Table S1. Candidate genes which when mutated conferred wild type swarming motility[1]

Gene	PS[2]	Class	Annotation[3]	Phenotype	Strain
ccpA	1	regulation	transcription factor	WT	DK8468
defB	1	translation	met/cys deformylase	WT	DK8617
dltA	1	envelope	teichoic acid biosynthesis	WT	DK8532
dltB	1	envelope	teichoic acid biosynthesis	WT	DK7865
dltC	1	envelope	teichoic acid biosynthesis	WT	DK8533
dltD	1	envelope	teichoic acid biosynthesis	WT	DK8461
ggaB	1	envelope	teichoic acid biosynthesis	WT	DK8422
gidA	1	translation	tRNA modification	WT	DS5804
glpD	1	metabolism	glycerol-3-P DH	WT	DK8466
immR	1	regulation	transcription factor	WT	DK8464
lgt	1	envelope	lipoprotein diacyltransferase	WT	DK8465
secG	1	secretion	preprotein translocase	WT	DK9260
ybaE	1	unknown	putative transporter	WT	DK8415
ybxA	1	unknown	putative transporter	WT	DK8414
yneQ	1	unknown	unknown	WT	DK8418
yvrH	1	regulation	TCS sensor kinase	WT	DK8420
ywzH	1	unknown	unknown	WT	DK8531
cdaS	2	regulation	c-di-AMP synthease	WT	DK8621
dnaK	2	translation	protein folding chaperone	WT	DS5801
galE	2	envelope	UDP-glucose isomerase	WT	DS2882
pgi	2	envelope	glucose 6-P isomerase	WT	DK8647
tagE	2	envelope	teichoic acid biosynthesis	WT	DK9164
yerH	2	unknown	unknown	WT	DK8616
yisU	2	unknown	unknown	WT	DK9255
ylbC	2	unknown	unknown	WT	DK9256
yodI	2	unknown	unknown	WT	DK8622
yrzL	2	envelope	proteolytic chaperone MurAA	WT	DK8257
ytxM	2	unknown	unknown	WT	DK8625
yvrG	2	regulation	TCS response regulator	WT	DK8529
abrB	3	regulation	transcription factor	WT	DS5342
bkdAB	3	metabolism	oxoisovalerate DH	WT	DK4654
bkdB	3	metabolism	oxoisovalerate DH	WT	DK8623
cshA	3	regulation	RNA helicase	WT	DK7353
cshB	3	translation	RNA helicase	WT	DK7307
cysK	3	metabolism	cysteine synthase	WT	DK9254
dnaJ	3	translation	activator of DnaK	WT	DS5762
lepA	3	translation	elongation factor	WT	DS2164
nusG	3	translation	transcription anti-termination	WT	DK5430
rnmV	3	translation	5S RNA maturation	WT	DK7864
sigX	3	regulation	sigma factor X	WT	DK8574
ugtP	3	metabolism	glucosyltransferase	WT	DS3141

yabA	3	replication	replication initiation	WT	DS2265
yjbl	3	unknown	unknown	WT	DK8569
ykaA	3	unknown	unknown	WT	DK7306
ylbN	3	unknown	unknown	WT	DK8618
ymdB	3	unknown	phosphodiesterase	WT	DS8546
ynzC	3	unknown	unknown	WT	DK9258
ypmA	3	unknown	unknown	WT	DK7862
ypmB	3	unknown	unknown	WT	DK7712
yqeY	3	unknown	unknown	WT	DK7621
ytpQ	3	unknown	unknown	WT	DK8624

[1]Table is sorted first by priority score and then alphabetically by gene name.

[2]Each gene was assigned a priority score (PS) based on data analysis. A score of "3" indicates that the gene passed the criteria of a ratio of 0.1 or lower and a statistical P value of <0.05 and could not be eliminated by manual evaluation of the data. A score of "2" indicates that the gene did not pass the ratio cutoff but was rescued because it appeared to have an insertion differential by visual scanning of the data set. A score of "1" indicates that the gene passed the quantitative criteria and also showed strong insertion differential by visual scanning of the data set.

[3]Annotation is a brief description of function with regards to why the gene has been shown to be required for swarming motility.

Table S2: Strains

Strain	Genotype
DK654	*dacA::kan*
DK1042	*wild type*
DK4654	*bkdAB::Tn10 spec*
DK4825	*yeaD::TnYLB kan*
DK4956	*ytlQ::kan*
DK5430	*nusG::spec (allele gift from Paul Babitzke, Penn State University)*
DK7305	*yvpB::spec*
DK7306	*ykaA::spec*
DK7307	*cshB::spec*
DK7353	*cshA::spec*
DK7621	*yqeY::TnLacJump kan*
DK7712	*ypmB::spec*
DK7713	*whiA::spec*
DK7763	*rnhC::erm*
DK7855	*gtaB::cat*
DK7862	*ypmA::spec*
DK7864	*rnmV::spec*
DK7865	*dltB::spec*
DK8056	*sweC::erm (allele gift from David Rudner, Harvard Medical School)*
DK8058	*sweD::kan (allele gift from David Rudner, Harvard Medical School)*
DK8257	*yrzL::kan (BGSC allele)*
DK8414	*ybxA::kan (BGSC allele)*
DK8415	*ybaE::kan (BGSC allele)*
DK8417	*yebA::kan (BGSC allele)*
DK8418	*yneQ::kan (BGSC allele)*
DK8419	*yolB::kan (BGSC allele)*
DK8420	*yvrH::kan (BGSC allele)*
DK8421	*rnr::kan (BGSC allele)*
DK8422	*ggaB::kan (BGSC allele)*
DK8424	*walH::kan (BGSC allele)*
DK8460	*prmC::kan (BGSC allele)*
DK8461	*dltD::kan (BGSC allele)*
DK8464	*immR::kan (BGSC allele)*
DK8465	*lgt::kan (BGSC allele)*
DK8466	*glpD::kan (BGSC allele)*
DK8468	*ccpA::kan (BGSC allele)*
DK8486	*yolB::kan amyE::P$_{hag}$-hag^{T209C} spec*
DK8525	*yeaC::kan (BGSC allele)*
DK8529	*yvrG::kan (BGSC allele)*
DK8531	*ywzH::kan (BGSC allele)*
DK8532	*dltA::kan (BGSC allele)*
DK8533	*dltC::kan (BGSC allele)*
DK8569	*yjbI::kan (BGSC allele)*
DK8570	*yjcN::kan (BGSC allele)*
DK8572	*yopQ::kan (BGSC allele)*
DK8574	*sigX::kan (BGSC allele)*
DK8614	*ybaF::kan (BGSC allele)*
DK8616	*yerH::kan (BGSC allele)*
DK8617	*defB::kan (BGSC allele)*

DK8618	*ylbN::kan (BGSC allele)*
DK8619	*glnA::kan (BGSC allele)*
DK8621	*cdaS::kan aka yojJ (BGSC allele)*
DK8622	*yodI::kan (BGSC allele)*
DK8623	*bkdB::kan (BGSC allele)*
DK8624	*ytpQ::kan (BGSC allele)*
DK8625	*ytxM::kan (BGSC allele)*
DK8626	*yvcJ::kan (BGSC allele)*
DK8647	*pgi::kan (BGSC allele)*
DK9164	*tagE::kan (BGSC allele)*
DK9233	*rsiX::TnYLB kan*
DK9234	*lpdV::kan (BGSC allele)*
DK9253	*yaaR::kan (BGSC allele)*
DK9254	*cysK::kan (BGSC allele)*
DK9255	*yisU::kan (BGSC allele)*
DK9256	*ylbC::kan (BGSC allele)*
DK9257	*ylbM::kan (BGSC allele)*
DK9258	*ynzC::kan (BGSC allele)*
DK9259	*greA::kan (BGSC allele)*
DK9260	*secG::kan (BGSC allele)*
DK9265	*fliT::TnYLB kan*
DK9348	*fliT::TnYLB kan amyE::P$_{hag}$-hag^{T209C} spec*
DK9658	*dltD::kan* (selected and maintained with high Mg^{2+})
DK9659	*ggaB::kan* (selected and maintained with high Mg^{2+})
DK9660	*tagE::kan* (selected and maintained with high Mg^{2+})
DK9661	*pgcA::kan* (selected and maintained with high Mg^{2+})
DK9663	*ugtP::spec* (selected and maintained with high Mg^{2+})
DK9664	*gtaB::spec* (selected and maintained with high Mg^{2+})
DS677	*ymcA::erm*
DS678	*ylbF::erm*
DS1138	*secDF::Tn10 spec*
DS1240	*ftsE::Tn10 spec*
DS1242	*ftsX::Tn10 spec*
DS1342	*ksgA::Tn10 spec amyE::P$_{epsA}$-lacZ cat*
DS1916	*amyE::P$_{hag}$-hag^{T209C} spec*
DS2164	*lepA::kan*
DS2265	*yabA::cat*
DS2802	*pgcA::Tn10 spec*
DS2882	*galE::Tn10 spec*
DS3141	*ugtP::spec*
DS5334	*mecA::mls*
DS5342	*abrB::cat*
DS5762	*dnaJ::TnYLB kan*
DS5801	*dnaK::TnYLB kan*
DS5804	*gidA::TnYLB kan*
DS6674	*yaaT::Tn10 spec*
DS8546	*ymdB::TnKRM kan*
DS8875	*yjbH::TnKRM kan*

Table S3: Primers

Primer	Genotype
3250	ACGACTCACTATAGGGCGAATTG
3251	CTCACTAAAGGGAACAAAAGCTGG
5418	CTGTTGCGTTTGATTTTGCATA
5419	CTTTTGTTCCCTTTAGTGAGTAAACAAAAAGGCTATTGGACAT
5420	TTCGCCCTATAGTGAGTCGTTTAAAAGGCACCTTCCTTATGA
5421	GCTTATGAGCCGCTTCCCG
5769	TTGCTTTATATCCGCGTGCT
5770	CAATTCGCCCTATAGTGAGTCGTGAGTGGCTGGAGTAAAAGAG
5771	CCAGCTTTTGTTCCCTTTAGTGAGGATTTGTGCCATGTTTGTTTCT
5772	TCTCTCCTCCAAACACGTTTA
6853	AGAATGTCCCACAGCGCCT
6854	TTCGCCCTATAGTGAGTCGTTGTTTTCATCTTTATCTCCT
6855	CTTTTGTTCCCTTTAGTGAGATTCAAAAGTAAACACCTGAAGC
6856	GGCGGAAATCCGATGAGGA
6861	CGCATAATGATTATCAGCTTCAG
6862	TTCGCCCTATAGTGAGTCGTATTTACCACTTATAAAACTGC
6863	CTTTTGTTCCCTTTAGTGAGAAATCTTACTAATTTGATCGATTC
6864	TCACAAGCTTAAAGATTTGCTG
6865	AAGCCGTAATCAAACGGCTC
6866	TTCGCCCTATAGTGAGTCGTTTCTTTCATTTTTCCCAGCTCC
6867	CTTTTGTTCCCTTTAGTGAGAAAAGAAAGTAGGGGAACCTG
6868	ATTACATCGAAAGAAACAGCTC
6876	TTTAGCTACGTTGTTTGCTTATG
6877	TTCGCCCTATAGTGAGTCGTTCTTATCATTAAAAACCCTCCAT
6878	CTTTTGTTCCCTTTAGTGAGAAAAACGACTAACGAGGGGT
6879	AAATAAAGTAGGCGATGGCAC
7142	GAGCAGCTTACTGGAATCTCTAATC
7143	CAATTCGCCCTATAGTGAGTCGTCTTGCCGAGACAAGAAGTACTGC
7144	CCAGCTTTTGTTCCCTTTAGTGAGGTTATGTGGACTTTACAACTGGAAAG
7145	CGCATTCTTCAATAAACAGAAAGCC
7146	CATGAGGAAAACGGCAAAGTCGG
7147	CAATTCGCCCTATAGTGAGTCGTCCTTCACTTCCAGATTCGTTAATTC
7148	CCAGCTTTTGTTCCCTTTAGTGAGCTTGACGAAATCGCAGAACAGC
7149	CAAGCGCGTCTCATGTCCCTG
7167	CATTTCCGTACAAAACGTGTTACAC
7168	CAATTCGCCCTATAGTGAGTCGTCAGATCATCAGAATGCTCTACATTAATTG
7169	CCAGCTTTTGTTCCCTTTAGTGAGCCAAGCCGTTTTTACCATTTATCGTAC
7170	GCGTCAGAATCCAGTCCATGTTC
7175	CACTGCCTGAACCGGCTTAGGC
7176	CAATTCGCCCTATAGTGAGTCGTGATCGTCGGCAGAAGAAGAATGC
7177	CCAGCTTTTGTTCCCTTTAGTGAGCAGGAAAACCATTTCATCACCACC
7178	CGGACAAATTCACTTTCAACAGCG
7179	CGAGCCATTGTTCCCTGCTTTAG
7180	CAATTCGCCCTATAGTGAGTCGTGTGTCTGCATCAACAGCCAATTTGATG
7181	CCAGCTTTTGTTCCCTTTAGTGAGGAGTGCGCTCGATACCGTTATGC
7182	CATTTCGACTTCATGATAGCCATCC
7238	CACATATGTTTCCATCATACTTG
7239	TTCGCCCTATAGTGAGTCGTCACTGAATGGGACACGTAATA
7240	CTTTTGTTCCCTTTAGTGAGGATAAAAAACGTTCATAGAAAAAAG
7241	GGAACTACAGGCGGATTAACG

Table S4. TnSeq samples used in this study.

Sample name	Figure	Reference	Identifier
Sanchez_Kearns_TnSeq_WT	1, 2, S1	this study	SAMN27609185
Sanchez_Kearns_TnSeq_swarm_rep1	1, 2, S1	this study	SAMN27609186
Sanchez_Kearns_TnSeq_swarm_rep2	S1	this study	SAMN27609187
Sanchez_Kearns_TnSeq_swarm_rep3	S1	this study	SAMN27609188
Sanchez_Kearns_TnSeq_swarm_rep4	S1	this study	SAMN27609189
Sanchez_Kearns_TnSeq_swarm_rep5	S1	this study	SAMN27609190

Table S5. Excel datasheet containing raw TnSeq data and priority score assignments.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1: TnSeq priority classification of candidate genes defective in swarming motility. Sample genes with transposon insertion density graph generated using Artemis software. Transposon insertions oriented such that the spectinomycin resistance cassette is co-oriented with the inserted gene colored red, while insertions that counter-orient the resistance gene are colored blue. Y-axis indicates the number of insertions indicates the number of sequencing reads at that insertion position. Gene length and orientation indicated as a gray arrow. Top dataset is from the control library, and next 5 data sets are from the five motility-enriched pools respectively to show reproducibility. Raw data annotation is included below to see actual gene location. Black bar indicates 1 kb in X-axis genome distance. Priority 1 represented genes that passed mathematical criteria for differential insertion and the difference was readily visible on manual inspection. Priority 2 were those genes that failed at least one of the mathematical criteria but were rescued by a clear insertion differential by manual inspection. Priority 3 genes were those that passed mathematical criteria but did not appear to have a dramatic insertion differential by manual inspection. Priority 4 genes were those genes that passed mathematical criteria but were discarded for having few, if any, transposon insertions in the control dataset. Priority 5 genes were those genes that passed neither mathematical nor manual inspection criteria and were discarded.

Figure S2: Some mutants exhibit a "terracing" phenotype. Shown are images of Petri plates containing LB fortified with 0.7% agar, centrally inoculated with the indicated strain and incubated overnight at 37°C. Terracing is the result of premature swarm cessation and re-initiation at a later time point. The period of immobility may consume nutrients at the region adjacent to the swarm edge such that upon re-initiation, the swarm density fails to reach a high

of a yield thereby demarking the location of the original stopping point. As a result, the swarm

tends to have a bullseye appearance with a mutant-specific pattern.
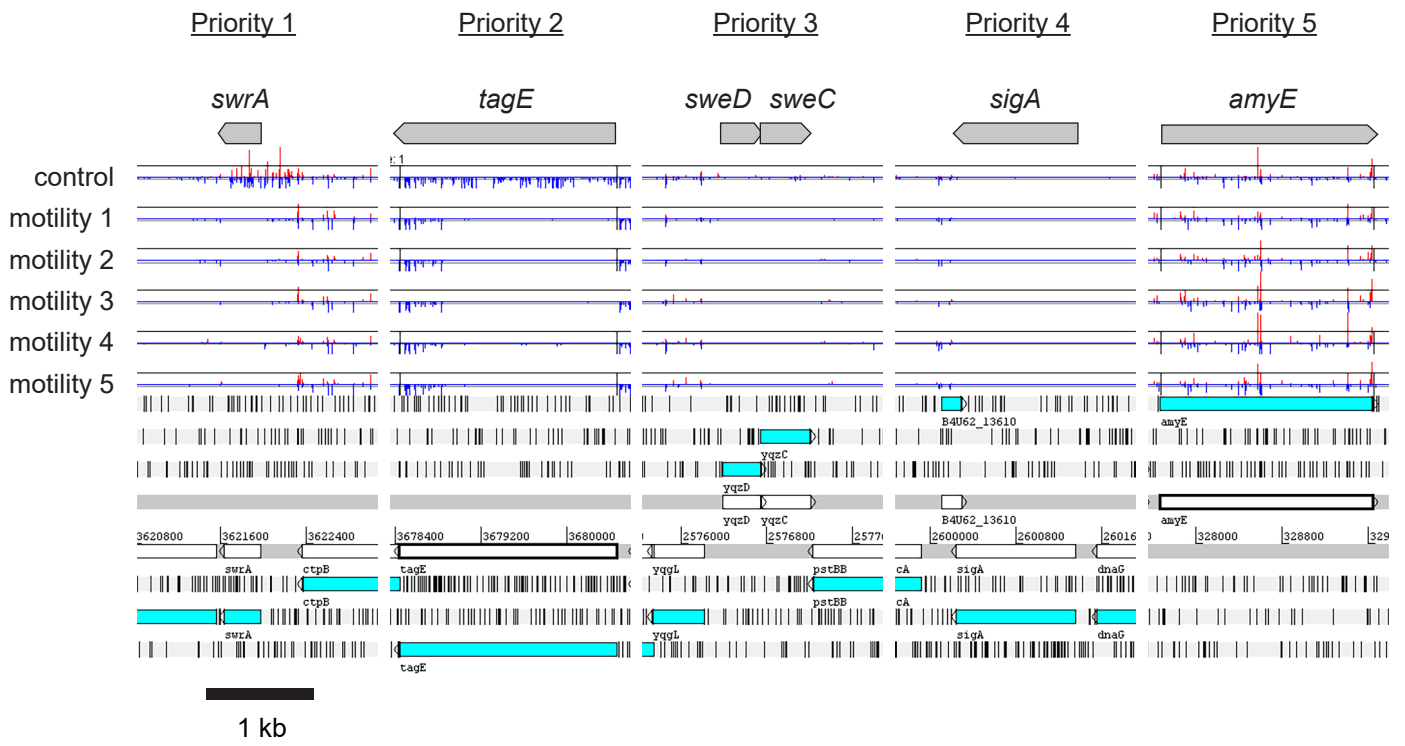
Figure S1

WT   *yvpB*   *yjbH*   *walH*   *ftsX*