

***Francisella tularensis* subsp. *novicida*,  
“Two-Allele” Transposon Mutant Library,  
Plate 33****Catalog No. NR-10484****For research use only. Not for human use.****Contributor:**

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**Product Description:**

A comprehensive 16508-member transposon mutant library<sup>1</sup> of sequence-defined transposon insertion mutants of *Francisella tularensis* subsp. *novicida*, strain U112 was prepared to allow the systematic identification of virulence determinants and other factors associated with *Francisella* pathogenesis. Genes refractory to insertional inactivation helped define the genes essential for viability of the organism.

To facilitate genome-scale screening using the mutant collection, a “two-allele” single-colony purified sublibrary, made up of approximately two purified mutants per gene, was assembled.

Several strains in the “two-allele” 3050-member sublibrary were not available at the time of the release of Plates 1-32 (BEI Resources NR-8035 through NR-8066) due to quality issues. NR-10484 contains 18 of these strains. Eleven strains remain unavailable. Detailed information for the 18 available strains is shown in Tables 1-3. Information about specific clones may also be accessed through the [Francisella Tularensis Genome Research](#) homepage.

*Francisella tularensis* subsp. *novicida*, strain U112 is excluded from Select Agent status. Please see [CDC Select Agent Program, Notification of Exclusion](#).

**Material Provided:**

Each well of the 96-well plate contains approximately 0.25 mL of bacterial culture in 0.7X Tryptic Soy Broth containing 0.1% L-cysteine supplemented with 5% glycerol. The mutant clones in wells A04, A05 and A06 are erythromycin-resistant and were grown on media without antibiotic. All other clones are kanamycin-resistant and were grown on media containing 10 µg/mL kanamycin.

**Note:** Production in the 96-well format has a potential for cross-contamination. Individual mutants should be checked by the recipient prior to use.

**Packaging/Storage:**

NR-10484 was packaged aseptically in 96-well plates. The product is provided frozen and should be stored at -60°C or

colder immediately upon arrival. For long-term storage, the vapor phase of a liquid nitrogen freezer is recommended. Freeze-thaw cycles should be avoided.

**Growth Conditions:****Media:**

Tryptic Soy Agar containing 0.1% L-cysteine and 10 µg/mL kanamycin

**Incubation:**

Temperature: 37°C

Atmosphere: Aerobic with 5% CO<sub>2</sub>

**Propagation:**

1. Scrape top of frozen well with a pipette tip and streak onto agar plate.
2. Incubate the plate at 37°C for 24 to 48 hours.

**Citation:**

Acknowledgment for publications should read “The following reagent was obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: *Francisella tularensis* subsp. *novicida*, “Two-Allele” Transposon Mutant Library, Plate 33, NR-10484.”

**Biosafety Level: 2**

Appropriate safety procedures should always be used with this material. Laboratory safety is discussed in the following publication: U.S. Department of Health and Human Services, Public Health Service, Centers for Disease Control and Prevention, and National Institutes of Health. [Biosafety in Microbiological and Biomedical Laboratories](#). 5th ed. Washington, DC: U.S. Government Printing Office, 2007; see [www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm](http://www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.htm).

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**References:**

1. Gallagher, L. A., et al. "A Comprehensive Transposon Mutant Library of *Francisella novicida*, A Bioweapon Surrogate." Proc. Natl. Acad. Sci. USA 104 (2007): 1009-1014. PubMed: 17215359.

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**Table 1 - Transposon Type and Mutated Gene**

Strain Name	NR-10484	Transposon type	Gene	Description	Function Class
tnfn1_pw060328p02q123, NR-8044 (G03)	A01	T20	-	conserved protein of unknown function	unknown function - conserved
tnfn1_pw060328p03q104, NR-8045 (D01)	A02	T20	cydC	ABC-type transport ATP-binding protein CydC	transport
tnfn1_pw060328p08q123, NR-8050 (G03)	A03	T20	aceF	pyruvate dehydrogenase complex, E2 component, dihydrolipoamide acyltransferase	carbohydrate metabolism - degradation, utilization, assimilation
tnfn1_pw060418p01q10, NR-8051 (A01)	A04	T15	atpH	ATP synthase, F1 sector, subunit delta	energy metabolism
tnfn1_pw060418p01q102, NR-8051 (B01)	A05	T15	-	beta-lactamase class A	other metabolism - degradation, utilization, assimilation
tnfn1_pw060418p01q103, NR-8051 (C01)	A06	T15	-	conserved protein of unknown function	unknown function - conserved
tnfn1_pw060418p01q105, NR-8051 (E01)	A07	<KAN-2>	-	transcriptional regulator, ArsR family	signal transduction and regulation
tnfn1_pw060418p01q106, NR-8051 (F01)	A08	<KAN-2>	-	protein of unknown function	unknown function - novel
tnfn1_pw060418p01q124, NR-8051 (H03)	A09	T20	iscS	cysteine desulfurase	amino acid metabolism - biosynthesis
tnfn1_pw060418p01q151, NR-8051 (C07)	A10	T20	panD	aspartate 1-decarboxylase	cofactors, prosthetic groups, electron carriers metabolism
tnfn1_pw060418p01q152, NR-8051 (D07)	A11	T20	fadA	acetyl-CoA acetyltransferase	other metabolism - degradation, utilization, assimilation
tnfn1_pw060418p01q158, NR-8051 (B08)	A12	T20	-	sugar transporter, MFS superfamily	transport - carbohydrates (sugars, polysaccharides)
tnfn1_pw060418p01q179, NR-8051 (G10)	B01	T18	-	protein of unknown function	unknown function - novel
tnfn1_pw060418p02q148, NR-8052 (H06)	B02	T20	-	glycosyl transferase, family 8	cell wall / LPS / capsule
tnfn1_pw060420p01q127, NR-8059 (C04)	B03	T20	-	serine-type D-Ala-D-Ala carboxypeptidase	cell wall / LPS / capsule
tnfn1_pw060420p04q175, NR-8062 (C10)	B04	T20	-	transcriptional regulator	signal transduction and regulation
tnfn1_pw060510p02q103, NR-8064 (C01)	B05	T20	priC	oligopeptidase A	amino acid metabolism
tnfn1_pw060510p03q138, NR-8065 (F05)	B06	T18	-	transcriptional regulator, LysR family	signal transduction and regulation

**Table 2 - Sequencing and Insertion Location**

Strain Name	NR-10484	Sequence confirmation (C, Confirmed; U, unconfirmed)	Effective Genome Position of Insertion	Direction of insertion (F, forward relative to genome; R, reverse)	Locus Tag	ORF left end	ORF right end	Direction of ORF (F, forward relative to genome; R, reverse)	Length of ORF (codons)	Effective position of insertion in ORF [nucleotide of insertion (length of ORF in nucleotides)]
tnfn1_pw060328p02q123, NR-8044 (G03)	A01	C	741261	F	FTN_0697	741230	741517	F	96	32(288)
tnfn1_pw060328p03q104, NR-8045 (D01)	A02	C	678748	F	FTN_0641	677863	679509	R	549	762(1647)
tnfn1_pw060328p08q123, NR-8050 (G03)	A03	C	1586468	F	FTN_1493	1586220	1588112	R	631	1645(1893)
tnfn1_pw060418p01q10, NR-8051 (A01)	A04	U	1764488	F	FTN_1649	1764017	1764538	R	174	51(522)
tnfn1_pw060418p01q102, NR-8051 (B01)	A05	U	1134802	R	FTN_1072	1134341	1135201	F	287	462(861)
tnfn1_pw060418p01q103, NR-8051 (C01)	A06	U	248954	F	FTN_0225	248455	249375	R	307	422(921)
tnfn1_pw060418p01q105, NR-8051 (E01)	A07	C	858810	R	FTN_0801	858600	858947	R	116	138(348)
tnfn1_pw060418p01q106, NR-8051 (F01)	A08	C	770015	F	FTN_0715	766940	770707	F	1256	3076(3768)
tnfn1_pw060418p01q124, NR-8051 (H03)	A09	C	1311156	R	FTN_1245	1310691	1311863	R	391	708(1173)
tnfn1_pw060418p01q151, NR-8051 (C07)	A10	C	1432485	R	FTN_1354	1432438	1432770	F	111	48(333)
tnfn1_pw060418p01q152, NR-8051 (D07)	A11	C	1528045	F	FTN_1439	1527712	1528896	F	395	334(1185)
tnfn1_pw060418p01q158, NR-8051 (B08)	A12	C	1070823	F	FTN_1011	1070332	1071615	R	428	793(1284)
tnfn1_pw060418p01q179, NR-8051 (G10)	B01	C	1384035	F	FTN_1311	1384032	1384406	F	125	4(375)
tnfn1_pw060418p02q148, NR-8052 (H06)	B02	C	1324223	R	FTN_1255	1323964	1324851	F	296	260(888)
tnfn1_pw060420p01q127, NR-8059 (C04)	B03	U	670284	F	FTN_0635	669592	670983	R	464	700(1392)
tnfn1_pw060420p04q175, NR-8062 (C10)	B04	C	905906	R	FTN_0850	905800	906207	F	136	107(408)
tnfn1_pw060510p02q103, NR-8064 (C01)	B05	C	424121	R	FTN_0425	423396	425399	R	668	1279(2004)
tnfn1_pw060510p03q138, NR-8065 (F05)	B06	C	391600	R	FTN_0392	390793	391710	R	306	111(918)

**Table 3 - Sequence Mapping Quality Metrics**

Strain Name	NR-10484	Junction information	Position in sequence read of last vector nucleotide	Length of match to transposon	Transposon match score	Average phred score for transposon match	Genome position information	Length of match to genome	Genome match score	Average phred score for genome match
tnfn1_pw060328p02q123, NR-8044 (G03)	A01	EXACT(0)	156	146	130	38	EXACT(0)	200	180	49
tnfn1_pw060328p03q104, NR-8045 (D01)	A02	EXACT(0)	155	145	129	51	EXACT(0)	200	188	67
tnfn1_pw060328p08q123, NR-8050 (G03)	A03	EXACT(0)	155	152	130	43	EXACT(0)	200	153	43
tnfn1_pw060418p01q10, NR-8051 (A01)	A04	EXACT(0)	196	107	88	33	EXACT(0)	200	188	59
tnfn1_pw060418p01q102, NR-8051 (B01)	A05	EXACT(0)	179	108	95	18	EXACT(0)	174	151	22
tnfn1_pw060418p01q103, NR-8051 (C01)	A06	EXACT(0)	107	107	101	45	EXACT(0)	134	131	55
tnfn1_pw060418p01q105, NR-8051 (E01)	A07	EXACT(0)	122	109	65	22	EXACT(0)	200	187	48
tnfn1_pw060418p01q106, NR-8051 (F01)	A08	EXACT(0)	121	106	96	31	EXACT(0)	200	194	51
tnfn1_pw060418p01q124, NR-8051 (H03)	A09	EXACT(0)	154	145	116	32	EXACT(0)	200	191	40
tnfn1_pw060418p01q151, NR-8051 (C07)	A10	EXACT(0)	155	152	130	42	EXACT(0)	200	144	37
tnfn1_pw060418p01q152, NR-8051 (D07)	A11	EXACT(0)	155	152	128	36	EXACT(0)	200	192	54
tnfn1_pw060418p01q158, NR-8051 (B08)	A12	EXACT(0)	157	145	129	32	EXACT(0)	200	180	65
tnfn1_pw060418p01q179, NR-8051 (G10)	B01	EXACT(0)	121	81	66	23	EXACT(0)	200	170	37
tnfn1_pw060418p02q148, NR-8052 (H06)	B02	EXACT(0)	156	153	137	40	EXACT(0)	200	186	57
tnfn1_pw060420p01q127, NR-8059 (C04)	B03	EXACT(0)	152	142	109	31	EXACT(0)	190	177	41
tnfn1_pw060420p04q175, NR-8062 (C10)	B04	EXACT(0)	156	152	127	35	EXACT(0)	200	183	56
tnfn1_pw060510p02q103, NR-8064 (C01)	B05	EXACT(0)	155	145	129	46	EXACT(0)	200	197	66
tnfn1_pw060510p03q138, NR-8065 (F05)	B06	EXACT(0)	119	119	98	39	EXACT(0)	200	189	56