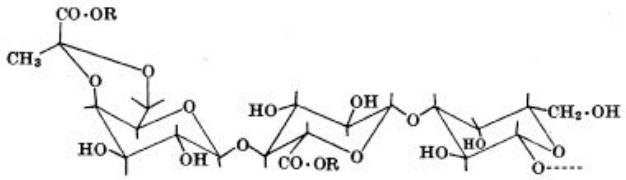
Colonic Acid Quantification (L-Fucose) Assay

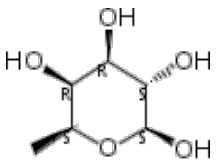
Prepared by Wisconsin iGEM Team 2010

Background

Colonic Acid is a polysaccharide containing a repeat unit with D-glucose, L-fucose, D-galactose, and D-glucuronate. Biological extracts often contain compounds, which under heating with H_2SO_4 yield brown products absorbing between 396 nm and 427 nm. Colonic acid can be estimated by measuring L-fucose content.

Structure of Colonic Acid (left) **and L-Fuctose** (right):





Materials

H_2SO_4/H_2O (6:1 v/v)
Cysteine hydrochloride
L-Frucose (calibration curve)

Cell Culture: (Final 50ml sample)

1. Transform plasmids for high protein production
2. Incubate plate overnight (do not place in fridge before next step)
3. Prepare liquid culture of each sample
4. Induce with 1mM IPTG at OD600 = 0.05
5. Grow cells to stationary phase

Protocol:

1. Prepare overnight liquid culture of **50 ml** sample
2. Measure OD600
3. To inactivate EPS-degrading enzymes and completely release EPS from cell surface:
    a. Boil sample for 15 min
    b. Cool to room temp
    c. Centrifuge at 14,000g for 30 min at 4°C
4. Add three volumes of 70% ethanol to 40 ml of supernatant fraction
5. Place in 4°C overnight
6. Centrifuge at 14,000g for 30 min at 4°C
7. Dissolve pellet in 1 ml of sterile distilled water

Quantifications: Use negative controls of glucose and sterile distilled water

1. Mix 4.5 ml of H_2SO_4/H_2O (6:1 v/v) with 50uL of 1ml sample
2. Heat mixture to 100°C for 20 min
3. Cool to room temperature
4. Measure absorbance at 396 nm and 427 nm
5. Add 100 μL of 1M cysteine hydrochloride
6. Measure absorbance at 396 nm and 427 nm
7. Difference in these measurements (after subtracted from pre-cysteine addition absorbance) can be directly correlated to methylpentose concentration by using a standard curve obtained with a fucose concentration ranging from 5 μg/ml to 100 μg/ml

Part Number	Plasmid	Strain	Function	Expression
K318500	pSB1AK3	MG1655	Produces Transcription Factor RcsA	IPTG
K318501	pSB1AK3	MG1655	Produces Transcription Factor RcsB	IPTG
K318502	pSB1AK3	MG1655	Produces Transcription Factors RcsA & RcsB	IPTG
K200021	pSB1AK3	MG1655	Empty Vector - Control	IPTG

L - Fucose – Standard Curve

- Concentrations of L-Fucose ranging from 0 μg/ml to 100 μg/ml
- L-Fucose was added to the appropriate amount of sterile distilled water to result in the following concentrations and 1ml of this preparation was taken from step 8(b) to 8(h) from Colonic Acid Quantification Assay (above)

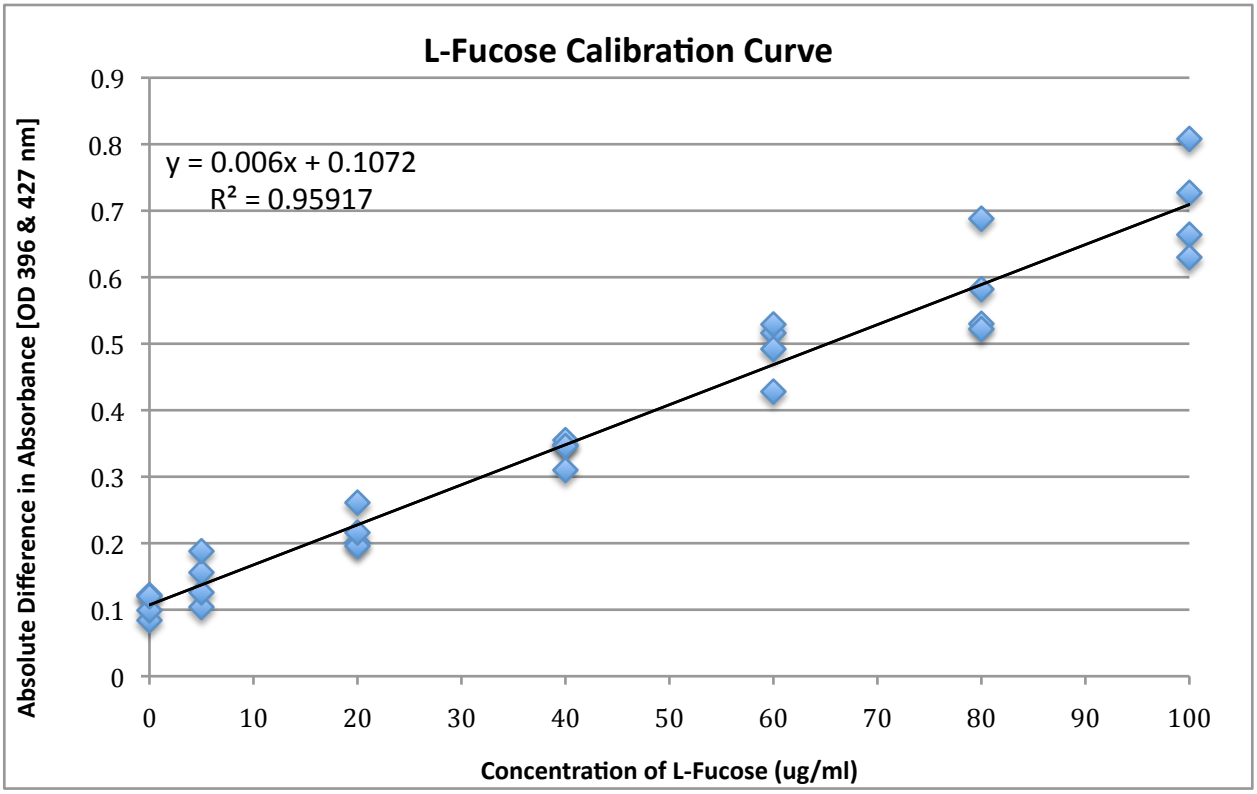


Figure 1 Calibration by Wisconsin iGEM Team 2010

References

1. Escherichia coli transcription factor YncC (McbR) regulates colanic acid and biofilm formation by repressing expression of periplasmic protein YbiM (McbA)
   Xue-Song Zhang, Rodolfo García-Contreras and Thomas K Wood

2. Influence of Tyrosine-Kinase Wzc Activity on Colanic Acid Production in Escherichia coli K12 Cells
   Brice Obadia, Soline Lacour, Patricia Doublet, Hélène Baubichon-Cortay, Alain J. Cozzone, and Christophe Grangeasse

3. Some Reactions and Derivatives of Sedoheptulosan
   W.T. Haskins, Raymond M. Hann, C.S. Hudson