Sequences

Type-1 singlet from *L. monocytogenes* (Lmo)
NC_002973.5/1352518-1352823

Lmo plasmid sequence
GGATCC*TAATACGACTCACTATA*GGCGGGTGAATGTAAGCAGAGAGACTG
CGAAAAGCGGCGCCGACGGGGAAAGCATGTATTATGTGAAACTCTCAGGCA
AAAGGATGTTTACGGGACGCAACTCTGGAGTCATTTTTGTGTTACGACAGG
G**AATTC**

pink – BamHI cut site
blue – T7 promoter
red – G inserted to improve transcription initiation
orange – EcoRI cut site

Lmo transcribed sequence
GGCGGGUGAAUGUAAGCAGAGAGACUGCGAAAAGCGGCGCCGACGGGGA
AAGCAUGUAUUAUGUGAAACUCUCAGGCAAAAGGAUGUUUACGGGACGCA
ACUCUGGAGUCAUUUUUGUGUUACGACAGGG**AAUU**

Type-2 singlet from *D. hafniens* (Dha)
NC_011830.1/1219347-1219484

Dha plasmid sequence
GGATCC*TAATACGACTCACTATA*GGCACTGGATGAGGTTTTCAGGAGAACA
GGGTAAGCTAACCATGATGAACTGAAAACGGACAGAACTCTGGAGAGTTCC
GCAAGGACGCCGAAGGGGCAAGACAGCAAAGCTGTTCAATCTCTCAGGCA
AAAGGACAGAGCG**AATTC**

Dha transcribed sequence
GGCACUGGAUGAGGUUUUCAGGAGAACAGGGUAAGCUAACCAUGAUGAA
CUGAAAACGGACAGAACUCUGGAGAGUUCCGCAAGGACGCCGAAGGGGC
AAGACAGCAAAGCUGUUCAAUCUCUCAGGCAAAAGGACAGAGCG**AAUU**

Lmo mutant transcribed sequences

Lmo mBS (U72A)

GGCGGGUGAAUGUAAGCAGAGAGACUGCGAAAAGCGGCGCCGACGGGGA
AAGCAUGUAUUAUGUGAAACUC**A**CAGGCAAAAGGAUGUUUACGGGACGCA
ACUCUGGAGUCAUUUUUGUGUUACGACAGGGAAUU

Lmo Trn (Δ101-129)

GGCGGGUGAAUGUAAGCAGAGAGACUGCGAAAAGCGGCGCCGACGGGGA
AAGCAUGUAUUAUGUGAAACUCUCAGGCAAAAGGAUGUUUACGGGACGCA
A – GAAUU

Lmo mutβ (A66C A67C A68C)

GGCGGGUGAAUGUAAGCAGAGAGACUGCGAAAAGCGGCGCCGACGGGGA
AAGCAUGUAUUAUGUG**CCC**CUCUCAGGCAAAAGGAUGUUUACGGGACGC
AACUCUGGAGUCAUUUUUGUGUUACGACAGGGAAUU

Lmo TL (Δ106-125 *Ins* GAGA)

GGCGGGUGAAUGUAAGCAGAGAGACUGCGAAAAGCGGCGCCGACGGGGA
AAGCAUGUAUUAUGUGAAACUCUCAGGCAAAAGGAUGUUUACGGGACGCA
ACUCUG **GAGA** CAGGGAAUU

Lmo PL (Δ106-125 *Ins* GAUAA)

GGCGGGUGAAUGUAAGCAGAGAGACUGCGAAAAGCGGCGCCGACGGGGA
AAGCAUGUAUUAUGUGAAACUCUCAGGCAAAAGGAUGUUUACGGGACGCA
ACUCUG **GAUAA** CAGGGAAUU

Dha mutant transcribed sequences

Dha mBS (U123A)

GGCACUGGAUGAGGUUUUCAGGAGAACAGGGUAAGCUAACCAUGAUGAA
CUGAAAACGGACAGAACUCUGGAGAGUUCCGCAAGGACGCCGAAGGGGC
AAGACAGCAAAGCUGUUCAAUCUC**A**CAGGCAAAAGGACAGAGCGAAUU

Dha Trn (Δ18-52 *Ins* UUCG)

GGCACUGGAUGAGGUUU **UUCG** AAACGGACAGAACUCUGGAGAGUUCC
GCAAGGACGCCGAAGGGGCAAGACAGCAAAGCUGUUCAAUCUCUCAGGC
AAAAGGACAGAGCGAAUU

Dha mutα (A117C A118C)

GGCACUGGAUGAGGUUUUCAGGAGAACAGGGUAAGCUAACCAUGAUGAA
CUGAAAACGGACAGAACUCUGGAGAGUUCCGCAAGGACGCCGAAGGGGC
AAGACAGCAAAGCUGUUC**CC**UCUCUCAGGCAAAAGGACAGAGCGAAUU

Dha HL (Δ26-46)

GGCACUGGAUGAGGUUUUCAGGAGA – GAACUGAAAACGGACAGAACUC
UGGAGAGUUCCGCAAGGACGCCGAAGGGGCAAGACAGCAAAGCUGUUCA
AUCUCUCAGGCAAAAGGACAGAGCGAAUU

Original Singlet Alignment (J. Barrick and R. Breaker, unpublished results)

Type-1 Singlets

Sau	Staphylococcus aureus subsp. aureus N315 DNA	NC_002745.2	1577113-1576807
Lmo	Listeria monocytogenes str. 4b F2365	NC_002973.5	1352518-1352823
Sep	Staphylococcus epidermidis RP62A	NC_002976.3	1147162-1146812
Lmo'	Listeria monocytogenes EGD-e chromosome	NC_003210.1	1372690-1372995
Lin	Listeria innocua Clip11262	NC_003212.1	1379476-1379781
Cte	Clostridium tetani E88	NC_004557.1	1925262-1925580
Tfu	Thermobifida fusca YX	NC_007333.1	1619770-1620134
Kra	Kineococcus radiotolerans SRS30216 strain	NZ_AAEF0200	6425-6800

Type-2 Singlets

Bli	Brevibacterium linens BL2 2662183_Cont246	WP_050773381.1	20952-20590
Spn	Streptococcus pneumoniae TIGR4	NP_344931.1	387343-387650
Oih	Oceanobacillus iheyensis HTE831		2942953-2942663
Smu	Streptococcus mutans UA159	NP_721560.1	1115812-1116128
Sth	Streptococcus thermophilus LMG 18311	WP_011225955.1	886124-885797
Dha	Desulfitobacterium hafniense DCB-2 ctg918	WP_011461681.1	5607-5354
Eba	Exiguobacterium sibiricum 255-15 ctg277	ZP_00539478.1	30315-30643
Xfa	Xylella fastidiosa 9a5c	WP_031337913.1	1336905-1337238
Baq	Bartonella quintana str. Toulouse	WP_011179698.1	1196121-1195790

Figure S1. Alignment of 8 type-1 singlets (part 1 of 2)

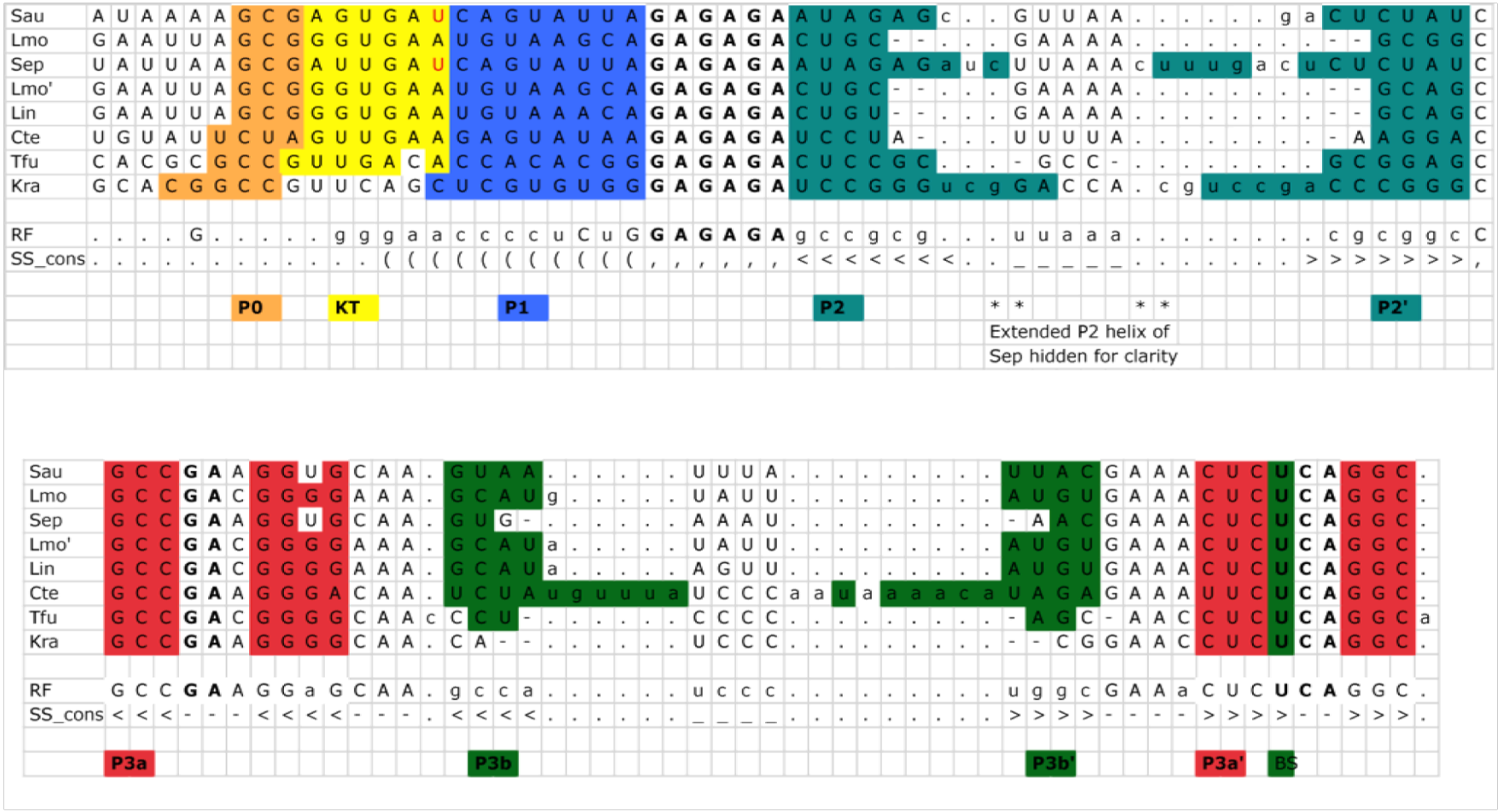


Figure S1. Alignment of 8 type-1 singlets (part 2 of 2)

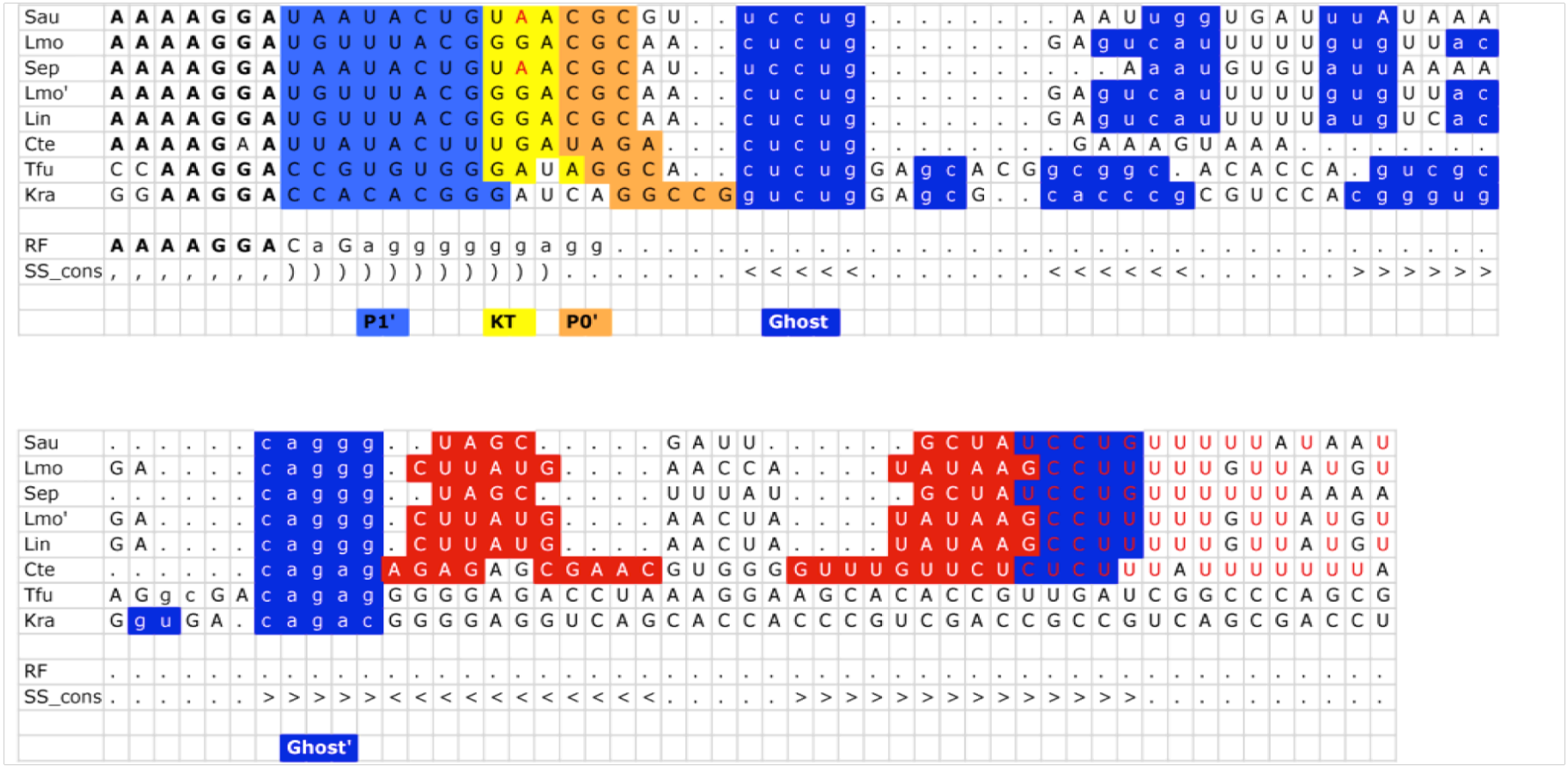


Figure S2. Alignment of 9 type-2 singlets (part 1 of 3)

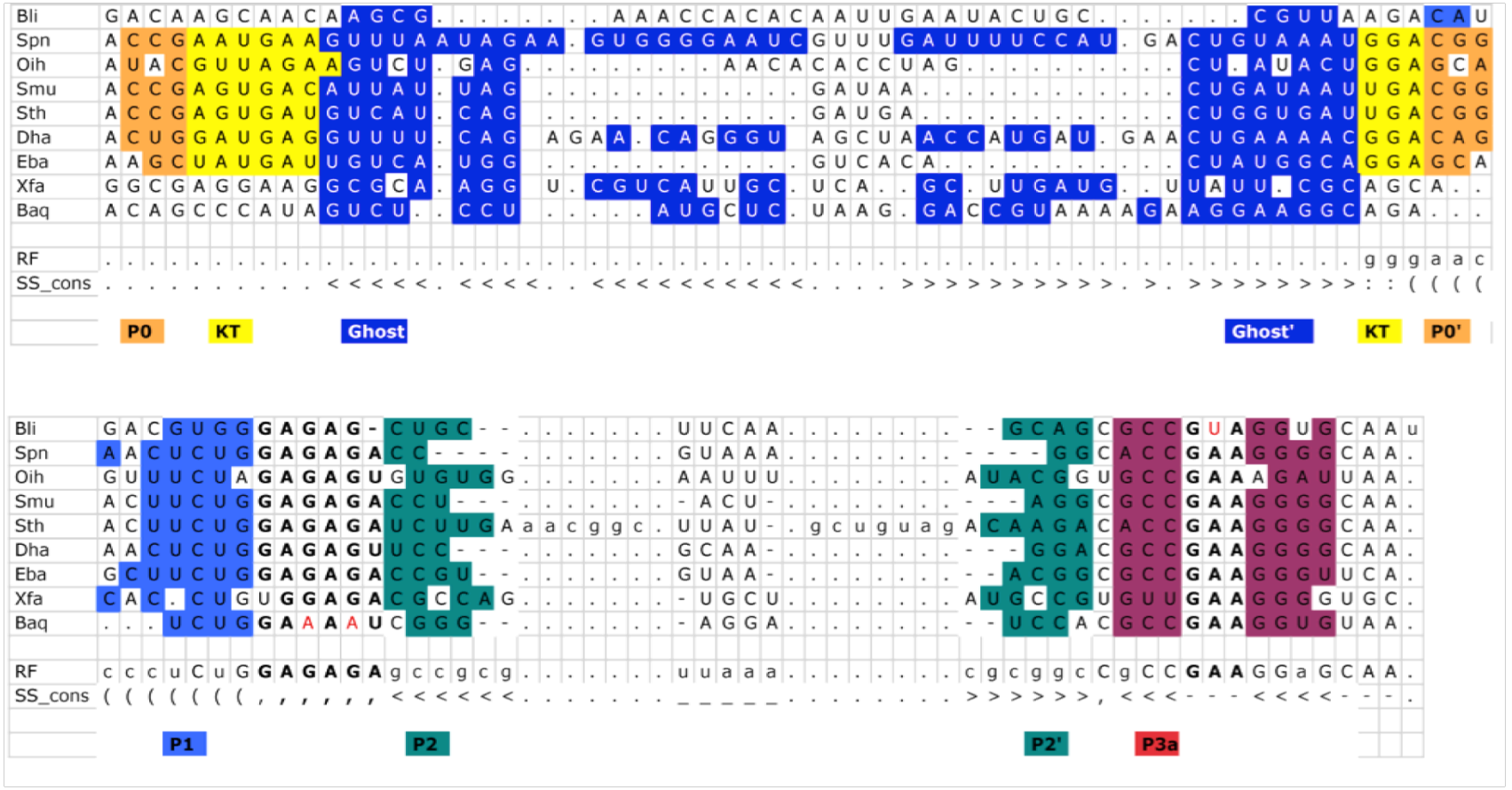


Figure S2. Alignment of 9 type-2 singlets (part 2 of 3)

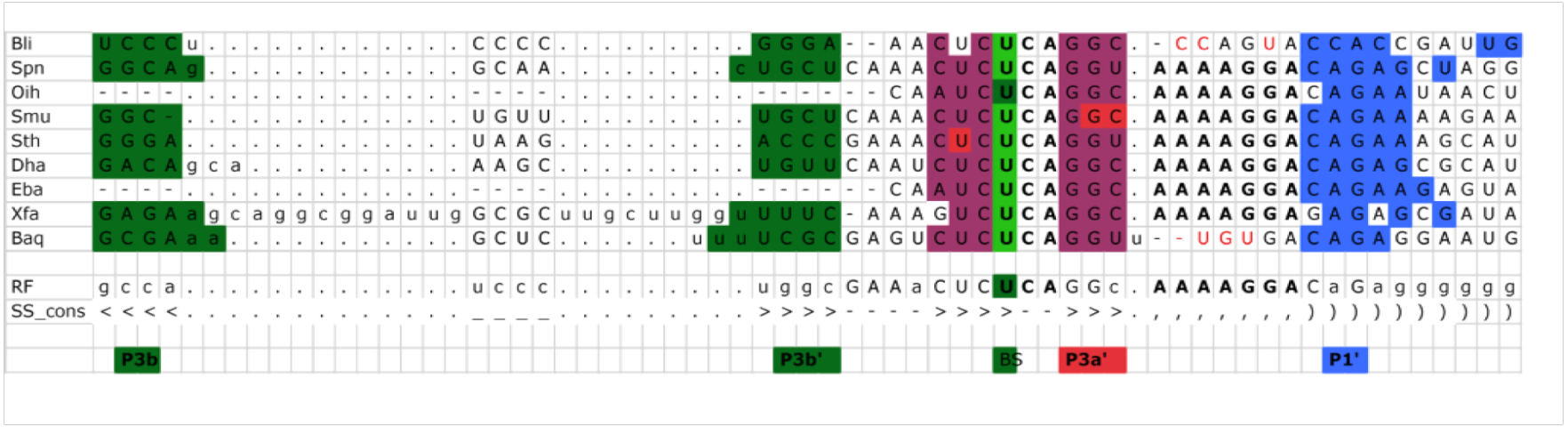
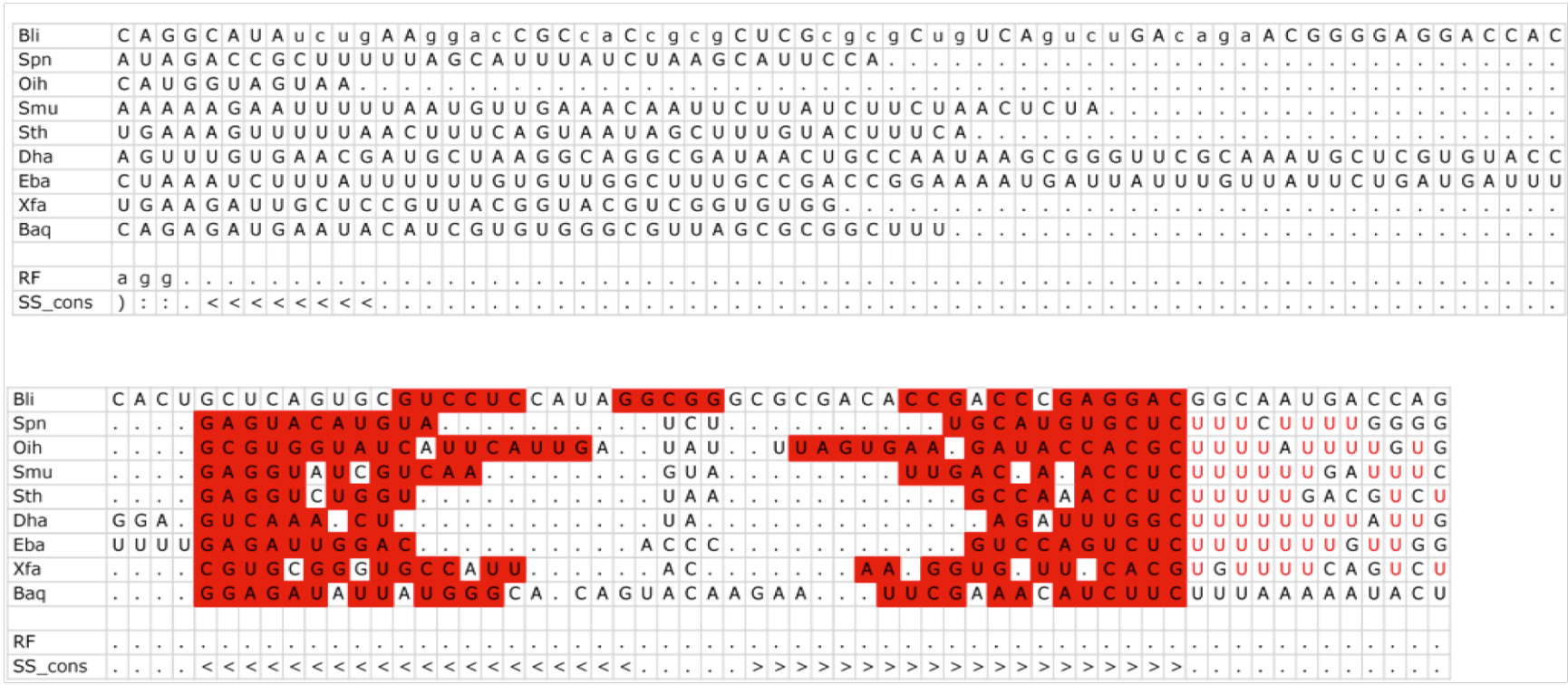


Figure S2. Alignment of 9 type-2 singlets (part 3 of 3)



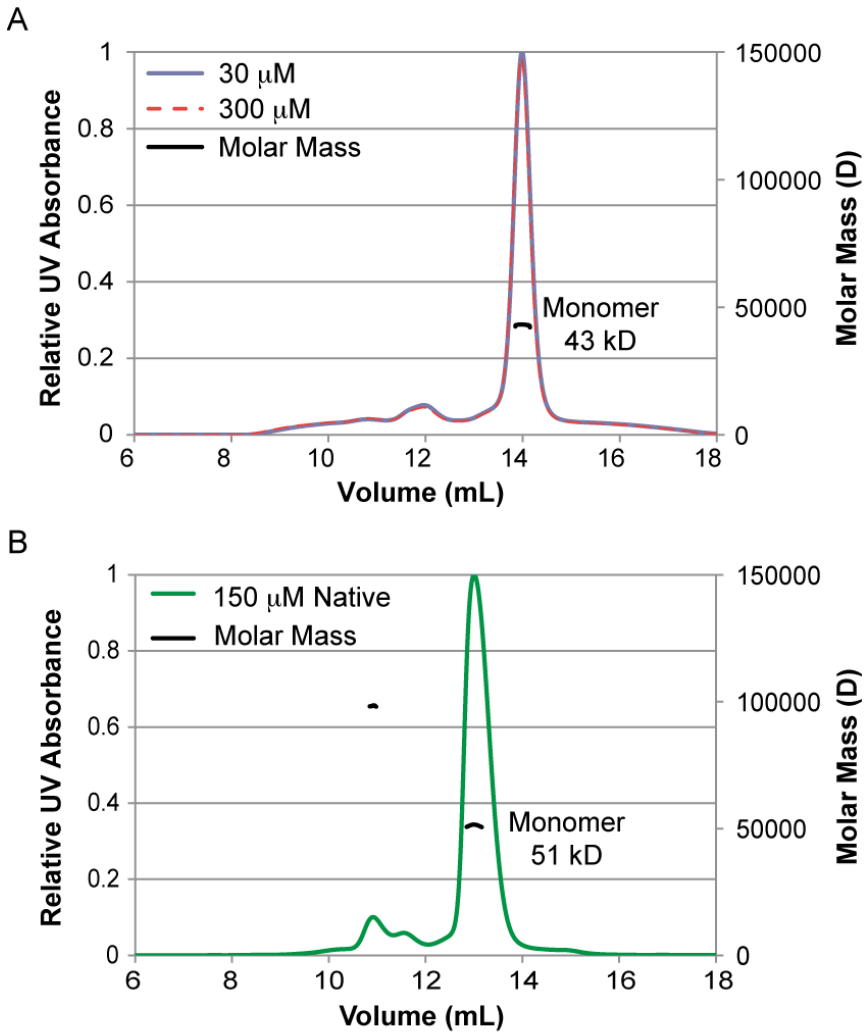


Figure S3: Singlet riboswitches are active as monomers. a) FPLC and MALS show that Lmo type-1 WT is greater than 95% monomer at both 30 and 300 μM. b) Dha type-2 WT is stable and greater than 90% monomer when purified using a native FPLC prep.

FPLC of Dha mutants

Dha WT that is heat-denatured and refolded forms some dimer, particularly at 300 μM RNA, the highest concentration tested (Figure S4). Intriguingly, refolding in the presence of saturating ligand substantially decreases the fraction of the pool in this alternative dimer state (47 ± 1% dimer in the absence of ligand versus 21 ± 1% in 5 mM glycine). Because binding and RNA folding are at equilibrium, this suggests that the monomeric form of Dha WT binds glycine with 4-fold stronger affinity than the dimer (corresponding to a .8 kcal/mol ΔΔG).

In contrast, the Dha trunctation mutant forms much more dimer in the presence of glycine, with the contrast appearing most significantly at lower RNA concentrations (Figure S4). At 30 μM RNA, the Dha truncation mutant forms only 19 ± 2% dimer in the absence of ligand, compared to 59 ± 2% in 5 mM glycine. Because binding and RNA folding are at equilibrium, this indicates that the dimeric form of Dha Trn binds glycine with 9-fold stronger affinity than the monomer (corresponding to a 1.3 kcal/mol ΔΔG).

Because a significant portion of the Dha Trn pool exists as dimer across the RNA concentrations where ligand binding occurs, and because the dimer binds ligand more tightly than monomer, we ascribe the unexpected ligand-binding activity of Dha Trn to a spurious dimer state.

The multimeric states of the other Dha mutants were also analyzed by FPLC (Figure S4). All form some dimer, particularly at the highest RNA concentrations tested, but in no other case did ligand binding promote formation of the dimer. Therefore, we attribute the observed ligand-binding affinities to monomer states of the other Dha mutants.

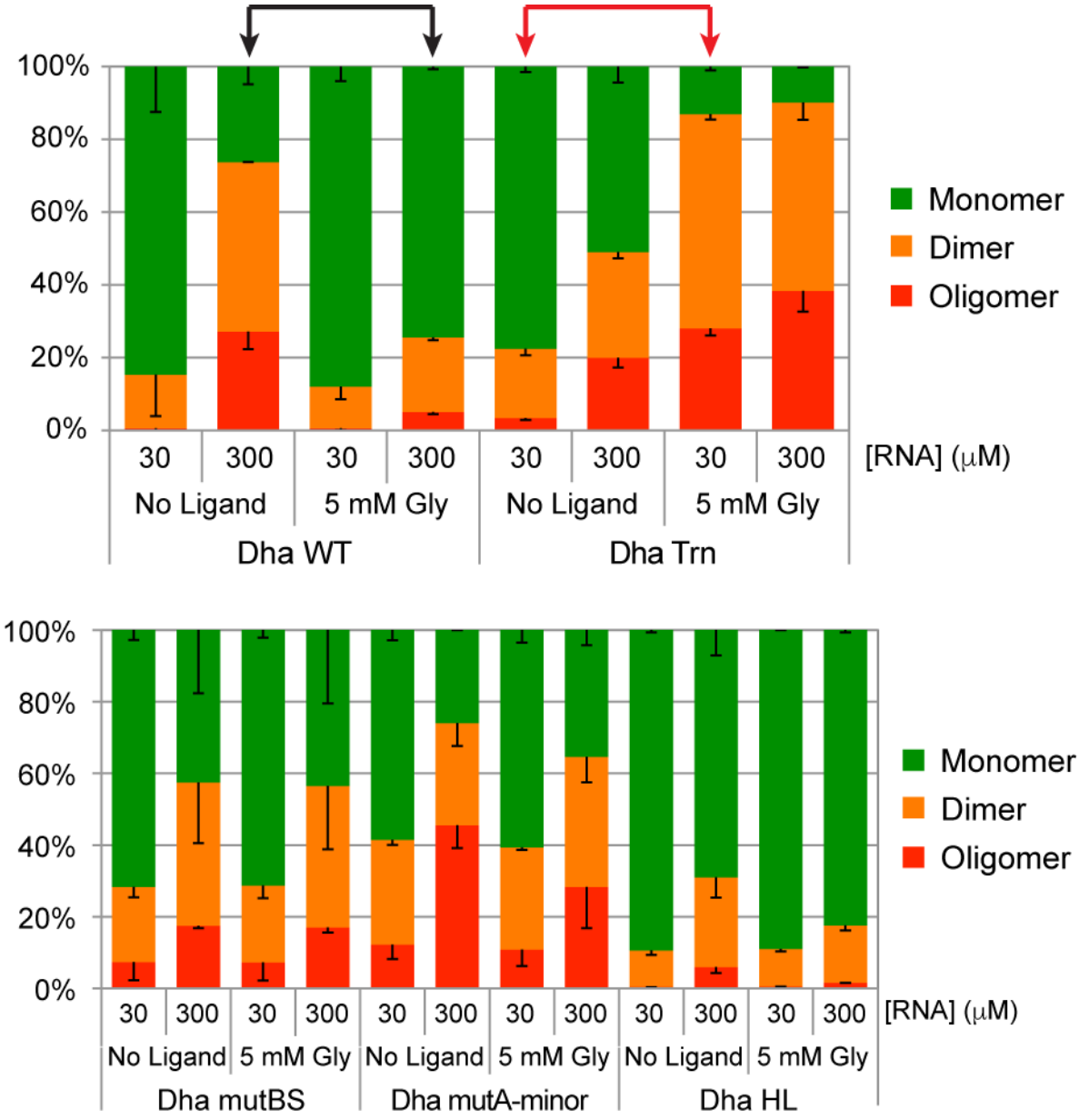


Figure S4: Analysis of multimeric state of Dha WT and mutants by size-exclusion FPLC. Moderate and high concentrations of each construct were refolded in the presence or absence of glycine. Ligand binding stabilizes the monomeric form of Dha WT (black arrows); in contrast, ligand binding stabilizes the dimeric form of Dha Trn (red arrows).

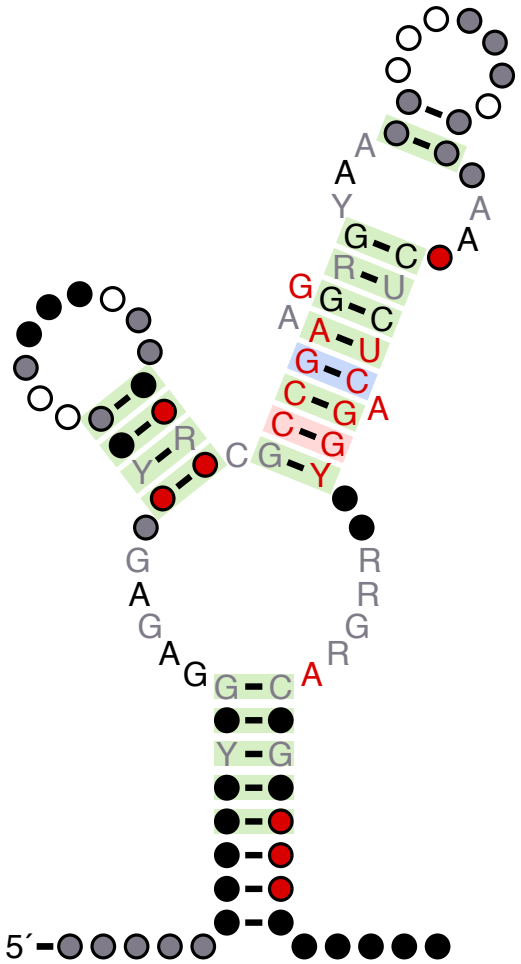


Figure S5: Minimal glycine aptamer consensus sequence used for RefSeq search.