

Additional file

Engineering *Escherichia coli* FAB system using synthetic plant genes for the production of long chain fatty acids. Elias Kassab¹, Monika Fuchs¹, Martina Haack¹, Norbert Mehlmer¹, Thomas B. Brueck^{1*}.

Table S1: Fatty acid distribution in $\mu\text{g/g}$ DCW of wild type *Escherichia coli* BL21 (DE3) at different time intervals. T=0: directly before induction with IPTG, t=12: 12 hours after induction with IPTG, t=24: 24 hours after induction with IPTG. All fatty acid values are the average of at least three biological replicates with the associated standard deviation indicated.

BL21 (DE3) pET28a-						
Fatty acid distribution ($\mu\text{g/g}$)	T=0		T=12		T=24	
C12:0	2	± 0.12	8	± 0.41	12	± 0.49
C14:0	4	± 0.40	12	± 0.65	20	± 1.12
C16:0	75	± 2.05	155	± 5.45	260	± 5.02
C16:1	27	± 1.25	20	± 1.41	30	± 1.10
C18:0	3	± 0.12	3	± 0.11	6	± 0.41
C18:1 (vaccenate)	57	± 1.25	83	± 1.48	135	± 2.96
C17:0c	1	± 0.00	1	± 0.00	1	± 0.00
C19:0c	3	± 0.22	36	± 1.87	69	± 2.50
total	172	± 5	318	± 11	533	± 14

Table S2: Fatty acid distribution in $\mu\text{g/g}$ DCW of *Escherichia coli* BL21 (DE3) expressing pET28aKASI at different time intervals. T=0: directly before induction with IPTG, t=12: 12 hours after induction with IPTG, t=24: 24 hours after induction with IPTG. All fatty acid values are the average of at least three biological replicates with the associated standard deviation indicated.

BL21 (DE3) pET28a KASI						
Fatty acid distribution ($\mu\text{g/g}$)	T=0		T=12		T=24	
C12:0	4	± 0.12	25	± 1.16	20	± 0.67
C14:0	5	± 0.05	24	± 1.06	18	± 0.59
C16:0	65	± 4.40	291	± 12.76	483	± 16.8
C16:1	27	± 1.27	80	± 3.37	132	± 5.80
C18:0	3	± 0.07	17	± 0.52	27	± 1.42
C18:1 (vaccenate)	47	± 1.95	228	± 7.29	349	± 8.62
C17:0c	1	± 0.00	2	± 0.10	5	± 0.88
C19:0c	3	± 0.09	21	± 0.30	34	± 1.20
Total	155	± 8	688	± 27	1068	± 36

Table S3: Fatty acid distribution in $\mu\text{g/g}$ DCW of *Escherichia coli* BL21 (DE3) ΔfadD pET28a- versus BL21 (DE3) ΔfadD expressing pET28aKASI and pET28aKASII separately. Samples were collected 48 hours after induction with IPTG. All fatty acid values are the average of at least three biological replicates with the associated standard deviation indicated.

BL21 (DE3)						
Fatty acid distribution ($\mu\text{g/g}$)	ΔfadD -		ΔfadD -KASI		ΔfadD -KASII	
C12:0	20	± 0.7	38	± 1.6	20	± 0.6
C14:0	44	± 1.2	66	± 2.3	42	± 1.3
C16:0	198	± 9.9	371	± 16.9	221	± 14.3
C16:1	5	± 0.4	168	± 5.8	90	± 3.8
C18:0	3	± 0.2	5	± 0.3	4	± 0.2
C18:1 (vaccenate)	20	± 1.1	188	± 4.7	88	± 3.6
C17:0c	122	± 4.0	63	± 2.7	20	± 0.8
C19:0c	60	± 2.4	13	± 0.4	67	± 1.2
total	471	± 20	912	± 34	557	± 26

Table S4: Fatty acid distribution in $\mu\text{g/g}$ DCW of *Escherichia coli* BL21 (DE3) ΔfabF pET28a- versus BL21 (DE3) ΔfadD expressing pET28aKASII. Samples were collected 48 hours after induction with IPTG. All fatty acid values are the average of at least three biological replicates with the associated standard deviation indicated.

BL21DE3				
Fatty acid distribution ($\mu\text{g/g}$)	ΔfabF		ΔfabF - KASII	
C12:0	13	± 0.3	12	± 0.3
C14:0	34	± 0.4	28	± 0.8
C16:0	164	± 1.2	148	± 4.3
C16:1	40	± 0.4	114	± 3.1
C18:0	3	± 0.1	7	± 0.2
C18:1 (vaccenate)	7	± 0.0	27	± 0.8
C17:0c	137	± 0.7	35	± 1.2
C19:0c	0	± 0.0	0	± 0.0
Total	397	± 2.8	370	± 10.7

Table S5: Fatty acid distribution in $\mu\text{g}/\text{mg}$ DCW of the 1.3 Liter fermentation of *Escherichia coli* BL21 (DE3) ΔfadD expressing an empty pET28a vector, pET28aKASI and pET28aKASII respectively. Samples were collected 48 hours after induction with IPTG. All fatty acid values are the average of at least three biological replicates with the associated standard deviation indicated.

BL21DE3						
Fatty acid distribution ($\mu\text{g}/\text{mg}$)	ΔfadD		ΔfadD -KASI		ΔfadD -KASII	
C12:0	7	± 0.2	12	± 0.2	9	± 0.1
C14:0	16	± 0.7	26	± 0.8	20	± 0.2
C16:0	78	± 2.7	137	± 2.9	104	± 1.1
C16:1	6	± 0.3	23	± 0.4	8	± 0.1
C18:0	6	± 0.1	18	± 0.1	9	± 0.1
C18:1 (vaccenate)	16	± 0.5	41	± 0.8	29	± 0.3
C17:0c	7	± 0.1	9	± 0.2	11	± 0.1
C19:0c	20	± 0.4	26	± 0.3	31	± 0.4
total	157	± 5	292	± 6	222	± 2

Table S6: Fatty acid distribution in $\mu\text{g}/\text{mg}$ DCW of the 1.3 Liter fermentation of *Escherichia coli* BL21 (DE3) ΔfabF expressing pET28aKASI and pET28aKASII respectively. Samples were collected 48 hours after induction with IPTG. All fatty acid values are the average of at least three biological replicates with the associated standard deviation indicated.

BL21DE3					
Fatty acid distribution ($\mu\text{g}/\text{mg}$)	ΔfabF -KASI		ΔfabF -KASII		
C12:0	10	± 0.28	4	± 0.14	
C14:0	20	± 0.51	15	± 0.29	
C16:0	97	± 0.81	105	± 0.47	
C16:1	17	± 0.12	12	± 0.09	
C18:0	12	± 0.04	5	± 0.05	
C18:1 (vaccenate)	3	± 0.06	9	± 0.18	
C17:0c	7	± 0.42	5	± 0.41	
C19:0c	2	± 0.04	6	± 0.09	
total	167	± 2.28	162	± 1.71	

Table S7: Growth analysis of M9 minimal media shake flask studies of wild type *Escherichia coli* BL21 (DE3) expressing pET28a-, pET28aKASI and pET28aKASII respectively. Absorbance values (OD600) are the average of at least three biological replicates.

M9 media	BL21 (DE3) pET28a-			BL21 (DE3) pET28a-KASI			BL21 (DE3) pET28a-KASII		
	Absorbance	Exponential growth	Specific growth rate	Absorbance	Exponential growth	Specific growth rate	Absorbance	Exponential growth	Specific Growth rate
0	0.186	-0.73048706	0.32850407	0.162	-0.79048499	0.42985656	0.178	-0.74958	0.29328433
1	0.25833333	-0.58781955	0.58921933	0.249	-0.60380065	0.62887636	0.23866667	-0.62220823	0.61774916
2	0.46566667	-0.33192485	0.685242	0.467	-0.33068312	0.68454498	0.44266667	-0.35392318	0.67337467
3	0.924	-0.03432803	0.44229647	0.926	-0.03338901	0.44568217	0.868	-0.06148027	0.48515327
4	1.438	0.15775889	0.21896236	1.446	0.16016829	0.23003538	1.41	0.14921911	0.23862592
5	1.79	0.25285303	0.25719407	1.82	0.26007139	0.23189634	1.79	0.25285303	0.20624174
6	2.315	0.364551	0.10643985	2.295	0.36078269	0.10536052	2.2	0.34242268	0.12783337
7	2.575	0.41077723	-0.00094884	2.55	0.40654018	-0.0021777	2.5	0.39794001	-0.00172118
23.5	2.535	0.40397796	-0.03410561	2.46	0.39093511	-0.04447024	2.43	0.38560627	-0.05715841
24.5	2.45	0.38916608	-0.00136193	2.353	0.37162193	-0.00085052	2.295	0.36078269	-0.0043716

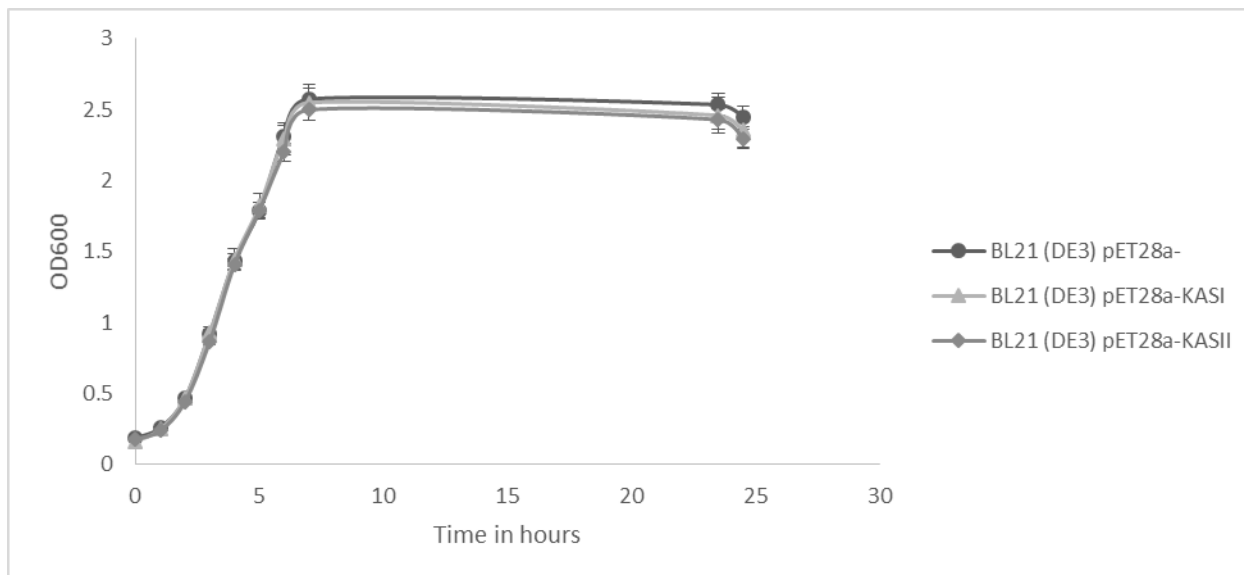


Figure S1: Growth curve of M9 minimal media shake flask studies of wild type *Escherichia coli* BL21 (DE3) expressing pET28a-, pET28aKASI and pET28aKASII respectively. Absorbance values (OD600) are the average of at least three biological replicates with the associated standard deviation indicated.