# Engineering and producing B-galactosidase

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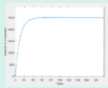
## Background

- 87% of people in China suffer from different levels of lactose intolerance
- The production of dairy product designed for people with lactose intolerance is limited by the high cost of the beta-galactosidase
- The activity of the acidic-preferring beta-galactosidase extracellularly expressed by A.Oryzae is limited in the neutral environment of dairy production

## Modeling

$$E + S \overset{K_d}{\leftrightarrow} ES \overset{k_{cat}}{\rightarrow} P + E$$



Our equation can predict the best time to check our target protein production. We add a analysis step to rank our outcome.

$$[ES] = \frac{([E_t] - [ES]) \cdot [S]}{K_s}$$

$$[ES] = \frac{[E_t] \cdot [S]}{K_s + [S]}$$

$$v_0 = \frac{k_{cat} \cdot E_t \cdot [S]}{K_s + [S]}$$




E: enzyme, S: substrate, ES: intermediate complex, P: product, Ks: dissociation equilibrium constant of ES, Kcat: Catalytic constant

M.Menten equation is an equation representing the relationship between the Initial velocity of reaction and the substrate concentration.

## Experiment

Strain building:

1.Use SalI to linearize pPICZ∂A-lacA

2.Transform linearized pPICZ∂A-lacA into X33

3.Cultivate X33-pPICZ∂A-lacA

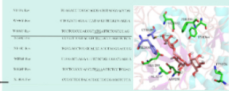
Determining enzyme activity.

1.Extract the crude enzyme from bacterial solution

2. Take 0.1 mL of crude enzyme solution and react with 0.9 mL 0.05 mol/L sodium phosphate buffer (pH 6) , 0.01 mol/L ONPG ,0.05mol/L Sodium phosphate buffer (pH 6.5). at 37°C for 10 min.

3. Add 1.5 mL of 0.4 mol/L Na2CO3 stop solution, and measure the concentration of ONP.

4. Determinate protein concentration by BCA Then do SDS-PAGE electrophoresis.



Site-directed mutation is applied on two sites-site 140 and site 806. A mutation at site 140 will cause the enzyme's T-S intermediate state to become less stable, but will improve its trans-glycoside ability. A 806 site mutation will improve the enzyme's hydrophobicity, and enhance its trans-glycoside ability by increasing its contact with sugar.

a random mutation based on error-prone PCR will be conducted on plasmid to form a mutant library, and a site directed random mutation will be applied to lacA with overlap extension PCR.

strain selection:

enzyme produced by each specimen in X33 will be measured by each specimen's bacterial solution 's ability to hydrolysis OPNG.

## Human practice

Current solution for alleviating lactose intolerance is either too expensive both for consumer and supplier or inefficient due to the limitations of enzymes. Our project, aiming at producing at a low cost and increasing efficacy, applies beta-galactosidase extracellularly expressed by A. oryzae. With the innovation of adding lactase into the packaging of dairy products and engineering the optimum pH of the lactase, lactose can be broken down faster during transportation as well as sale, and thus, symptoms of lactose intolerance will be relieved.

## Conclusion

Overall, from cost aspect, our project intends to mass-produced which lows down the cost; from efficiency aspect, the innovative use of enzyme accelerates decomposition rate. Meanwhile, people around us rarely pay attention to lactose intolerance and to people suffering from it because they don't even regard those kinds of symptoms as "sickness"propaganda aspect, we are hoping to draw more people's attention towards not only lactose intolerance, along with synthetic biology, but also some other mild but commonplace disease like lactose intolerance. From these aspects, the large scale of existent and potential "patient" suffering from lactose intolerance can also live with dairy products peacefully.