

DG.OTS Expression Optimization Experiment

Prepare Master Mixes

1. Make stock solutions of nonstandard amino acids (nsAAs).
2. Prepare arabinose master mixes at different concentrations.
3. Prepare nsAA master mixes for each experimental condition (controls, pAcF, ppAF, ppAF + HCl, etc.).

50X pAcF (50mM)	100X pPaF (100mM)						
1mL SMQ Water	1mL NaOH						
10.36 mg pAcF	21.92mg pPaF						
*	*						
Arabinose							
	0.4% ara	0.2% ara	0.02% ara	0.002% ara	0% ara		
	4900 uL LB	2500 uL LB	4500uL LB	4950 uL LB	5mL LB		
	100 uL 20% ara	2500 uL 0.4% ara	500uL 0.2% ara	50uL 0.2% ara	0uL 20% ara		
Volume	5mL	5mL	5mL	5mL	5mL		
Each well	73uL	73uL	73uL	73uL	73uL		
nsAA Master Mixes							
	No NsAA MM	DG.OTS MM	GFP (no TAG) MM	pAcF (1mM) MM	pPaF (1mM) MM	pPaF (HCl)	
	4990mL LB Media	4790 LB Media	4790 LB Media	4790 LB Media	4890 LB Media	4880 LB Media	
	10uL aTc	10 uL aTc	10 uL aTc	10 uL aTc	10 uL aTc	10uL aTc	*1000X aTc
		200uL 50X pAcF	200uL 50X pAcF	200uL 50X pAcF	100 uL 100X pPaF	100 uL 100X pPaF	
						20 uL HCL	
Volume	5mL	5mL	5mL	5mL	5mL	5mL	
Each well	73uL	73uL	73uL	73uL	73uL	73uL	

On a 96 well plate

4. Add arabinose master mixes to designated wells.
5. Add nsAA master mixes to the corresponding wells.
6. Inoculate wells with the appropriate cell strains (e.g., GFP with 1 TAG, GFP no TAG, no GFP controls).
7. Induce with aTc

Example Layout:

https://docs.google.com/spreadsheets/d/16TRJhXjuooCn597EwogBCTx_GKjCsm31sCifuJRg4Lg/edit?usp=sharing

Plate Reader Measurements

8. Incubate overnight at 37C shaking
9. Run a kinetic read on a plate reader (OD and fluorescence).
 - a. Record data over ~20 hours using double orbital shaking.
 - b. Compare Fluorescence/OD