DETECTION OF SUC2 GENE BY REAL TIME PCR IN *Saccharomyces cerevisiae*

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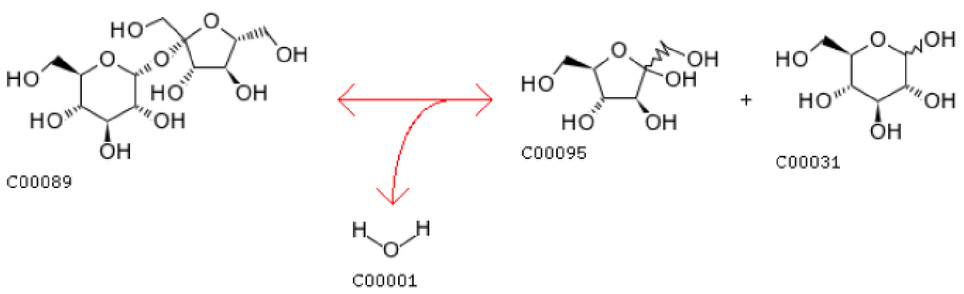
Abstract

Invertase hydrolyzes sucrose, a disaccharide, to glucose and fructose. This transformation is desired in the food industry. *Saccharomyces cerevisiae* is used which naturally secretes the enzyme. In this study, it is aimed to detection of SUC2, the gene responsible for the production of invertase enzyme in *Saccharomyces cerevisiae* strain.

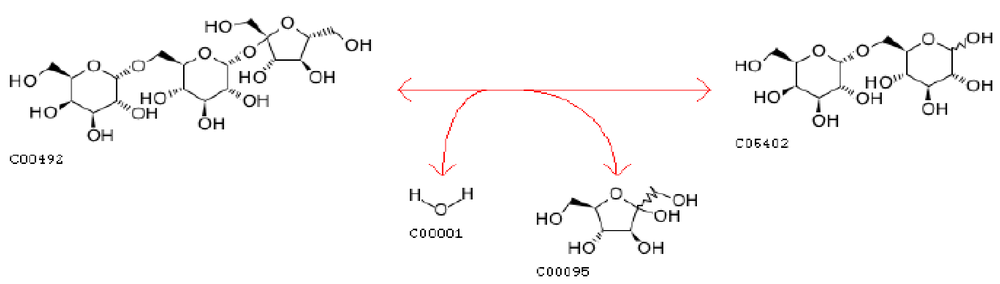
Saccharomyces cerevisiae HUF16M1C0004 was grown at 25℃ in YPD Broth medium (1% yeast extract, 2% peptone and 2% glucose, pH 5.3) for 48 hours. Samples were taken at the end of the 4th, 8th, 24th and 48th hours of growth. Total DNA extractions from 24 hours growth *Saccharomyces cerevisiae* HUF16M1C0004 were performed using the Eurx GeneMATRIX Bacterial and Yeast Genomic DNA Purification Kit (EURxLtd,Poland). Nucleic acid amount of DNA templates and A260 / 280 values were obtained with Thermo Scientific NanoDrop 2000 Spectrophotometer. For detection of SUC2 gene, RT-PCR analysis was carried out using LightCycler FastStart DNA Master SYBR Green I kit according to the manufacturer's protocol with primers which were designed by using Primer 3 software.

As a result of RT- PCR analysis, standard curve was created with log-concentration of dilution series versus known Cq values. In this study, it was determined that there was no cross-contamination, no primer-dimer or other non-specific substance was formed, and the DNA template contained the SUC2 gene due to amplification.

Introduction

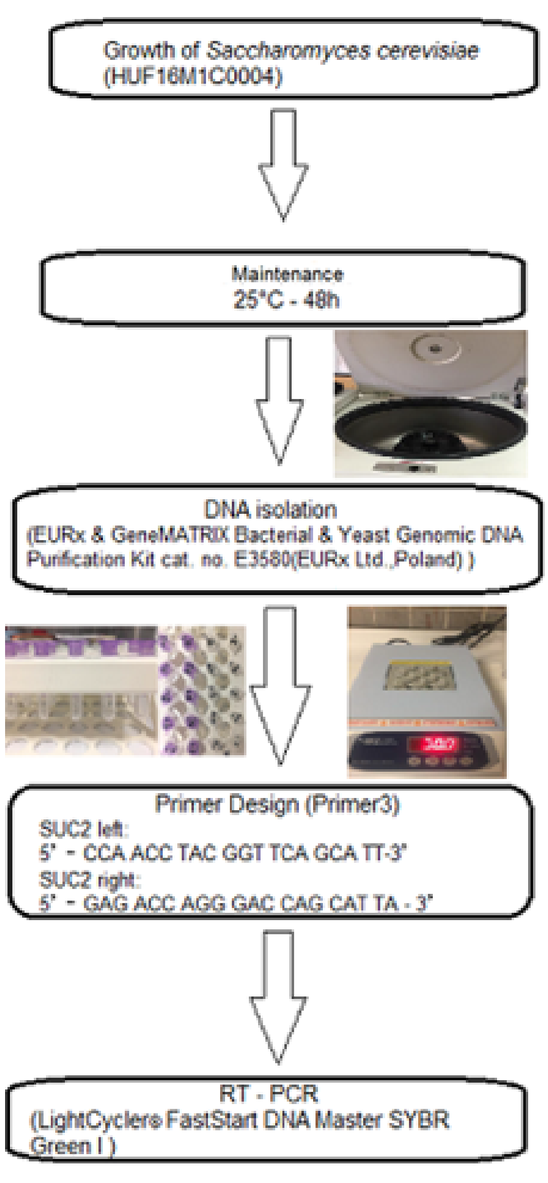


Sucroseglucohydrolase (EC 3.2.1.26):Hydrolysis of sucrose to D-glucose and D-Fructose under the catalase of invertase enzyme.

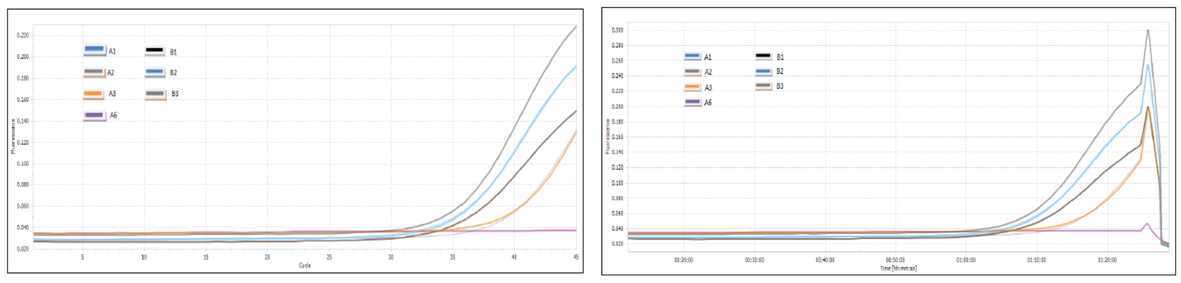


Rafinozfruktohidrolaz (EC 3.2.1.26):Hydrolysis of raffinose to melibiose and D-fructose under the catalase of invertase enzyme.

Methodology



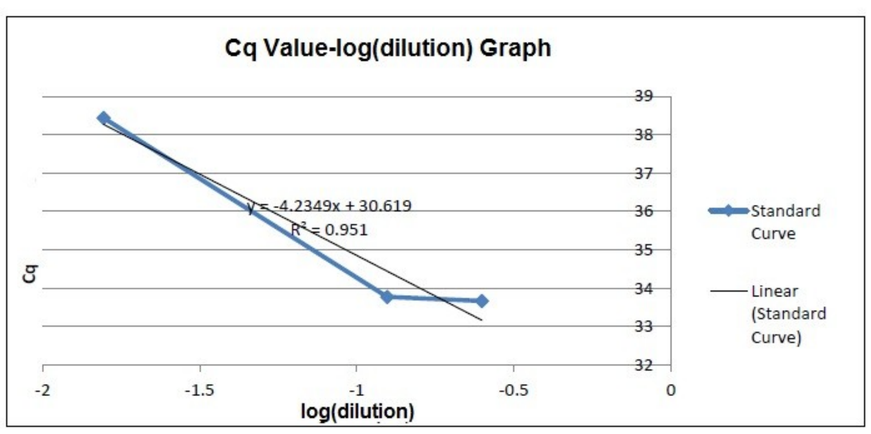
Results



Amplification curves and Fluorescence curves were obtained from RT-PCR analysis

Color	Position	Sample type	Sample code	Cq	Result	Cq Hata
	A1	Standard	24-HUF16M1C0004-1-1/4-1	33.60	+	0.00
	A2	Standard	24-HUF16M1C0004-1-1/8-1	33.75	+	0.00
	A3	Positive Control	PC	38.86	+	0.00
	A6	Negative Control	NC	-	-	0.00
	B1	Standard	24-HUF16M1C0004-1-1/4-2	33.74	+	0.00
	B2	Standard	24-HUF16M1C0004-1-1/8-2	33.80	+	0.00
	B3	Positive control	PC	38.01	+	0.00

Position	Sample type	Nucleic acid concentration
A1	1/4 dilution	103,2ng/20µl
A2	1/8 dilution	51,51ng/20µl
B1	1/4 dilution	103,2ng/20µl
B2	1/8 dilution	51,51ng/20µl
A6	negative control	0ng/20µl
A3	positive control	6,44ng/20µl
B3	positive control	6,44ng/20µl



Standard curve used to determine the efficiency of RT-PCR analysis.

Conclusion

In this study, Cq values of 1/4 and 1/8 dilutions, positive, negative controls and technical repeats were determined by using RT- PCR. To evaluate the PCR efficiency, standard curve was created with log-concentration of dilution series versus known Cq values. The PCR efficiency was calculated by the equation Yield= (10^{-1}/- $slope^{-1}$) x 100. When the slope obtained from the graph was replaced in the equation, the yield was found to be 72.25%. Primers with high stability melt at higher temperatures, increasing Cq values. Increased Cq values decrease the efficiency and decrease the reliability of the analysis as in the inhibition effect. As a result, when the results of the fluorescence curve, amplification curve and dissolution curve of the DNA template examined for the SUC2 gene in the context of RT-PCR analyses were evaluated together. It was determined that there was no cross-contamination, no primer-dimer or other non-specific substance was formed, and the DNA template contained the SUC2 gene due to amplification.

Acknowledgement

The study was carried out with dear technical assistants Büşra Bostan, Nilhan Beyza Erkeç.

References

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