

1 *Article*

2 **Advancing Genetic Tools in *Streptococcus***
3 ***pneumoniae***

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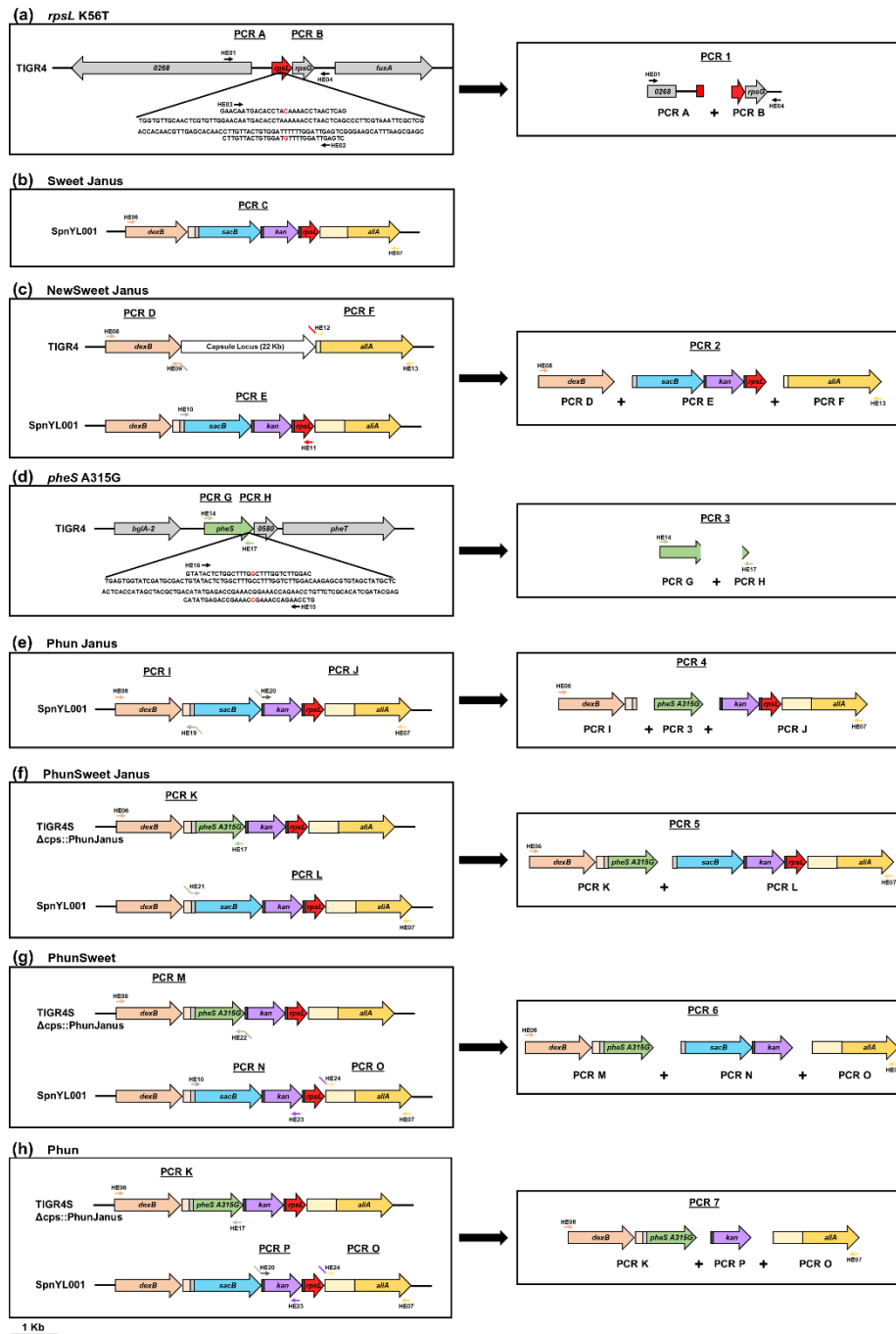
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10 **Supplementary Material**



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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 (h) 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.

CDC001SΔspxB::SJ, Clone 4	3600	4100	0	4100.00
CDC001SΔspxB::SJ, Clone 5	983	1170	0	1170.00
CDC001SΔspxB::SJ, Clone 6	3950	1550	2400	0.65
CDC001SΔspxB::SJ, Clone 7	1290	1218	72	16.92
CDC001SΔspxB::SJ, Clone 8	2750	3200	0	3200.00
