



1 Article

2 Advancing Genetic Tools in Streptococcus

3 pneumoniae

- 4 Haley Echlin 1 and Jason Rosch 1,*
- Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, TN, USA;
 jason.rosch@stjude.org
- 7 * Correspondence: jason.rosch@stjude.org; Tel.: +1-901-595-3408
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10 Supplementary Material

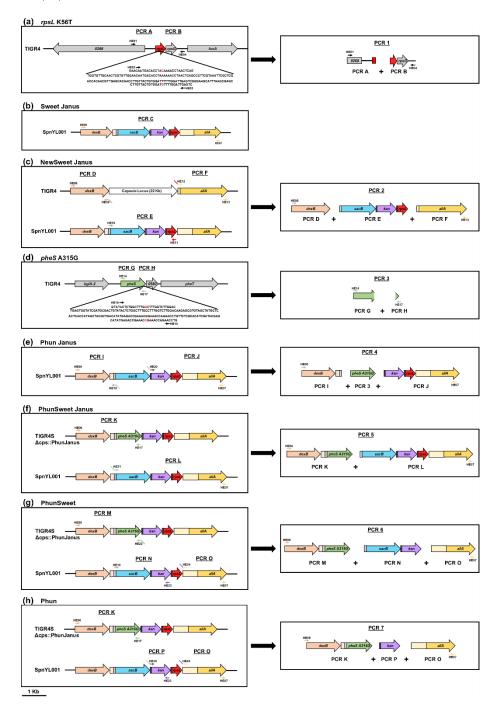


Figure S1. Schematic of PCR primers and template used to generate novel markerless cassettes for allelic replacement of capsule. (a) To confer streptomycin resistance in *S. pneumoniae*, mutation K56T was introduced into *rpsL*. (b) Sweet Janus and (b) NewSweet Janus were amplified from SpnYL001. (d) To confer chlorinated phenylalanine sensitivity, mutation A315G was introduced into *pheS*. (e) Phun Janus was generated from *pheS* A315G and SpnYL001. (f) PhunSweet Janus, (g) PhunSweet, and (f) Phun were created using Phun Janus and Sweet Janus as templates. Cassettes were spliced between the genes flanking the capsule loci, *dexB* (tan) and *aliA* (yellow). The cassettes are comprised of genes that confer sucrose sensitivity (*sacB*; blue), kanamycin resistance (*kan*, purple), streptomycin sensitivity (*rpsL*, red), and chlorinated phenylalanine sensitivity (*pheS* A315G, green). The light gray box represents the promoter from Sweet Janus and the dark gray box represents ribosome-binding site. The light-tan box represents the sequence downstream of *dexB* and the light-yellow box represents the sequence upstream of *aliA* from D39. The direction of the arrow indicates the gene orientation. Primers used in generation of each cassette are depicted.

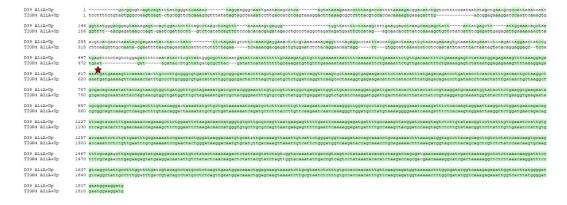


Figure S2. TIGR4 does not share homology with D39 for upstream sequence of *aliA*. Alignment of *aliA* plus upstream sequence from TIGR4 compared to that of D39. Red star indicates start codon of *aliA*.

Table S1. Number and ratio of true-positive to false-positive colonies of allelic replacement of spxB. Strain include TIGR4S Δ spxB::SweetJanus, D39S Δ spxB::SweetJanus,

ABCA31S Δ spxB::SweetJanus, and CDC001S Δ spxB::SweetJanus and were transformed with SOE PCR Δ spxB::Erythromycin. False positives were calculated by subtracting the number of true positive colonies (colonies on plates containing 1 μ g/ml erythromycin) from the number of total transformants (colonies on plates containing 800 μ g/ml streptomycin plus 10% sucrose). If there were more colonies on plates containing erythromycin than on those containing streptomycin plus sucrose, the number of false positive colonies was considered to be zero. SJ refers to SweetJanus.

#T-(-1 #T #T-1 ***					
	-	-	Ratio		
			0.07		
			0.08		
			0.26		
			3.25		
			0.01		
			0.32		
			0.89		
			0.85		
			0.20		
			92.50		
			4.23		
			0.80		
2700	1650		1.57		
470	460	10	46.00		
1043	13	1030	0.01		
1153	950	203	4.68		
95	35	60	0.58		
68	25	43	0.58		
788	533	255	2.09		
8450	9400	0	9400.00		
1280	1875	0	1875.00		
1115	1135	0	1135.00		
75	28	47	0.60		
23	63	0	63.00		
3100	2900	200	14.50		
3300	3400	0	3400.00		
1505	1863	0	1863.00		
	470 1043 1153 95 68 788 8450 1280 1115 75 23 3100 3300	Colonies Colonies 240 15 215 15 120 25 510 390 4100 50 240 58 53 25 290 133 18 3 935 925 68 55 18000 8000 2700 1650 470 460 1043 13 1153 950 95 35 68 25 788 533 8450 9400 1280 1875 1115 1135 75 28 23 63 3100 2900 3300 3400	Colonies Colonies 240 15 225 215 15 200 120 25 95 510 390 120 4100 50 4050 240 58 182 53 25 28 290 133 157 18 3 15 935 925 10 68 55 13 18000 8000 10000 2700 1650 1050 470 460 10 1043 13 1030 1153 950 203 95 35 60 68 25 43 788 533 255 8450 9400 0 1280 1875 0 1115 1135 0 75 28 47 23 63 0 310		

CDC001S∆spxB::SJ, Clone 4	3600	4100	0	4100.00
CDC001S∆spxB::SJ, Clone 5	983	1170	0	1170.00
CDC001S∆spxB::SJ, Clone 6	3950	1550	2400	0.65
CDC001S∆spxB::SJ, Clone 7	1290	1218	72	16.92
CDC001S∆spxB::SJ, Clone 8	2750	3200	0	3200.00