Convergent evolution of a modified, acetate-dependent TCA cycle in bacteria

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This document includes:

Supplementary Figures 1–3
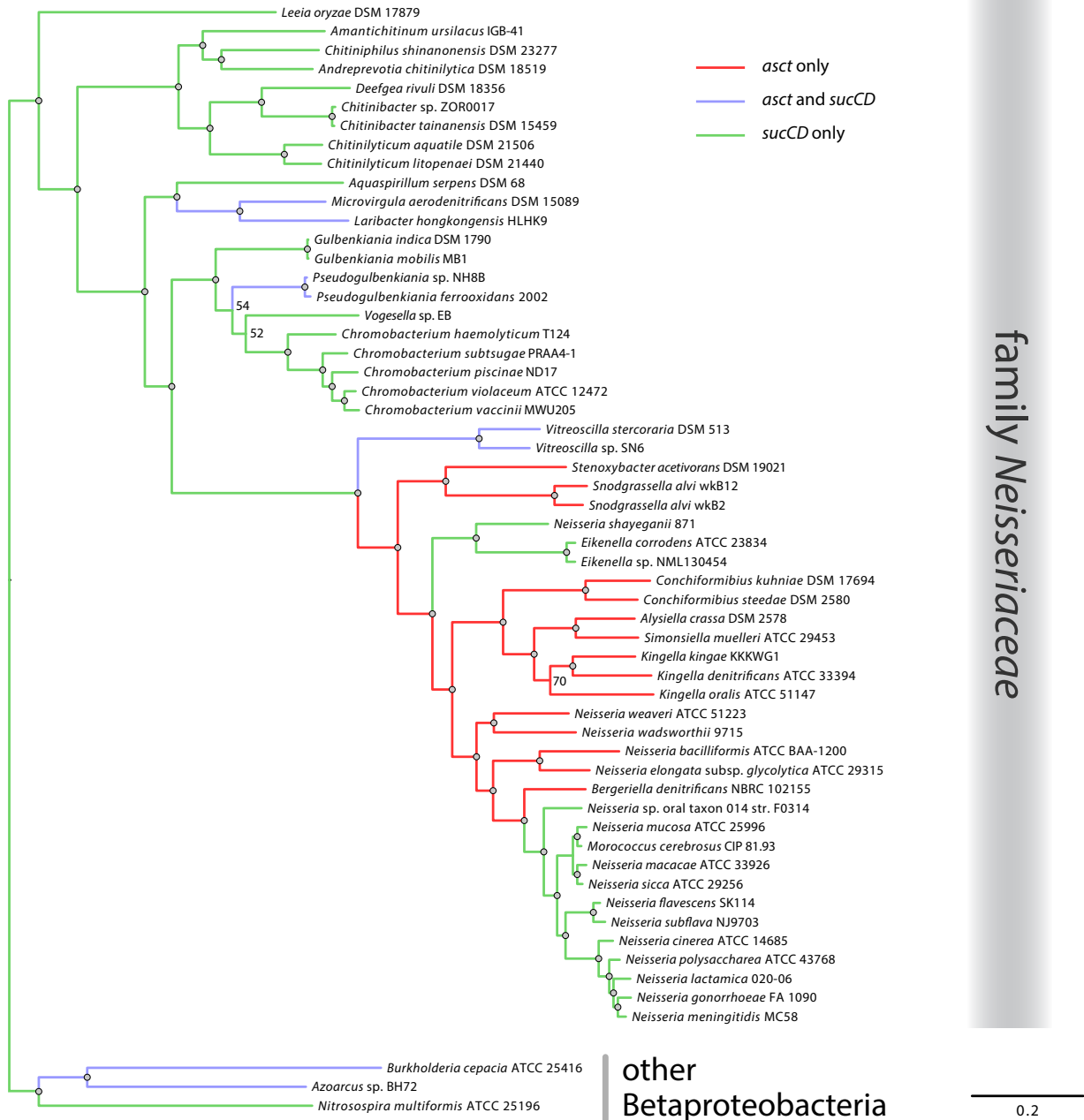
Supplementary Tables 1 & 2

Supplementary Figure 1. Growth effects of ASCT-complementation in different *E. coli* mutant backgrounds. Strains with both ASCT and SCS had slowest growth, suggesting competitive inhibition. Strains with *ΔsucC* backgrounds had fastest growth, but at the cost of achieving lower final densities. Strains were grown in M9 minimal media supplemented with 0.3% w/v sodium acetate and 75 mg/ml carbenicillin for plasmid maintenance. OD_{600} readings were taken hourly. Lines represent replicates (n = 3 for each strain).
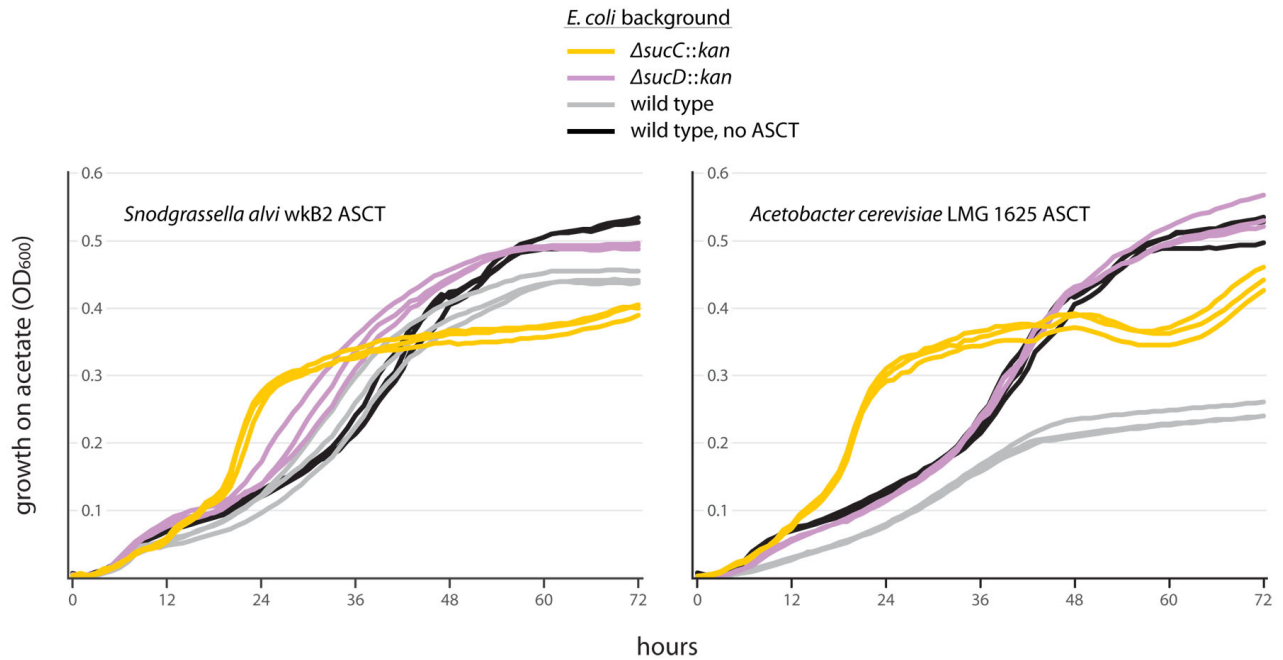
Supplementary Figure 2. Rectangular tree representation of *asct* phylogeny in Fig. 1c. Values at nodes indicate bootstrap support from maximum-likelihood analysis (1,000 replicates). Bar, substitutions per site.
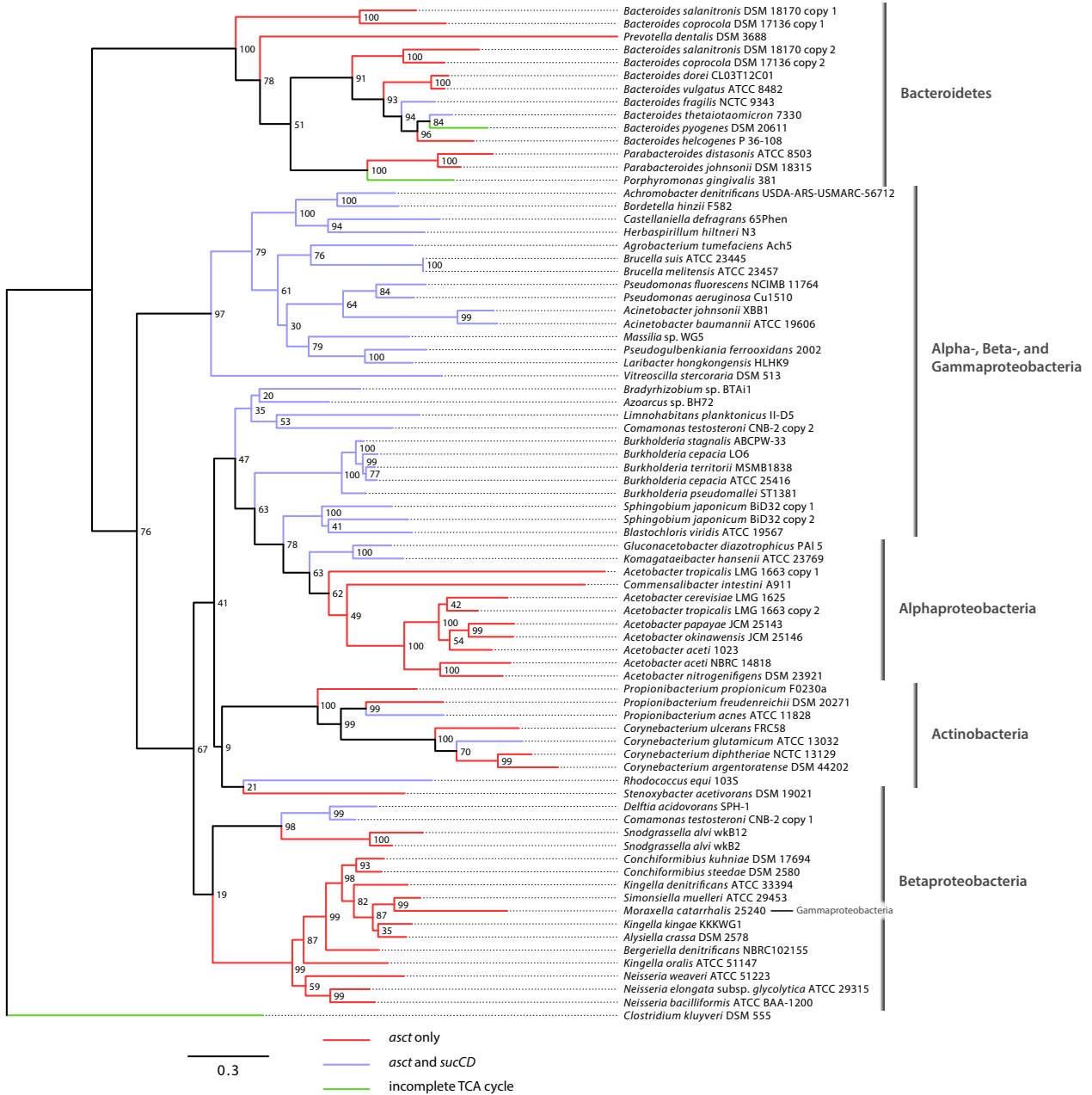
Supplementary Figure 3. Prevalence of *asct* in the *Neisseriaceae*. The maximum likelihood algorithm was used to construct a whole genome phylogeny, based on 363 orthologous genes (108,434 amino acid residues). Branches are colored based on presence of *asct* and *sucCD* in tip taxa, and extrapolated using parsimony to interior branches. Circles indicate nodes with 100% bootstrap support (1,000 replicates). Bar, substitutions per site.

Supplementary Table 1. Genes, plasmids, and strains used in this study.

	Description	Reference
Gene		
SALWKB2_RS03080	*asct* from *Snodgrassella alvi* wkB2	NZ_CP007446
AD928_RS13430	*asct* from *Acetobacter cerevisiae* LMG 1625	NZ_LHZA01000000
DIP_RS20725	*asct* from *Corynebacterium diphtheriae* NCTC 13129	NC_002935
DR90_RS06235	*asct* from *Moraxella catarrhalis* ATCC 25240	NZ_CP008804
F911_00479	*asct* from *Acinetobacter baumannii* ATCC 19606	NZ_APRG01000000
BF9343_RS00125	*asct* from *Bacteroides fragilis* NCTC 9343	NC_003228
Plasmid		
pBad-EBFP2 (control)	pBad backbone, EBFP2 (blue fluorescent protein) insert	[15]
pWK1	pBad backbone, SALWKB2_RS03080 insert	This study
pWK24	pBad backbone, DIP_RS20725 insert	This study
pWK25	pBad backbone, AD928_RS13430 insert	This study
pWK29	pBad backbone, DR90_RS06235 insert	This study
pWK34	pBad backbone, F911_00479 insert	This study
pWK39	pBad backbone, BF9343_RS00125 insert	This study
pWK31 (control)	pBad backbone, F911_00479 insert (reversed)	This study
Strain		
E. coli CGSC 7636	F-, *Δ(araD-araB)567*, *ΔlacZ4787*(::rrnB-3), λ⁻, *rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514*	[16]
E. coli CGSC 8788	F-, *Δ(araD-araB)567*, *ΔlacZ4787*(::rrnB-3), *ΔsucC777::kan*, λ⁻, *rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514*	[16]
E. coli CGSC 11810	F-, *Δ(araD-araB)567*, *ΔlacZ4787*(::rrnB-3), *ΔsucD778::kan*, λ⁻, *rph-1*, *Δ(rhaD-rhaB)568*, *hsdR514*	[16]

Supplementary Table 2. Growth curve data for Fig. 1b, means and standard deviations. Strain identities as follows: 7636, *E. coli* CGSC 7636; wkE9, pBad-EBFP2 in CGSC 8788; wkE11, pBad-EBFP2 in CGSC 11810; wkE5, pWK1 in CGSC 8788; wkE3, pWK1 in CGSC 11810; wkE17, pWK24 in CGSC8788; wkE15, pWK24 in CGSC 11810; wkE21, pWK25 in CGSC 8788; wkE23, pWK25 in CGSC 11810; wkE27, pWK29 in CGSC 8788; wkE29, pWK29 in CGSC 11810; wkE33, pWK34 in CGSC 8788; wkE34, pWK34 in CGSC 11810; wkE35, pWK39 in CGSC 8788; wkE36, pWK39 in CGSC 11810.

OD_{600} means

Hour	7636	wkE9	wkE11	wkE5	wkE3	wkE17*	wkE15*	wkE21	wkE23	wkE27	wkE29	wkE33	wkE34	wkE35	wkE36
0	0.00929	0.00593	0.00759	0.00839	0.00686	0.00831	0.00396	0.00749	0.00589	0.00756	0.00533	0.00678	0.01148	0.00701	0.00511
1	0.01526	0.00973	0.01253	0.01029	0.01126	0.01376	0.00651	0.01209	0.00956	0.01186	0.00819	0.01185	0.01638	0.01148	0.00745
2	0.02206	0.01443	0.01479	0.01179	0.01853	0.02066	0.01081	0.01833	0.01533	0.01359	0.01286	0.01461	0.02441	0.01848	0.01125
3	0.02596	0.01486	0.01436	0.01546	0.02319	0.02376	0.01771	0.02029	0.02086	0.01476	0.01973	0.01618	0.03715	0.02768	0.01721
4	0.03269	0.01449	0.01426	0.02029	0.02709	0.02551	0.02326	0.02356	0.02379	0.01666	0.02649	0.02098	0.04895	0.03171	0.02735
5	0.03979	0.01419	0.01413	0.02629	0.03496	0.02916	0.02711	0.02986	0.02799	0.01826	0.02803	0.02671	0.05891	0.03425	0.04311
6	0.04653	0.01393	0.01406	0.03323	0.04453	0.03366	0.03421	0.03779	0.03346	0.02136	0.03056	0.03355	0.06691	0.04158	0.05438
7	0.05416	0.01359	0.01399	0.04069	0.05349	0.04006	0.04401	0.04459	0.03953	0.02349	0.03399	0.03808	0.07255	0.04945	0.06281
8	0.06083	0.01329	0.01363	0.04973	0.06129	0.04756	0.05406	0.05389	0.04616	0.02679	0.03786	0.04471	0.07648	0.05555	0.07088
9	0.06783	0.01296	0.01333	0.06576	0.06823	0.05256	0.06421	0.06703	0.05413	0.03089	0.04269	0.05341	0.08291	0.06688	0.07848
10	0.07466	0.01276	0.01329	0.07746	0.07466	0.06931	0.07516	0.07999	0.06186	0.03693	0.04783	0.06115	0.08898	0.07688	0.08498
11	0.08176	0.01256	0.01313	0.08609	0.07979	0.07741	0.08346	0.08793	0.06949	0.04456	0.05403	0.07301	0.09225	0.09025	0.08978
12	0.08846	0.01226	0.01303	0.09479	0.08516	0.08586	0.09011	0.09546	0.07693	0.05696	0.06083	0.08091	0.09635	0.09955	0.09475
13	0.09563	0.01219	0.01286	0.10463	0.08973	0.09446	0.09631	0.10249	0.08453	0.07756	0.06869	0.09178	0.10045	0.11001	0.09898
14	0.10296	0.01203	0.01263	0.11426	0.09549	0.10181	0.10506	0.11036	0.09086	0.08659	0.07536	0.09701	0.10435	0.11938	0.10291
15	0.11056	0.01189	0.01249	0.12513	0.10036	0.10921	0.10866	0.11809	0.09573	0.09383	0.08079	0.10385	0.11138	0.13028	0.10595
16	0.11843	0.01186	0.01246	0.14623	0.10653	0.11691	0.11371	0.13146	0.10146	0.10146	0.08646	0.11811	0.11665	0.14668	0.10928
17	0.12503	0.01169	0.01229	0.18089	0.11383	0.12726	0.11931	0.15506	0.10689	0.10939	0.09289	0.13878	0.12338	0.17348	0.11261
18	0.13306	0.01156	0.01223	0.21343	0.12366	0.14196	0.12736	0.18873	0.11309	0.11966	0.09919	0.16695	0.13098	0.20291	0.11738
19	0.13933	0.01153	0.01213	0.23439	0.13006	0.16651	0.13631	0.22149	0.11893	0.13273	0.10583	0.20298	0.13885	0.22918	0.12295
20	0.14619	0.01139	0.01206	0.24639	0.13916	0.20081	0.15011	0.24809	0.12456	0.15223	0.11169	0.23801	0.14641	0.24831	0.13038
21	0.15293	0.01133	0.01199	0.25656	0.14863	0.22846	0.16716	0.26743	0.13073	0.17826	0.11706	0.26305	0.15481	0.26078	0.13965
22	0.16039	0.01119	0.01176	0.26529	0.16013	0.24691	0.18256	0.28323	0.13633	0.20309	0.12329	0.28278	0.16078	0.27158	0.14665
23	0.16743	0.01113	0.01173	0.27066	0.17203	0.25941	0.20121	0.29149	0.14283	0.21859	0.12879	0.29488	0.16675	0.27838	0.15431
24	0.17436	0.01109	0.01166	0.27566	0.18453	0.26726	0.21501	0.29903	0.15009	0.22986	0.13423	0.30271	0.18011	0.28561	0.16441
25	0.18123	0.01109	0.01163	0.28129	0.19423	0.27406	0.22871	0.30749	0.15646	0.23693	0.13913	0.31088	0.19115	0.29128	0.17458

Standard deviations

Hour	7636	wkE9	wkE11	wkE5	wkE3	wkE17*	wkE15*	wkE21	wkE23	wkE27	wkE29	wkE33	wkE34	wkE35	wkE36
0	0.00069	0.00082	0.00005	0.00060	0.00022	0.00075	0.00050	0.00076	0.00057	0.00022	0.00012	0.00255	0.00233	0.00141	0.00025
1	0.00093	0.00127	0.00026	0.00031	0.00037	0.00110	0.00085	0.00087	0.00091	0.00043	0.00024	0.00268	0.00265	0.00190	0.00046
2	0.00114	0.00167	0.00050	0.00038	0.00061	0.00060	0.00135	0.00127	0.00152	0.00019	0.00045	0.00258	0.00345	0.00314	0.00081
3	0.00164	0.00136	0.00057	0.00045	0.00049	0.00070	0.00185	0.00103	0.00151	0.00022	0.00069	0.00255	0.00379	0.00369	0.00128
4	0.00250	0.00126	0.00036	0.00068	0.00121	0.00115	0.00250	0.00135	0.00193	0.00022	0.00074	0.00286	0.00475	0.00331	0.00189
5	0.00215	0.00126	0.00054	0.00131	0.00294	0.00070	0.00245	0.00184	0.00285	0.00029	0.00070	0.00298	0.00678	0.00375	0.00282
6	0.00209	0.00108	0.00043	0.00153	0.00419	0.00090	0.00205	0.00246	0.00402	0.00059	0.00094	0.00284	0.00777	0.00636	0.00332
7	0.00259	0.00111	0.00040	0.00019	0.00464	0.00120	0.00075	0.00100	0.00586	0.00040	0.00109	0.00294	0.00891	0.00735	0.00696
8	0.00287	0.00111	0.00045	0.00183	0.00421	0.00100	0.00110	0.00275	0.00716	0.00049	0.00128	0.00335	0.00887	0.00512	0.01048
9	0.00295	0.00099	0.00033	0.00045	0.00372	0.00200	0.00345	0.00106	0.00822	0.00045	0.00148	0.00360	0.00962	0.00590	0.01416
10	0.00287	0.00099	0.00042	0.00202	0.00291	0.00275	0.00600	0.00182	0.00866	0.00103	0.00186	0.00433	0.01025	0.00548	0.01660
11	0.00276	0.00085	0.00029	0.00249	0.00223	0.00425	0.00740	0.00140	0.00876	0.00120	0.00224	0.00475	0.00951	0.00351	0.01692
12	0.00263	0.00085	0.00025	0.00366	0.00143	0.00400	0.00705	0.00144	0.00792	0.00057	0.00257	0.00594	0.00886	0.00513	0.01607
13	0.00225	0.00083	0.00029	0.00540	0.00110	0.00510	0.00645	0.00156	0.00777	0.00188	0.00252	0.00661	0.00796	0.00680	0.01588
14	0.00170	0.00087	0.00033	0.00711	0.00171	0.00625	0.00500	0.00075	0.00742	0.00304	0.00304	0.00791	0.00695	0.00774	0.01434
15	0.00227	0.00090	0.00037	0.00921	0.00159	0.00785	0.00410	0.00074	0.00675	0.00363	0.00192	0.00992	0.00727	0.00906	0.01377
16	0.00179	0.00085	0.00033	0.01270	0.00208	0.00865	0.00405	0.00016	0.00685	0.00448	0.00279	0.01147	0.00689	0.00829	0.01298
17	0.00195	0.00083	0.00029	0.01656	0.00275	0.01110	0.00475	0.00176	0.00672	0.00550	0.00288	0.01403	0.00652	0.00812	0.01257
18	0.00156	0.00085	0.00033	0.01795	0.00503	0.01250	0.00520	0.00335	0.00660	0.00719	0.00288	0.01656	0.00644	0.00656	0.01303
19	0.00116	0.00080	0.00033	0.01832	0.00553	0.01315	0.00595	0.00454	0.00706	0.00852	0.00278	0.01645	0.00763	0.00687	0.01350
20	0.00083	0.00083	0.00033	0.01707	0.00771	0.01245	0.00415	0.00336	0.00718	0.01072	0.00309	0.01470	0.00890	0.00785	0.01570
21	0.00068	0.00080	0.00034	0.01622	0.00891	0.01120	0.00930	0.00090	0.00716	0.01136	0.00269	0.01292	0.00924	0.00978	0.01972
22	0.00137	0.00075	0.00033	0.01622	0.01144	0.01025	0.01480	0.00392	0.00741	0.01090	0.00217	0.01238	0.00899	0.01001	0.02102
23	0.00163	0.00080	0.00017	0.01609	0.01326	0.01075	0.01765	0.00148	0.00815	0.01211	0.00259	0.01353	0.01075	0.01048	0.02220
24	0.00203	0.00075	0.00022	0.01474	0.01594	0.01030	0.01365	0.00252	0.00894	0.01254	0.00250	0.01383	0.01508	0.01184	0.02619
25	0.00231	0.00075	0.00012	0.01633	0.01849	0.01000	0.01195	0.00423	0.00958	0.01263	0.00224	0.01400	0.01698	0.01109	0.02944

* n = 3 for each strain, except wkE17 and wkE15, where n = 2.