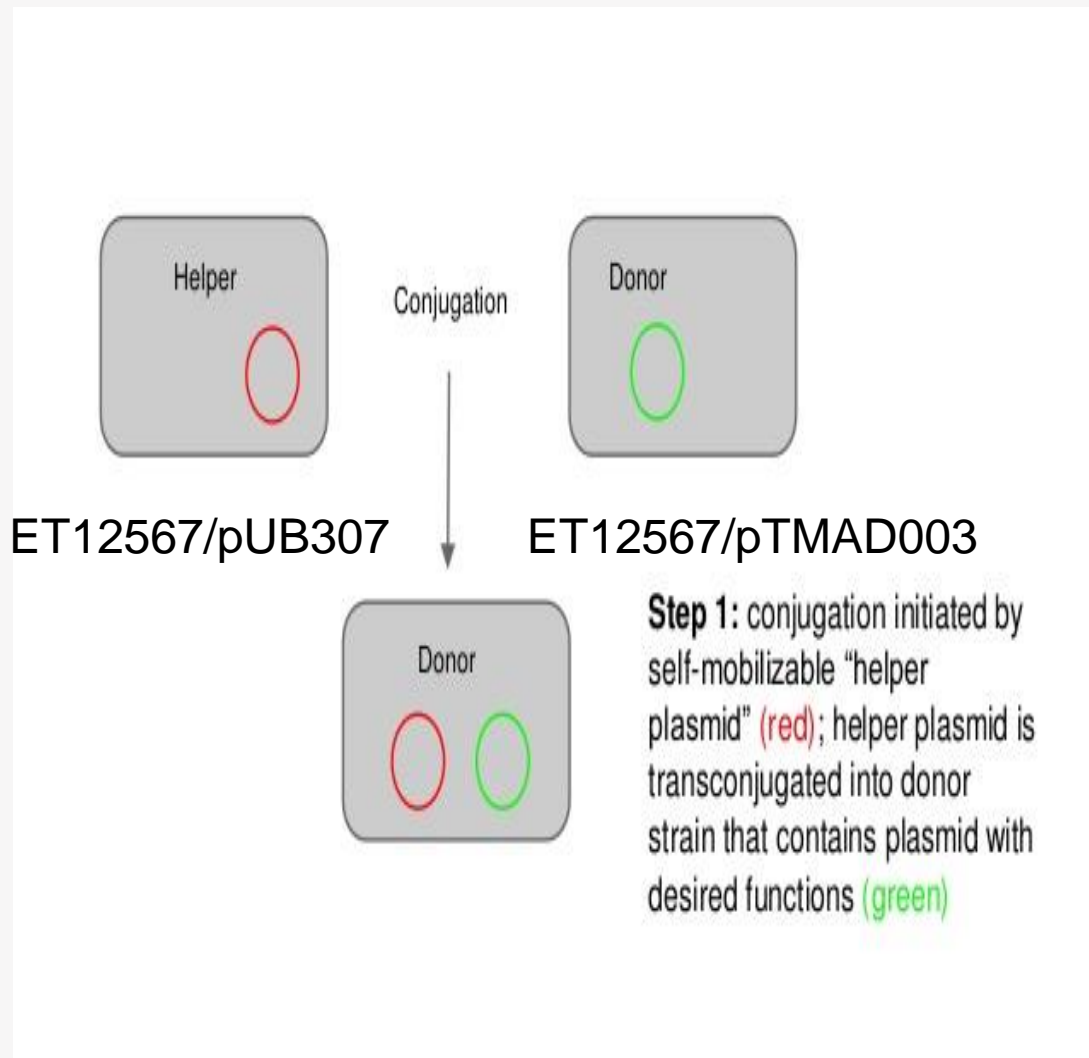


E. coli ET12567/pUZ8002

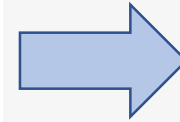
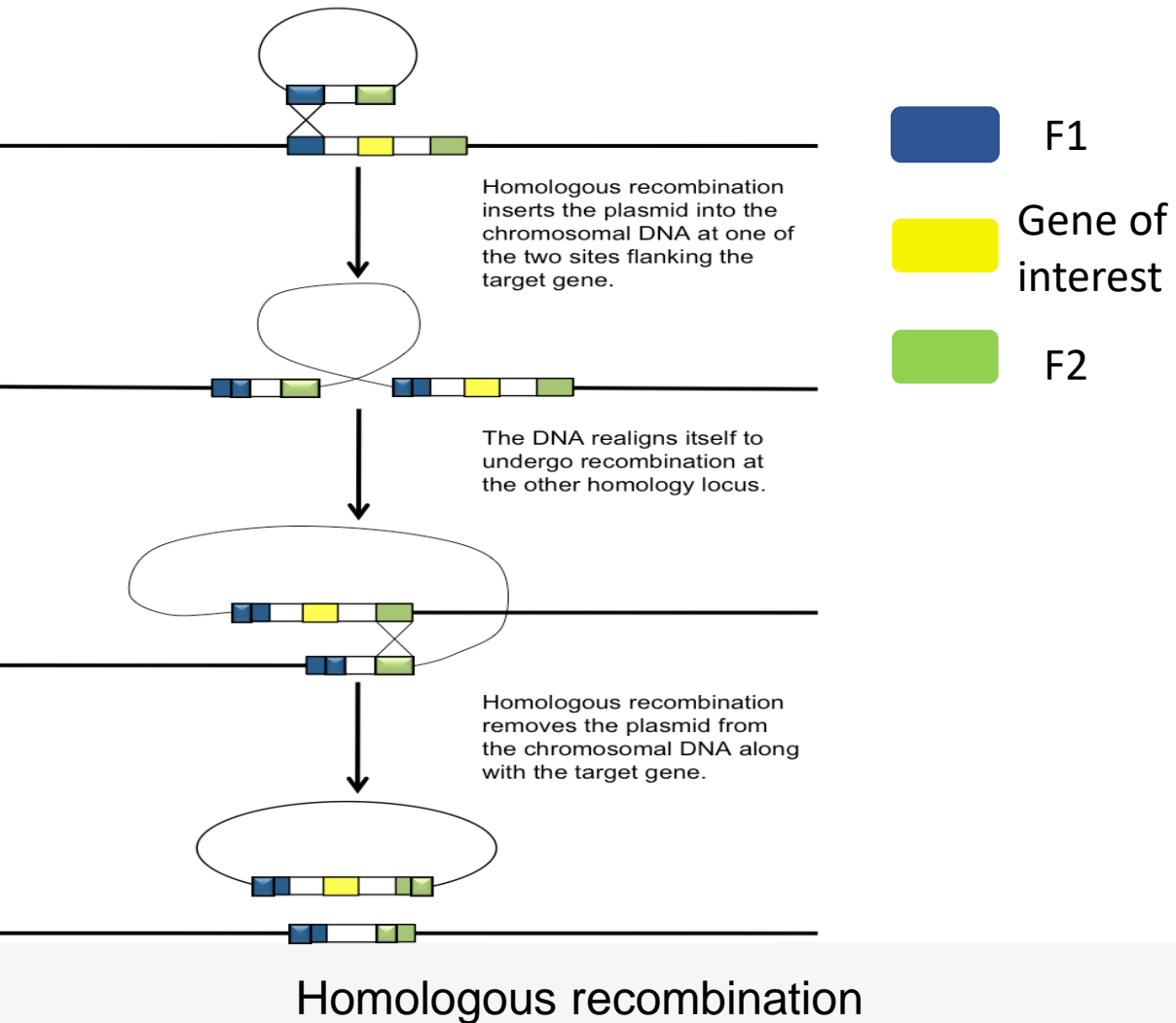


Conjugation between *E. coli* ET12567/pUZ8002/pTMAD003 and *Streptomyces dimorphogenes*

Future work: Triparental Mating



Once the plasmid enters *Streptomyces dimorphogenes* homologous recombination will take place



Compare the metabolic profiles of the wild-type and the mutant strains of *S. dimorphogenes*

Summary

- Rate of heart disease, diabetes and obesity is on the rise
- Trestatins can be developed as a useful therapeutic for the treatment of diabetes and obesity
- Only little information available on mode of formation in nature

Summary

- Trestatin biosynthetic gene cluster is thought to contain one glycosyltransferases and one pseudoglycosyltransferase
- pTMAD003 and pTMAD004 were successfully constructed
- Future work focus on alternative transformation systems

References

1. Golay, Alain, et al. "Effect of trestatin, an amylase inhibitor, incorporated into bread, on glycemic responses in normal and diabetic patients." *The American journal of clinical nutrition* 53.1 (1991): 61-65.
2. Watanabe, K., Furumai, T., Sudoh, M., Yokose, K., & Maruyama, H. B. (1984). New Alpha-amylase Inhibitor, Trestatins IV Taxonomy of the Producing Strains and Fermentation of Trestatin A. *The Journal of antibiotics*, 37(5), 1983: 479-486.
3. YOKOSE, K., OGAWA, M., & OGAWA, K. (1984). New. ALPHA.-amylase inhibitor, trestatins. III. Structure determination of new trestatin components Ro 09-0766, Ro 09-0767 and Ro 09-0768. *The Journal of antibiotics*, 37(2), 182-186.
4. Tappy, L., Buckert, A., Griessen, M., Golay, A., Jequier, E., & Felber, J. (1986). Effect of trestatin, a new inhibitor of pancreatic alpha-amylase, on starch metabolism in man. *International journal of obesity*, 10(3), 185-192.

Acknowledgements

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Thank You!

Questions?

