

Ethanol Promoter Test

1. Prepare LC's overnight for desired bacterial strains
 - a. LB (- control)
 - b. Pconst_alcR (K2539300) only
 - c. PalcA_GFP (K2539500) only
 - d. GFP only (+ control)
 - e. alcR + PalcA + GFP (K2539550) +1g ethanol
 - f. alcR + PalcA + GFP (K2539550) +0.5g ethanol
 - g. alcR + PalcA + GFP (K2539550) +0.3g ethanol
 - h. alcR + PalcA + GFP (K2539550) +0.1g ethanol
 - i. alcR + PalcA + GFP (K2539550) +0.01g ethanol

1. Take 100ul of each liquid culture and fill a 96 well plate (12 replicates per variable)
2. Take fluorescence measurement (T=0) using plate reader
3. Add ethanol into liquid cultures and dilute the rest with water to maintain equal volume
 - a. Add 1270ul of ethanol into the liquid cultures called (1g)
 - i. No water needs to be added
 - b. Add 127ul of ethanol into the liquid culture called (0.1g)
 - i. Add 1143ul of ddH2O into liquid culture to maintain equal volume
 - c. Add 12.7ul of ethanol into the liquid culture called (0.01g)
 - i. Add 1257.3ul of ddH2O into liquid culture to maintain equal volume
 - d. Add 1.27ul of ethanol into the liquid culture called (0.001g)
 - i. Add 1268.73ul of ddH2O into liquid culture to maintain equal volume
4. Screw cap to avoid evaporation and let cultures shake in the incubator at 37C and 60 RPM for 24 hours
5. Aliquot 100ul of liquid culture into 96 well plate
6. Take fluorescence measurement using plate reader (T=24 hours)
7. Check for change in fluorescence to see if alcR and ethanol induced PalcA

Table #1: 8/23: 0 Hour fluorescence absorbance

	8/23: Fluorescence Absorbance at 0 hour											
LB	3.571	3.068	3.924	4.071	3.238	4.29	3.7723	4.256	2.979	3.987	4.575	3.612
alcR only	5.206	4.708	3.812	4.785	5.189	4.815	6.519	3.558	3.81	4.43	4.77	4.717
PalcA												
GFP only	5.019	5.438	4.017	5.244	5.028	4.801	5.184	4.405	4.289	4.565	4.534	3.986
1g	6.656	6.277	6.577	6.632	6.632	6.436	7.378	5.854	7.521	6.491	6.141	7.338
0.5g	6.515	6.016	5.671	6.388	6.047	5.218	5.471	6.293	5.944	6.091	4.843	4.692
0.3g	6.281	6.281	6.195	6.098	6.784	5.418	5.528	6.659	4.966	5.425	5.928	6.625
0.1g	6.823	6.44	5.969	7.692	5.387	5.801	6.101	6.49	5.524	6.984	5.91	5.304
0.01g	6.117	5.593	5.447	6.561	5.615	6.897	5.172	6.488	5.711	4.843	5.547	5.161

Table #2: 8/24: 24 Hour Fluorescence Absorbance

	8/24: Fluorescence Absorbance at 24 hour											
LB	3.6105	4.19706	4.05815	5.1462	3.66763	4.97463	4.35599	3.90667	4.19242	4.07024	4.29803	3.55029
alcR only	7.69063	6.68425	6.8709	8.79508	6.77018	6.96874	6.25966	5.39583	5.58363	7.27014	7.21752	7.83738
PalcA												
GFP only	7.95072	7.37697	7.86931	8.3381	8.29157	6.51481	6.60425	6.88357	8.68275	6.15114	7.22086	6.90837
1g	7.5543	6.19686	5.93256	6.38631	7.17041	6.6706	6.76152	6.75527	7.86846	5.64737	5.61004	6.40124
0.5g	4.69876	6.53374	6.72771	4.87381	4.35178	6.64097	3.98819	5.20164	6.03792	4.45993	5.20848	4.1367
0.3g	19.074	21.7636	25.4015	21.6987	24.0231	22.8917	20.6037	23.3237	23.5443	22.7428	21.6994	23.1812
0.1g	4.13209	4.74489	4.27665	5.21021	5.62314	3.96729	5.34196	4.17476	5.94669	5.06098	3.68969	4.44417
0.01g	8.05858	7.78385	11.7038	8.88555	8.54751	9.02991	8.67423	6.83242	8.05415	8.92037	8.3434	9.18369

Table #3: Relative Change in Fluorescence

	Relative Change in Absorbance											
LB	1.143213	1.618821	1.019925	1.493758	1.351496	1.426881	0.641076	0.800718	0.917885	0.862509	0.779645	1.007877
alcR only	0.994292	1.539995	1.165472	1.663032	1.652754	1.352021	1.820695	1.381442	1.465774	1.267544	1.893948	1.305578
PalcA GFP only	1.766685	1.695835	1.104801	1.581373	1.474646	1.408058	1.313684	1.38661	1.201781	1.007185	1.12871	1.316792
1g	0.818354	0.709314	1.152377	1.096196	1.362408	0.947168	1.706009	1.313694	0.890808	0.729573	0.963216	0.938313
0.5g	0.897432	0.902539	0.924766	0.788484	0.859591	0.882525	1.065007	0.648172	0.536642	1.028786	0.803142	0.992142
0.3g	0.691338	0.828047	0.57469	0.880295	0.645398	0.65107	0.766674	0.716134	0.821325	0.839813	0.853969	0.823544
0.1g	1.673751	2.754295	2.464601	2.275475	2.065243	2.529353	2.412075	2.676469	2.083291	2.642781	2.367389	2.403153
0.01g	1.121536	1.824952	1.158802	1.261486	1.330893	1.386227	1.435365	1.204742	1.144698	1.118431	0.987132	1.564586

Table #4: 9/20: 0 Hour Fluorescence Absorbance

	Fluorescence Absorbance at 0 Hour											
LB	4.697	3.665	3.883	2.85	3.769	3.373	5.392	3.801	4.056	3.686	4.935	5.157
alcR only	6.538	5.555	5.044	4.888	4.996	5.768	5.223	6.186	5.251	6.27	5.121	4.733
PalcA GFP only	4.299	4.694	5.976	4.333	5.032	5.13	5.032	5.383	6.765	7.311	6.226	5.209
1g	6.645	8.109	6.404	5.573	4.032	6.624	4.032	4.938	7.004	8.539	6.785	6.16
0.5g	6.012	6.412	6.448	6.845	6.409	5.401	5.894	8.814	8.205	5.635	6.689	6.003
0.3g	7.316	7.288	7.604	7.843	8.729	7.563	6.939	7.951	6.376	6.957	6.384	7.74
0.1g	6.903	5.588	5.808	6.952	8.24	6.03	6.799	5.053	7.463	5.853	6.139	6.597
0.01g	7.671	5.03	8.537	6.983	7.484	7.239	6.88	7.931	6.822	7.048	9.215	5.962

Table #5: 9/21: 24 Hour Fluorescence Absorbance

	9/21: Fluorescence Absorbance at 24 Hours											
LB	5.36967	5.93298	3.96037	4.25721	5.09379	4.81287	3.45668	3.04353	3.72294	3.17921	3.84755	5.19762
alcR only	6.50068	8.55467	5.87864	8.1289	8.25716	7.79846	9.50949	8.5456	7.69678	7.9475	9.69891	6.1793
PalcA GFP only	7.59498	7.96025	6.60229	6.85209	7.42042	7.22334	6.61046	7.46412	8.13005	7.36353	7.02735	6.85917
1g	5.43796	5.75183	7.37982	6.1091	5.49323	6.27404	6.87863	6.48702	6.23922	6.22982	6.53542	5.78001
0.5g	5.39536	5.78708	5.96289	5.39717	5.50912	4.76652	6.27715	5.71299	4.40315	5.79721	5.37222	5.95583
0.3g	5.05783	6.03481	4.36994	6.90415	5.63368	4.92404	5.31995	5.69398	5.23677	5.84258	5.45174	6.37423
0.1g	11.5539	15.391	14.3144	15.8191	17.0176	15.252	16.3997	13.5242	15.5476	15.4682	14.5334	15.8536
0.01g	8.6033	9.17951	9.89269	8.80896	9.9604	10.0349	9.87531	9.55481	7.80913	7.8827	9.09642	9.32806

Table #6: Relative Change in absorbance

	Relative Change in Absorbance											
LB	1.011061	1.368012	1.034187	1.264112	1.132684	1.159587	1.154731	0.917921	1.407325	1.020878	0.93946	0.982915
alcR only	1.477263	1.419764	1.80244	1.838052	1.304718	1.447298	0.960218	1.516535	1.46552	1.641115	1.513107	1.661518
PalcA GFP only	1.584124	1.356559	1.959002	1.590027	1.649079	1.356969	1.273968	1.562672	2.024423	1.347457	1.592603	1.733159
1g	1.134961	0.987233	0.902016	0.962954	1.081184	1.036451	0.916443	1.153958	1.046199	0.870031	0.913539	0.872341
0.5g	0.721222	1.086061	1.186336	0.762963	0.719659	1.272704	0.728969	0.826576	1.015801	0.732216	1.075466	0.88165
0.3g	3.036778	3.46499	4.100323	3.558331	3.541141	4.22512	3.727153	3.502583	4.741099	4.192221	3.660493	3.499049
0.1g	0.605612	0.736784	0.716477	0.677354	1.043835	0.683898	0.875588	0.64326	1.076519	0.724653	0.624313	0.83789
0.01g	1.317407	1.391713	2.148669	1.354298	1.522264	1.309252	1.677152	1.053086	1.410287	1.84191	1.504128	1.77944

Table #7: Average change, Standard Deviation and Standard Error of fluorescence among 24 sets of data points

	average change of fluorescence	STDEV	SEM
LB	1.102361544	0.246302	0.061576
alcR only	1.481253909	0.245866	0.061466
PalcA GFP only	1.47567514	0.257355	0.064339
1g	1.021030792	0.21582	0.053955
0.5g	0.889118791	0.176364	0.044091
0.3g	2.264232352	1.572925	0.393231
0.1g	1.566419132	0.846873	0.211718
0.01g	1.4103523	0.284323	0.071081

Graph #1: Relative fluorescence of *E. coli* cultures carrying Pconst_alcR+PalcA_GFP (BBa_K2539550) with varying amounts of ethanol

