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SUPPORTING INFECTIOUS DISEASE RESEARCH

Francisella tularensis subsp. *tularensis*, Strain SCHU S4, Gateway[®] Clone Set, Recombinant in *Escherichia coli*, Plate 19

Catalog No. NR-19476

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Contributor:

Pathogen Functional Genomics Resource Center at the J. Craig Venter Institute

Manufacturer:

BEI Resources

Product Description:

The Francisella tularensis (F. tularensis) subsp. tularensis, strain SCHU S4, Gateway[®] clone set consists of 19 plates which contain 1693 sequence validated clones from *F. tularensis* subsp. *tularensis*, strain SCHU S4 cloned in *Escherichia coli* (*E. coli*) DH10B-T1 cells. Each open reading frame was constructed in vector <u>pDONRTM221</u> (InvitrogenTM) with a native start codon and no stop codon. The sequence was validated by full length sequencing of each clone with greater than 1X coverage and a mutation rate of less than 0.2%. Detailed information about each clone is shown in Table 1.

Information related to the use of Gateway[®] Clones can be obtained from <u>Invitrogen</u>[™]. Recombination was facilitated through an *att*B substrate (*att*B-PCR product or a linearized *att*B expression clone) with an *att*P substrate (pDONR[™]221) to create an *att*L-containing entry clone. The entry clone contains recombinational cloning sites, *att*L1 and *att*L2 to facilitate gene transfer into a destination vector, M13 forward and reverse priming sites for sequencing and a kanamycin resistance gene for selection. Please refer to the Invitrogen[™] Gateway[®] Technology Manual for additional details.

Material Provided:

Each inoculated well of the 96-well plate contains approximately 60 μ L of *E. coli* culture (strain DH10B-T1) in Luria Bertani (LB) Broth containing 50 μ g/mL kanamycin supplemented with 15% glycerol.

<u>Note:</u> Production in the 96-well format has increased risk of cross-contamination between adjacent wells. Individual clones should be purified (e.g. single colony isolation and purification using good microbiological practices) and sequence-verified prior to use. BEI Resources cannot confirm or validate any clone not identified on the plate information table.

Packaging/Storage:

NR-19476 was packaged aseptically in a 96-well plate. The

product is provided frozen and should be stored at -80°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:

LB Broth or Agar containing 50 µg/mL kanamycin.

Incubation: Temperature: *E. coli*, strain DH10B-T1 clones should be grown at 37°C.

Atmosphere: Aerobic

Propagation:

- 1. Scrape top of frozen well with a pipette tip and streak onto agar plate.
- 2. Incubate the plates at 37°C for 18 to 24 hours.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Francisella tularensis* subsp. *tularensis*, Strain SCHU S4, Gateway[®] Clone Set, Recombinant in *Escherichia coli*, Plate 19, NR-19476."

Biosafety Level: 1

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. <u>Biosafety in</u> <u>Microbiological and Biomedical Laboratories</u>. 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see <u>www.cdc.gov/biosafety/publications/bmbl5/index.htm</u>.

Disclaimers:

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References:

- Larsson, P., et. al. "The Complete Genome Sequence of *Francisella tularensis*, the Causative Agent of Tularemia." <u>Nat. Genet.</u> 37 (2005): 153-159. PubMed: 15640799.
- Pandya, G. A., et. al. "Whole Genome Single Nucleotide Polymorphism Based Phylogeny of *Francisella tularensis* and its Application to the Development of a Strain Typing Assay." <u>BMC Microbiology</u> 9 (2009): 213. PubMed: 19811647.

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Clone	Well Position	Locus ID	Description	ORF Length	Accession Number	Average Depth of Coverage
9487	A01	NT06FT0331	valyI-tRNA synthetase	2794	CAG44932.1	3.43486
9490	A02	NT06FT1035	isoleucyl-tRNA synthetase	2842	CAG45548.1	4.56193
9491	A03	NT06FT0081	2-oxoglutarate dehydrogenase, E1 component	2848	CAG44709.1	3.20611
9496	A04	NT06FT0071	hypothetical protein	2851	CAG44699.1	2.26377
9498	A05	NT06FT1281	cyanophycin synthetase	2857	CAG45763.1	4.55128
9499	A06	NT06FT1495	excinuclease ABC, A subunit	2857	CAG45945.1	4.35492
9507	A07	NT06FT0116	hydrophobe/amphiphile efflux family protein	3148	CAG44738.1	3.65311
9509	A08	NT06FT0454	alpha-dextran endo-1,6-alpha- glucosidase	3247	CAG45045.1	4.90176
9513	A09	NT06FT1535	PdpB	3316	CAG45978.1	2.5953
9515	A10	NT06FT1965	PdpB	3316	CAG46333.1	3.8468
9523	A11	NT06FT0443	DNA polymerase III, alpha subunit, form 1	3514	CAG45035.1	3.31417

Table 1: Francisella tularensis subsp. tularensis, Strain SCHU S4, Gateway[®] Clone Set, Plate 19 (ZFTLC)