Result for glgC assay.

Glycogen concentration is measured by absorbance. Because the recommended wavelength by the assay kit manufacturer is 570. Based on wavelength/absorbance plot provided by the manufacturer, we determined that the out of the wavelength filters that we have, the 540 nm is most ideal. So we used the absorbance measured at 540 nm to reproduce a plot.

Wavelength	540 nm	1	2	3	4	5	6
1st measurement		1% Glu	5% Glu	10% Glu	1% Fru	5% Fru	10% Fru
	glgC	0.937	2.496	2.622	0.118	0.389	0.779
	Control	0.279	0.333	0.455	0.403	1.422	0.179
2nd measurement		1% Glu	5% Glu	10% Glu	1% Fru	5% Fru	10% Fru
	glgC	1.028	2.572	2.612	0.121	0.415	0.848
	Control	0.281	0.332	0.431	0.418	1.432	0.195

Table 1. Absorbance readings of K12+glgC and Jump (control) treated with sugar solution of various concentrations after 2 hrs. All these values have been normalized. The two measurements were taken between 30 secs. 10% glucose solution treatment has the most significant effect on glycogen synthesis. Compared to glucose, fructose nearly has no effect on glycogen synthesis. Theoretically, fructose should be taken up by levansucrase encoded by sacB gene in our design.

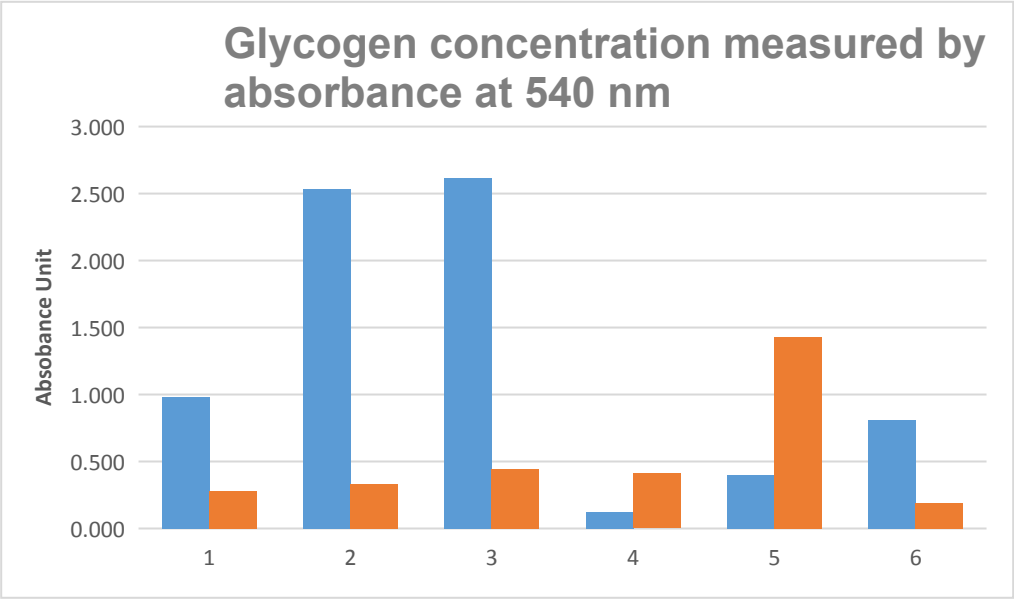


Figure 1. Relative Glycogen concentration measured by absorbance. Averaged values from the two measurements in Table 1 are used to construct the plot. As we can see the trend is as we expected. Higher starting

glucose concentration results in higher absorbance readings. K12+glgc treated with glucose generated higher readings than that treated with fructose and also significantly higher than the controls.

	1% G	5%G	10% G	1% F	5% F	10% F
glgC	0.379	0.982	1.014	0.043	0.153	0.313
Control	0.105	0.126	0.169	0.156	0.551	0.069
Concentration	0	0.04	0.08	0.12	0.16	0.2 (ug/well)
Duplicate 1	0.044	0.139	0.411*	0.393	0.483	0.527
Duplicate 2	0.082	0.211	0.293	1.133*	0.526	0.564
Normalized	0.000	0.095	X	0.349	0.439	0.483
	0.000	0.129	0.211	X	0.444	0.482
Averaged	0.000	0.112	0.211	0.349	0.442	0.483

Table 2. Absorbance readings on glycogen samples of known concentration. These samples are made from the glycogen standard (2mg/mL) provided by the assay kit. Asterisk sign (*) means abnormal values, probably due to air bubbles in the well. Thus these values are removed when normalizing. The first row denotes the diluted final concentration of the samples in ug/well.

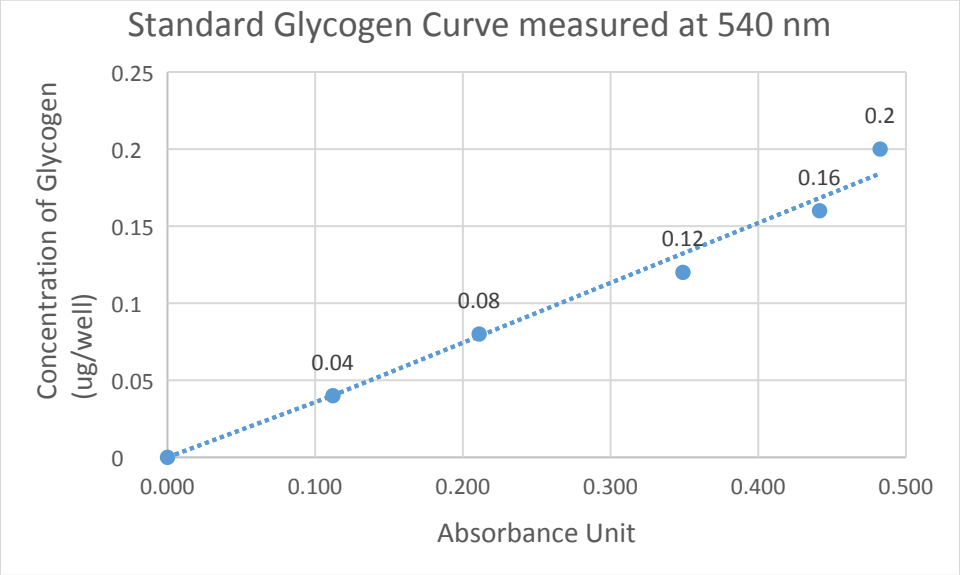


Figure 2. Standard Glycogen Curve measured at 540 nm. This standard curve is produced based on Table 2 above. As expected, the standard curve is linear and is fitted with $y = 0.3888x + 0.0034$, with a $R^2 > 0.98$.

If we plug the measured absorbance readings to the equation $y = 0.3888x + 0.0034$, then we have the estimated concentrations of glycogen in each sample.

	1% G	5%G	10% G	1% F	5% F	10% F
glgC	0.379	0.982	1.014	0.043	0.153	0.313
Control	0.105	0.126	0.169	0.156	0.551	0.069

Table 3. Estimated concentration of glycogen in each sample based on standard curve. The unit is ug/well.

Let's assume bacteria normally produce some stable level of glycogen, then we can assume this level is the average of the glycogen concentration of the controls and the fructose-treated ones, with two obvious outliers removed (0.313 and 0.551). The average is then 0.117.

Subtract this value from the first three entries in the first row and intuitively with no glucose, no glycogen will be produced. Thus we have the following table.

	No glucose	1% G	5%G	10% G
Normalized	0	0.262	0.865	0.897

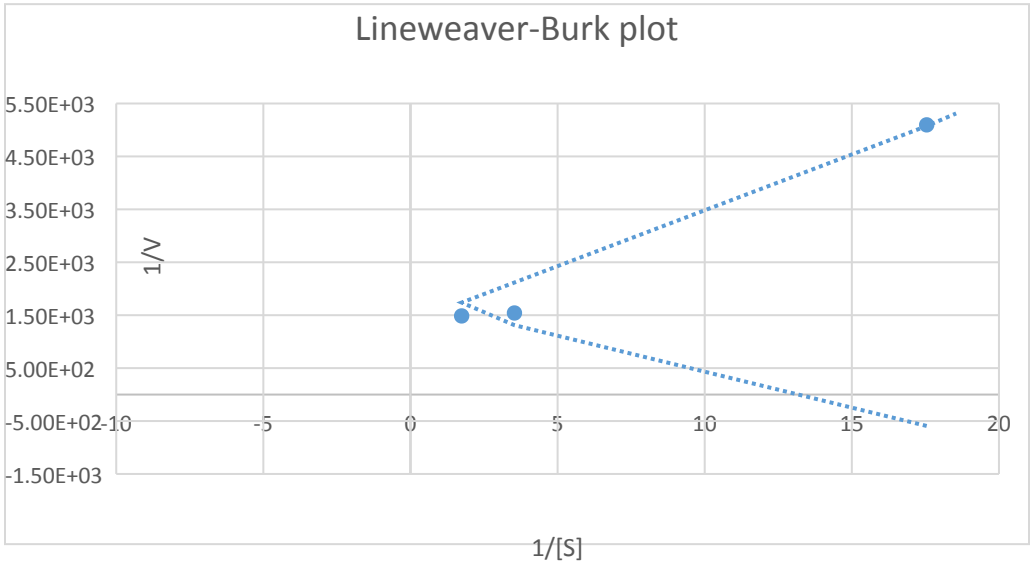
Table 4. Estimated concentration of glycogen synthesized from different concentration of substrate, glucose. The unit is ug/well.

We can then estimate the enzymatic velocity of glgC since this is measured after 2 hrs and the volume of each well is 100 uL and MW of glycogen is 666.578 g/mol

Conc. S, [S]	No Glucose	0.057M	0.283M	0.575M
Velocity, Vi	0	1.96E-04	6.49E-04	6.73E-04
1/[s]	X	17.54386	3.533569	1.73913
1/Vi	X	5.10E+03	1.54E+03	1.49E+03

Table 5. Estimated enzymatic velocity of glgC in our system in umol/hr/ 2.4 x 10^8 cells. Substrate concentration is in molar, M.

Now we can estimate the Vmax and Km of glgC using Lineweaver-Burk plot if we assume the reaction obeys Michaelis-Menten model.



So we have,