iGEM 2016: Team Pittsburgh
Collaboration with William & Mary

Materials
E. coli S30 Extract System for Circular DNA from Promega
Product Information Quick Protocol Complete Protocol
8 RBS variants from William & Mary team

Procedure
Transformation
Add 5 uL plasmid to 50 uL competent cells
Spin down tubes [lids of F, G, and H snapped off, but fairly certain they were correctly relabeled]
Incubate on ice for 30 min
Heat shock at 42 degrees for 1 min
Recover on ice for 2 min
Add 200 uL SOC media
Incubate in a shaker at 37 degrees for 1.5 hours
Plate 100 uL on CM plate [plated 200 uL of A - C]
Incubate at 37 degrees overnight

Liquid cultures
To 5 mL of LB inoculated with chloramphenicol, add 2 colonies from each plate labeled A1, A2, B1, B2, …, H1, H2
Incubate in a shaker at 37 degrees overnight

Miniprep

Concentrations (ng/uL):	A1: 258.9	A2: 195.2	B1: 297.0	B2: 343.0
	C1: 304.1	C2: 302.3	D1: 283.9	D2: 255.3
	E1: 262.9	E2: 276.0	F1: 228.9	F2: 266.0
	G1: 257.1	G2: 227.6	H1: 260.5	H2: 262.5

Cell-free expression, first time

DNA	2 ug		
Amino Acids Mix without Methionine	5 uL		80 uL
Premix without Amino Acids	20 uL	x 16 reactions =	320 uL
S30 Extract for Circular DNA	15 uL		240 uL
Water to volume	50 uL		

Sample	DNA volume (uL)	Water volume (uL)
Negative control	0.00	10.00
A1	7.72	2.28

A2	10.25	0
B1	6.73	3.27
B2	5.83	4.17
C1	6.58	3.42
C2	6.62	3.38
D1	7.05	2.96
D2	7.83	2.17
E1	7.61	2.39
E2	7.25	2.75
F1	8.74	1.26
F2	7.52	2.48
G1	7.78	2.22
G2	8.79	1.21
H1	7.68	2.32
H2	7.62	2.38

1. Add appropriate amount of DNA into 1.5 mL Eppendorf tube
2. Combine Amino Acids Mix minus Methionine, Premix without Amino Acids, and S30 Extract for Circular DNA for 16 reactions separately; spin down
3. Add 40 uL of the mix from Step 2 from the DNA from Step 1
4. Add appropriate amount of water to each tube; spin down
5. Divide each of the 16 50-uL tubes into 3 16.66-uL aliquots; spin down
6. Incubate at 37 degrees for 1 hour
7. Make negative control about ten minutes later, adding water in Step 1
8. Quench reactions on ice for a few minutes

Cell expression, second time

DNA	2 ug
Amino Acids Mix without Methionine	5 uL
Premix without Amino Acids	20 uL
S30 Extract for Circular DNA	15 uL
Water to volume	50 uL

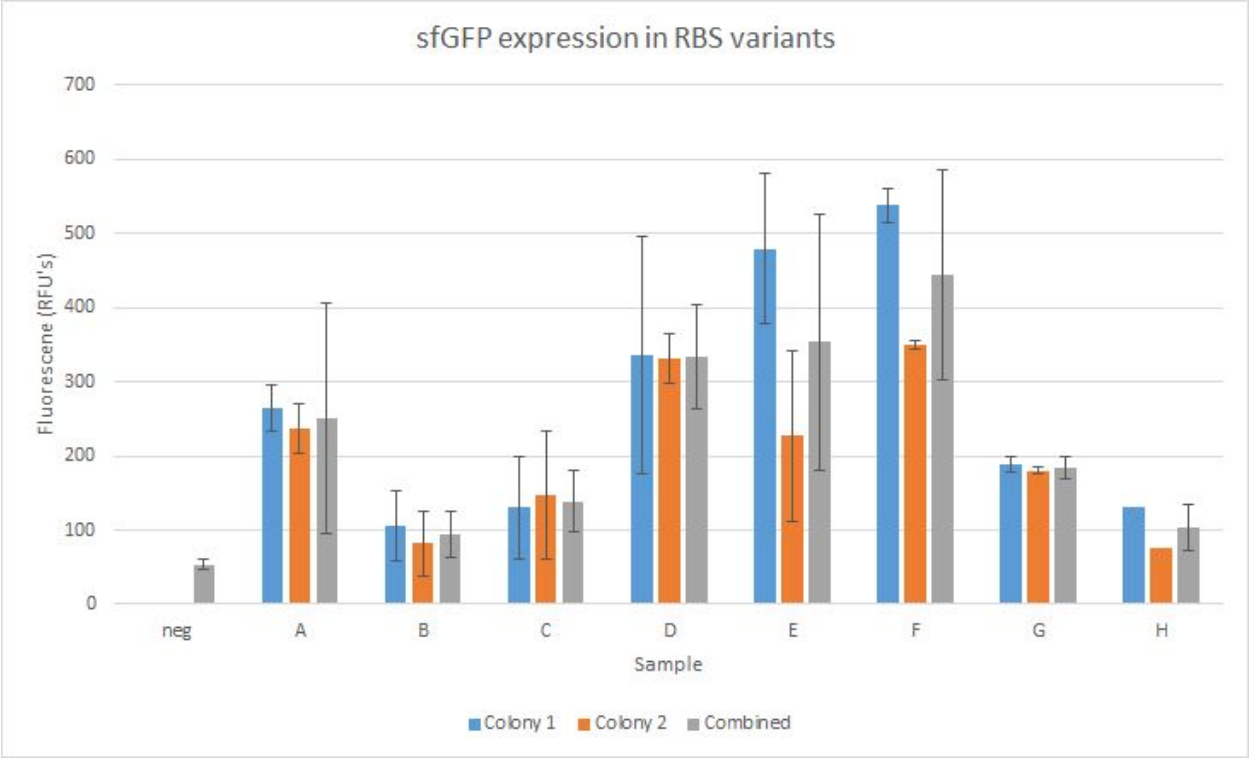
Sample	DNA volume (uL)	Water volume (uL)
Negative control	0.00	10.00
A1	7.72	2.28
B1	6.73	3.27
C1	6.58	3.42
D1	7.05	2.96
E1	7.61	2.39
F1	8.74	1.26
G1	7.78	2.22
H1	7.68	2.32

1. Add appropriate amounts of DNA and water into 1.5 mL Eppendorf tube
2. Add Amino Acids Mix minus Methionine to each tube
3. Add Premix without Amino Acids to each tube
4. Add S30 Extract for Circular DNA to each tube
5. Pipette up and down; spin
6. Divide each of the 8 50-uL tubes into 3 16.66-uL aliquots; spin down
7. Incubate at 37 degrees for 1 hour
8. Quench reactions on ice for five minutes; spin down

Reading fluorescence
Add 15 uL of each reaction into black, clear-bottom 384-well plate
Plate Reader: SpectraMax M2
Excitation: 485 nm Emission: 515 nm Cutoff: 595 nm

Results
First time

Sample	Colony 1					Colony 2					Combined	
	Replicate 1	Replicate 2	Replicate 3	Average	St Dev	Replicate 1	Replicate 2	Replicate 3	Average	St Dev	Average	St Dev
neg	45.798	59.426	54.426								53.21666667	6.894016343
A	518.911	116.255	159.943	265.0363333	220.9443824	351.27	148.001	211.91	237.0603333	103.9421764	251.0483333	155.1868404
B	138.98	101.79	77.914	106.228	30.77394957	118.23	53.418	75.149	82.26566667	32.98687685	94.24683333	31.405828
C	91.412	182.484	116.734	130.21	47.0077571	102.289	189.556	151.515	147.7866667	43.7528019	138.9983333	41.74082563
D	296.777	292.849	415.261	334.9623333	69.56841379	390.287	231.692	371.598	331.1923333	86.67500753	333.0773333	70.32206141
E	315.194	487.806	634.543	479.181	159.849113	192.83	257.282	230.684	226.932	32.38939956	353.0565	172.4215539
F	576.818	421.422	613.221	537.1536667	101.8658854	219.824	437.542	393.086	350.1506667	115.0341863	443.6521667	141.1908129
G	184.221	167.934	212.428	188.1943333	22.51154242	186.177	178.489	174.624	179.7633333	5.880977498	183.9788333	15.42292786
H	125.651	124.512	142.954	131.039	10.3343964	75.381	70.383	79.511	75.09166667	4.570873148	103.0653333	31.46598615

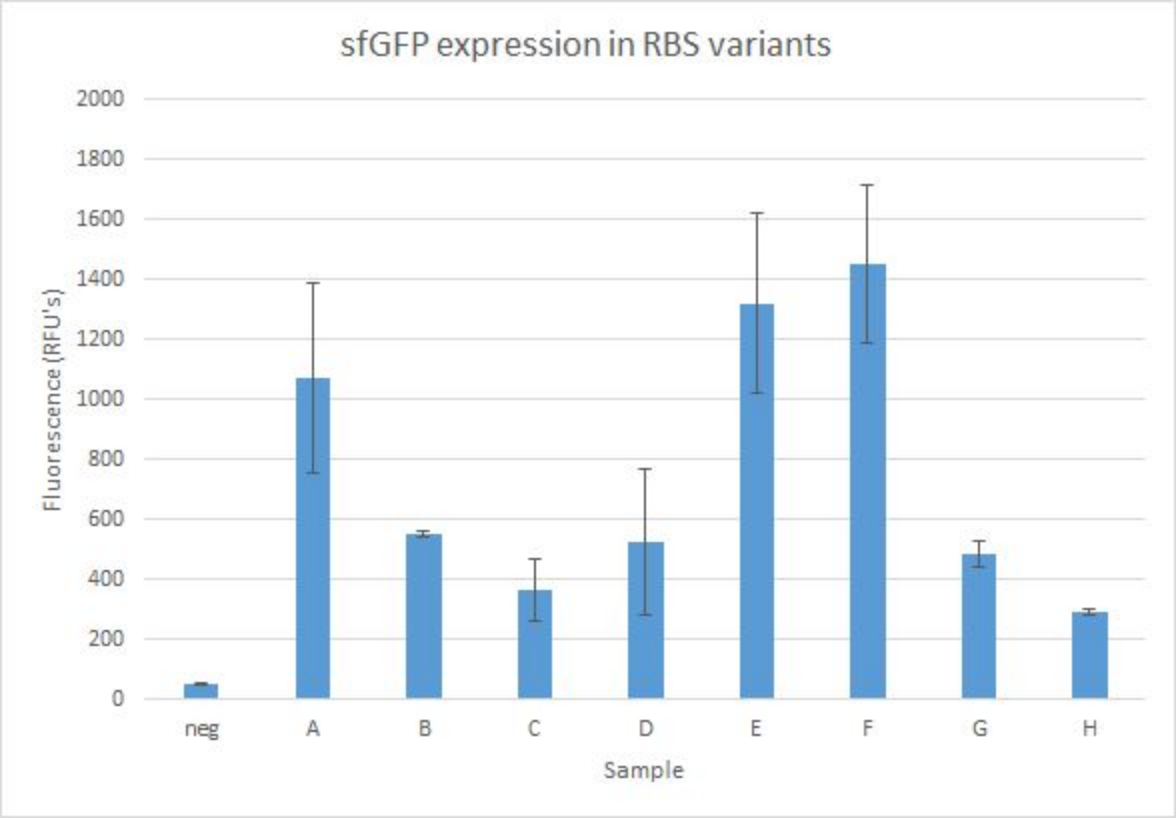


Average fluorescence of each RBS variant expressed in *E. Coli* S30 Extract System. Error bars represent standard deviation.

Second time

Sample	Replicate 1	Replicate 2	Replicate 3	Average	St Dev
neg	50.769	44.443	49.099	48.10367	3.278351
A	847.783	486.65	1296.949	1072.366	317.6083
B	537.716	557.127	557.224	550.689	11.23505
C	371.092	466.864	254.967	364.3077	106.1113
D	789.768	308.461	480.132	526.1203	243.9268
E	1378.061	997.871	1585.216	1320.383	297.8903
F	1620.387	1146.405	1585.266	1450.686	264.0995
G	530.228	443.971	477.78	483.993	43.46284
H	305.129	287.992	279.504	290.875	13.0535

Sample A, Replicate 2 was omitted as an outlier. Sample A, Replicate 1 contained less than 15 uL, so the experimental reading is low. Thus, Replicate 2 was determined to be the outlier.



Average fluorescence of each RBS variant expressed in *E. Coli* S30 Extract System. Error bars represent standard deviation.