

SUPPLEMENTARY INFORMATION

Elucidation of a Mechanism of Oxidative Stress Regulation in

***Francisella tularensis* Live Vaccine Strain**

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Running title: Regulation of oxidative stress resistance in *Francisella tularensis*

Table S1

		Hydrogen Peroxide (-)		Hydrogen Peroxide (+)	
		Mean	SD	Mean	SD
Stress related protein					
FTL_1015	AhpC/TSA family protein	-0.37	0.104651804	-0.352	0.101823376
FTL_1791	Superoxide dismutase	-0.368	0.424264069	-0.32	0.028284271
FTL_1571	Thioredoxin reductase	0.024	0.158391919	-0.248	0.135764502
FTL_1714	Chaperonin GroEL	-0.044	0.113137085	-0.038	0.308298557
FTL_1715	Co-chaperonin GroES	0.096	0.011313708	-0.344	0.073539105
FTL_1191	Molecular chaperone Dnak	-0.016	0.141421356	0.416	0.214920461
FTL_0094	ClpB protein	0.136	0.022627417	0.344	0.231931024
FTL_1957	Heat shock protein	-0.438	0.608111832	0.53	0.647709812
FTL_0457	Cold Shock Protein	0.01	0.353553391	-0.386	0.110308658
FTL_1392	Cold-shock DEAD-box protein A	-0.382	0.51194531	-0.034	0.195161472
FTL_0166	Universal Stress Protein	0.084	0.220617316	-0.488	0.271529004
FTL_1413	(p)ppGpp synthase/guanosine-3',5'-bis(diphosphate) 3'-pyrophosphohydrolase	-0.015	0.06363961	-0.77	0.834386002
Translation					
FTL_1236	Translation Initiation factor IF-1	0.046	0.330925974	-0.146	0.053740115
FTL_1751	Elongation Factor Tu	-0.32	0.18667619	0.004	0.254558441
FTL_0234	Elongation Factor G	-0.006	0.01979899	0.236	0.045254834
FTL_0225	Elongation Factor Ts	-0.056	0.005656854	0.234	0.1668772
FTL_0768	GTP binding translational elongation factor Tu and G family protein	-0.016	0.197989899	0.518	0.421435642
Transcription					
FTL_1474	Transcription elongation factor GreA	0.056	0.118793939	0.11	0.376180808
FTL_1810	Transcription elongation factor NusA	-0.026	0.098994949	0.308	0.016970563
FTL_0610	Transcription termination factor Rho	-0.016	0.045254834	-0.322	0.042426407
Transport proteins					
FTL_1870	Putative ABC transporter ATP-binding protein	-0.044	0.073539105	-0.13	0.031112698
FTL_1229	ABC transporter ATP-binding protein	0.046	0.110308658	0.314	0.01979899
FTL_1065	ABC transporter ATP-binding protein	-0.014	0.042426407	-0.286	0.070710678
FTL_0679	Polyamine Transporter	-0.016	0.141421356	-0.218	0.036769553
Regulatory proteins					
FTL_0895	Histone-like protein HU form B	0.04	0.277185858	-0.22	0.073539105
FTL_1179	Sigma 54 modulation protein	-0.08	0.152735065	0.182	0.025455844
FTL_0851	RNA polymerase factor sigma-32	0.014	0.036769553	0.082	0.065053824
Metabolism and others					
FTL_0454	Glucosamine-fructose-6-phosphate aminotransferase	0.416	0.305470129	-1.206	0.217788889
FTL_0114	Intracellular growth locus, subunit D	-0.05	0.336582828	0.136	0.056568542
FTL_1801	F0F1 ATP synthase subunit alpha	-0.008	0.050911688	-0.182	0.025455844
FTL_1800	F0F1 ATP Synthase subunit gamma	-0.056	0.169705627	-0.606	0.025455844
FTL_0519	Septum site-determining protein MinD	-0.022	0.251730014	0.19	0.127279221
FTL_1789	Citrate synthase	0.008	0.175362482	-0.134	0.002828427
FTL_1490	Phosphoglyceromutase	0.078	0.065053824	-0.34	0.237587878
FTL_0538	(3R)-hydroxymyristoyl-ACP dehydratase	-0.018	0.132936075	-0.31	0.01979899
FTL_0592	dTDP-glucose 4,6-dehydratase	0.006	0.1668772	-0.218	0.008485281
FTL_1166	Hypothetical protein	0.05	0.093338095	0.45	0.461033621
FTL_1164	Hypothetical protein	0.08	0.118793939	0.276	0.14707821
FTL_0984	bifunctional guaredoxin/ribonucleoside-diphosphate reductase subunit beta	0.038	0.172534055	0.126	0.031112698
FTL_1118	Hypothetical protein	-0.046	0.093338095	-0.154	0.031112698
FTL_0423	Hypothetical protein	-0.01	0.1668772	-0.254	0.24607316
FTL_0582	Hypothetical protein	0.016	0.152735065	0.332	0.028284271
FTL_0537	UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase	0.08	0.056568542	0.338	0.132936075
FTL_0449	Hypothetical protein	-0.052	0.096166522	-0.394	0.087681241
FTL_0097	Hypothetical protein	0.056	0.169705627	0.182	0.014142136
FTL_0596	UDP-glucose/GDP-mannose dehydrogenase	-0.054	0.076367532	0.24	0.079195959
FTL_1546	Pyridoxal biosynthesis lyase PdxS	0.056	0.050911688	-0.162	0.053740115
FTL_1139	3-oxoacyl-(acyl-carrier-protein) reductase	0.032	0.084852814	-0.26	0.056568542
FTL_1310	Nucleoside diphosphate kinase	0	0.113137085	-0.194	0.127279221
FTL_1785	Succinate dehydrogenase iron-sulfur subunit	-0.032	0.096166522	0.324	0.03959798
FTL_1272	Biotin synthase	-0.022	0.036769553	0.292	0.045254834
FTL_0479	Glycine dehydrogenase subunit 1	0.002	0.132936075	-0.176	0
FTL_1784	2-oxoglutarate dehydrogenase E1 component	-0.04	0.016970563	-0.122	0.031112698
FTL_0480	Glycine dehydrogenase subunit 2	0.002	0.138592929	0.236	0.073539105
FTL_0421	Lipoprotein	0.022	0.01979899	0.274	0.200818326
FTL_0424	Lipoprotein	-0.036	0.028284271	-0.57	0.115965512
FTL_0405	Menaquinone biosynthesis methyltransferase	-0.024	0.045254834	0.27	0.20647518
FTL_0157	Hypothetical protein	0.036	0.028284271	0.47	0.240416306
FTL_1899	Glutamine synthetase	0.022	0.110308658	-0.18	0.022627417
FTL_1060	D-alanyl-D-alanine carboxypeptidase	-0.002	0.014142136	0.144	0.101823376
FTL_0453	UDP-N-acetylglucosamine pyrophosphorylase/glucosamine-1-phosphate N-acetyltransferase	0.022	0.042426407	0.178	0.025455844
FTL_1266	Lipase/esterase	-0.066	0.042426407	0.326	0.025455844
FTL_0717	Ribonuclease E	-0.034	0.002828427	0.15	0.048083261
FTL_0886	(Dimethylallyl) adenosine tRNA methyltransferase	0.014	0.115965512	-0.566	0.172534055
FTL_0987	Malate dehydrogenase	-0.01	0.01979899	0.249	0.091923882
FTL_0916	Ketol-acid reductoisomerase	0.084	0.164048773	0.162	0.036769553
FTL_0030	Carbamoyl phosphate synthase small subunit	-0.074	0.05939697	0.226	0.01979899
FTL_0028	Aspartate carbamoyltransferase	0.032	0.056568542	-0.378	0.161220346
FTL_1842	Aspartyl/glutamyl-tRNA amidotransferase subunit A	0.042	0.042426407	0.132	0.022627417
FTL_1018	Phosphoserine aminotransferase	0.046	0.093338095	-0.402	0.098994949
FTL_0739	tRNA uridine 5-carboxymethylaminomethyl modification protein GidA	-0.086	0.008485281	-0.118	0.036769553
FTL_1407	Threonyl-tRNA synthetase	-0.028	0.056568542	0.216	0.050911688
FTL_1861	Amidophosphoribosyltransferase	-0.03	0.144249783	0.276	0.130107648
FTL_1775	Coproporphyrinogen III oxidase	0.006	0.01979899	-0.206	0.065053824
FTL_0536	Outer membrane protein OmpH	-0.006	0.070710678	0.286	0.189504617
FTL_0073	Hypothetical protein	-0.082	0.172534055	0.436	0.033941125
FTL_1936	Periplasmic solute binding family protein	-0.014	0.031112698	0.302	0.042426407

FTL_1332	HesB family protein	-0.102	0.234759451	0.222	0.031112698
FTL_0306	Tryptophanyl-tRNA synthetase	-0.004	0.079195959	-0.242	0.110308658
FTL_0015	Propionate kinase	0.014	0.048083261	-0.114	0.002828427
FTL_0049	D-tyrosyl-tRNA(Tyr) deacylase	-0.028	0.130107648	0.208	0.062225397
FTL_0674	3-methyl-2-oxobutanoate hydroxymethyltransferase	-0.072	0.237587878	0.266	0.053740115
FTL_0439	Hypothetical protein	-0.068	0.175362482	0.308	0.090509668
FTL_0261	DNA-directed RNA polymerase subunit alpha	-0.106	0.008485281	-0.03	0.132936075
FTL_0937	Hypothetical protein	0.078	0.05939697	-0.156	0.118793939
FTL_1537	Polynucleotide phosphorylase/polyadenylase	0	0.124450793	-0.134	0.161220346
FTL_0336	Peptidoglycan-associated lipoprotein	0.032	0.016970563	-0.132	0.152735065
FTL_1554	Succinyl-CoA synthetase, alpha subunit	0.032	0.011313708	0.104	0.101823376
FTL_0960	Soluble pyridine nucleotide transhydrogenase	0.036	0.011313708	0.172	0.135764502
FTL_1678	Hypothetical protein	-0.004	0.022627417	-0.732	1.210566809
FTL_1420	Carbohydrate/purine kinase pfkB family protein	-0.012	0.113137085	0.294	0.104651804
FTL_1286	Hypothetical protein	0.026	0.048083261	0.136	0.079195959
FTL_0582	Hypothetical protein	0.016	0.152735065	0.334	0.025455844
Ribosomal proteins					
FTL_0239	50S L2	-0.036	0.203646753	-0.108	0.379009235
FTL_1912	30S S1	0.038	0.313955411	0.153	0.371938167
FTL_0242	30S S3	-0.148	0.03959798	0.068	0.305470129
FTL_0247	50S L24	-0.206	0.048083261	0.254	0.053740115
FTL_1748	50S L11	-0.188	0.005656854	-0.032	0.005656854
FTL_0236	50S L3	-0.164	0.181019336	-0.498	0.161220346
FTL_0456	30S S21	0	0.248901587	-0.51	0.09338095
FTL_1404	50S L20	-0.178	0.958836795	-0.072	0.231931024
FTL_0241	50S L22	-0.023	0.188090404	-0.226	0.01979899
FTL_0224	30S S2	-0.044	0.135764502	-0.78	0.820243866
FTL_0251	50S L6	-0.04	0.107480231	-0.158	0.025455844
FTL_0238	50S L23	-0.04	0.45254834	-0.226	0.070710678
FTL_0235	30S S10	0.02	0.101823376	0.532	0.446891486
FTL_0260	30S S4	0.074	0.01979899	0.13	0.014142136
FTL_0246	50S L14	-0.008	0.011313708	0.108	0.022627417
FTL_1746	50S L10	0	0.028284271	-0.116	0.028284271
FTL_0245	30S S17	-0.106	0.098994949	0.132	0.610940259
FTL_1745	50S L7/L12	-0.024	0.050911688	0.34	0.345068109
FTL_0250	30S S8	-0.114	0.20647518	-0.27	0.223445743
FTL_1538	30S S15	-0.032	0.079195959	0.148	0.090509668
FTL_0258	30S S13	-0.036	0.124450793	0.17	0.234759451
FTL_1453	50S L21	-0.012	0.033941125	0.308	0.18667619
FTL_1452	50S L27	-0.202	0.353553391	0.254	0.082024387
FTL_1026	50S L9	0.054	0.002828427	0.376	0.311126984
FTL_0950	50S L25	-0.034	0.002828427	0.138	0.149906638
FTL_0237	50S L4	0.042	0.025455844	0.18	0.022627417
FTL_0521	50S L33	-0.018	0.070710678	-0.362	0.01979899
FTL_0243	50S L16	-0.026	0.014142136	0.354	0.002828427
FTL_1047	30S S21	-0.152	0.164048773	-0.25	0.042426407

Table S1: Differential induction of proteins in the $\Delta oxyR$ mutant determined by iTRAQ analysis. To be identified as being significantly differentially produced, a protein was quantified in at least three spectra (allowing generation of a P -value), have a P -value of <0.05 , and have a Log_2 ratio fold change of >1.3 or <0.7 in replicates from two independent experiments. A ratio of >0.11 indicated elevation, while a ratio of <-0.15 indicated suppression of a protein. The values shown are cumulative of two independent iTRAQ experiments, each conducted with two biological replicates.

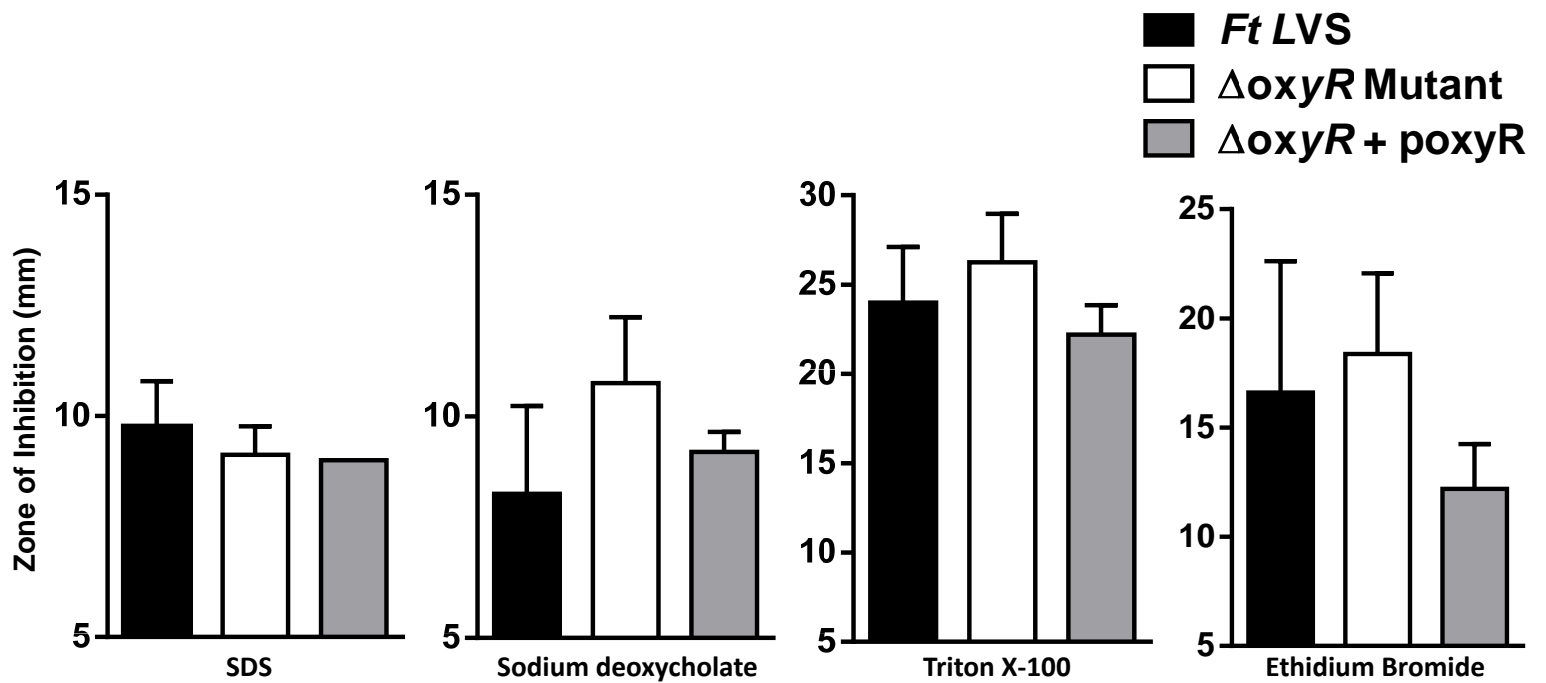


Figure S2: OxyR of *F. tularensis* does not contribute to bacterial membrane integrity. Disk diffusions assay with Sodium dodecyl sulfate (SDS; 750 $\mu\text{g}/\text{disk}$), Sodium deoxycholate (100 $\mu\text{g}/\text{disk}$), Triton X-100 (2.5% solution) and Ethidium bromide (5 $\mu\text{g}/\text{disk}$). The results are expressed as zone of inhibition around the discs in millimeters.

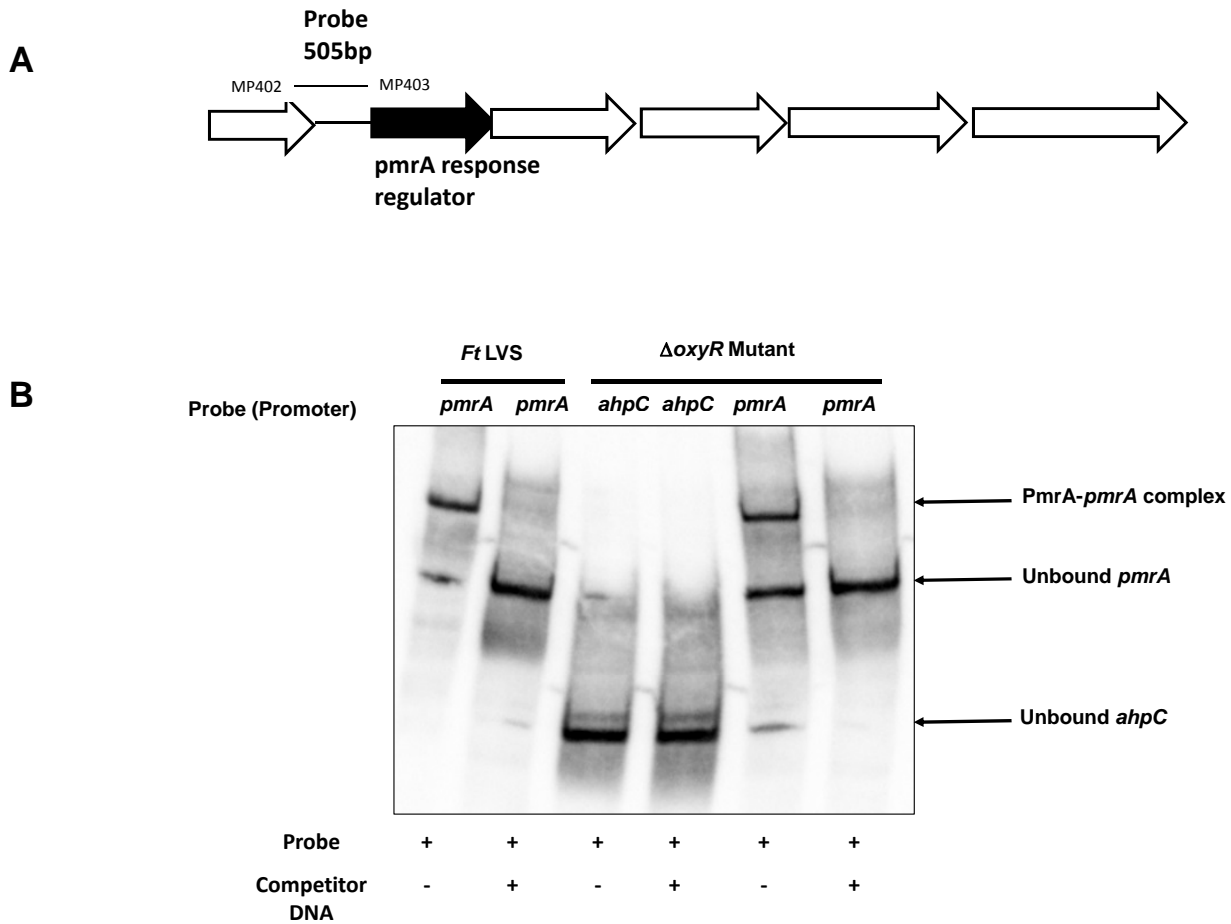


Figure S3: (A) Genomic organization of the *pmrA* gene and location of primers used for generation of biotinylated and competitor DNA. (B) The activity of lysates from the Δ *oxyR* mutant was determined by binding of transcriptional regulator PmrA to its putative *pmrA* promoter region by EMSA. Biotinylated *pmrA* promoter region was used as probe, whereas unlabeled promoter region were used as controls. *ahpC* biotinylated probe and competitor DNA were also used. Results shown are representative of two independent experiments.

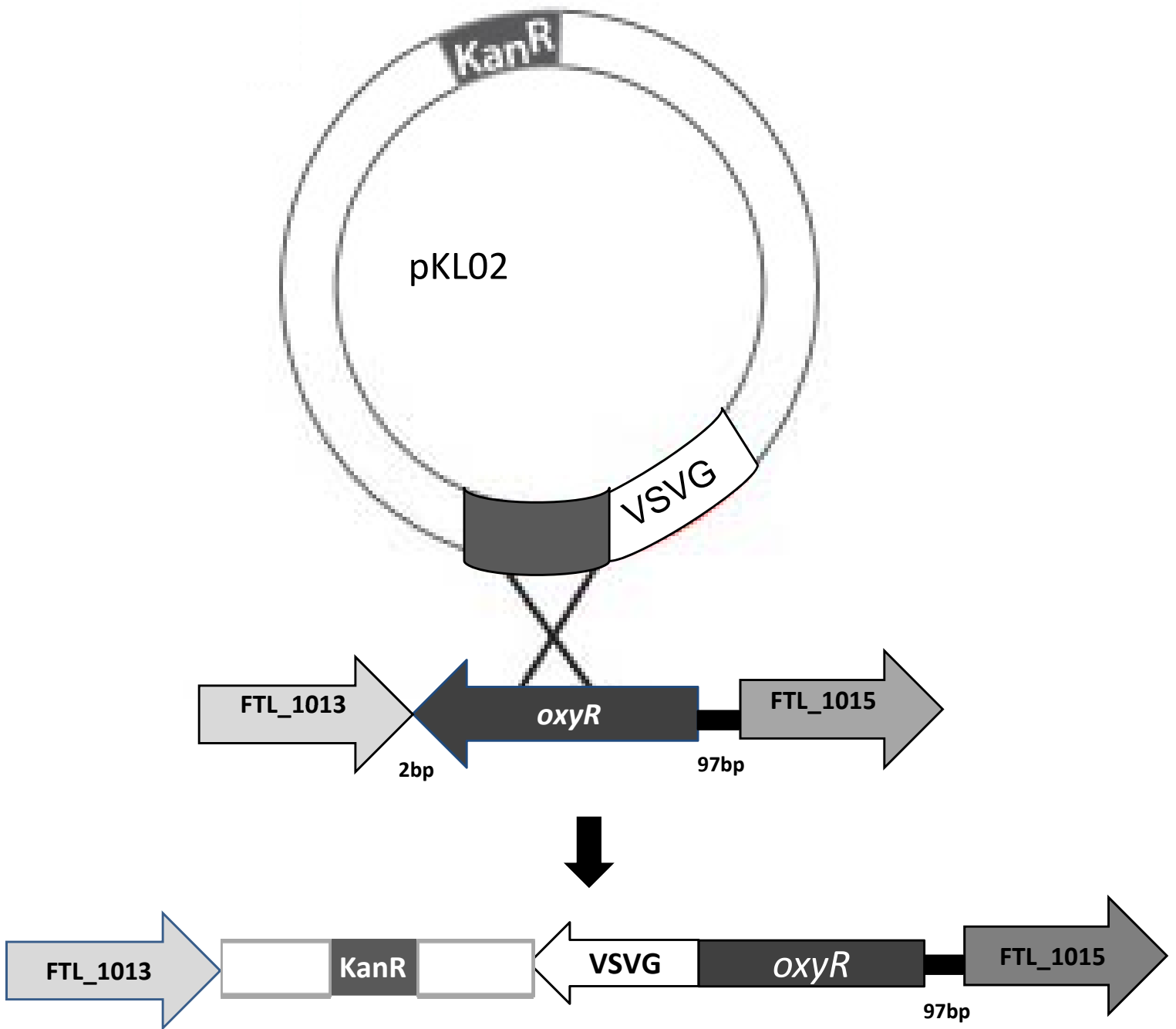


Figure S4: OxyR-VSV-G construct. The *oxyR* gene, minus the stop codon was amplified with primers MP316/317 using pfx Taq-DNA polymerase. The 5' primer has *SalI* site and the 3' primer included a *NotI* site. The fragment was cloned *in-frame* with 11 amino acid long VSV-G epitope tag into pKL02 shuttle vector. After purification, the plasmid was electroporated into wild type *F. tularensis* LVS. The positive clones were selected on the MH-chocolate agar plates containing kanamycin and confirmed by PCR and DNA sequencing.