

***Aspergillus fumigatus* Knockout Gateway®
Clone Set, Recombinant in *Escherichia coli***

Catalog No. NR-19437

This reagent is the tangible property of the U.S. Government.

For research use only. Not for human use.

Contributor:

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

Manufacturer:

BEI Resources

Product Description:

Clone plates are replicated using a BioMek® FX robot. 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 only confirms the clone plate orientation and viability of randomly picked clones. BEI Resources does not confirm or validate individual clone identities provided by the contributor.

The *Aspergillus fumigatus* (*A. fumigatus*), Gateway® clone set consists of 1 plate which contains 70 sequence validated knockout clones from *A. fumigatus*, strain A1163 with ampicillin selectable pyrG selection gene, cloned in *Escherichia coli* (*E. coli*) DH10B-T1 cells. Each open reading frame was constructed in vector pDEST™ 17 (Invitrogen™). Successful cloning was determined using a restriction digestion approach. Detailed information about each clone is shown in Table 1.

Information related to the use of Gateway® Clones can be obtained from [Invitrogen™](http://www.invitrogen.com). Using the three fragment MultiSite Gateway® strategy the pyrG selectable marker was assembled with chromosomal amplicons (~1Kb) of the regions flanking the targeted gene. The three fragments (left flank, pyrG, right flank) were amplified and cloned into entry vector pDONR™221 P1-P4, pDONR™221 P4r-P3r, and pDONR™221 P3-P2 respectively. The flanking clones were confirmed by PCR, the pyrG clone was sequence verified. The final assembled construct is created using LR Clonase™ II Plus to recombine the three clones with each other and the destination vector, pET-DEST-TIGR02. Linearized vector can be used to transform chemically competent protoplasts. Upon homologous recombination the pyrG gene complements an auxotrophic mutant A1163 strain. Please refer to the [Invitrogen™ Gateway® Technology Manual](#) for additional details.

Plate orientation and viability were confirmed for NR-19437.

Material Provided:

Each inoculated well of the 96-well plate contains approximately 60 µL of *E. coli* culture (strain DH10B-T1) in Luria Bertani (LB) broth containing 100 µg/mL ampicillin supplemented with 15% glycerol.

Packaging/Storage:

NR-19437 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 containing 100 µg/mL ampicillin

LB agar containing 100 µg/mL ampicillin

Incubation:

Temperature: 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 24 hours.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Aspergillus fumigatus* Knockout Gateway® Clone Set, Recombinant in *Escherichia coli*, NR-19437."

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. [Biosafety in Microbiological and Biomedical Laboratories](#). 5th ed. Washington, DC: U.S. Government Printing Office, 2009; see www.cdc.gov/biosafety/publications/bmb15/index.htm.

Disclaimers:

You are authorized to use this product for research use only. It is not intended for human use.

Use of this product is subject to the terms and conditions of the BEI Resources Material Transfer Agreement (MTA). The MTA is available on our Web site at www.beiresources.org.

While BEI Resources uses reasonable efforts to include accurate and up-to-date information on this product sheet, neither ATCC® nor the U.S. Government makes any warranties or representations as to its accuracy. Citations from scientific literature and patents are provided for informational purposes only. Neither ATCC® nor the U.S. Government warrants that such information has been confirmed to be accurate.

This product is sent with the condition that you are responsible for its safe storage, handling, use and disposal. ATCC® and the U.S. Government are not liable for any damages or injuries arising from receipt and/or use of this product. While reasonable effort is made to ensure authenticity and reliability of materials on deposit, the U.S. Government, ATCC®, their suppliers and contributors to BEI Resources are not liable for damages arising from the misidentification or misrepresentation of products.

Use Restrictions:

This material is distributed for internal research, non-commercial purposes only. This material, its product or its derivatives may not be distributed to third parties. Except as

performed under a U.S. Government contract, individuals contemplating commercial use of the material, its products or its derivatives must contact the contributor to determine if a license is required. U.S. Government contractors may need a license before first commercial sale.

References:

1. Fedorova, N. D., et al. "Genomic Islands in the Pathogenic Filamentous Fungus *Aspergillus fumigatus*." *PLoS Genet.* 4 (2008): e1000046. PubMed: 18404212.

ATCC® is a trademark of the American Type Culture Collection.



Table 1: *Aspergillus fumigatus* Knockout Gateway® Clone Set, Recombinant in *Escherichia coli*¹

Well Position	Locus ID	Description	Accession Number
A01	AFUB_000380	AraC-like ligand binding domain protein, putative	EDP55348.1
A02	AFUB_000820	polyketide synthase, putative	EDP55391.1
A03	AFUB_001440	conserved hypothetical protein	EDP55449.1
A05	AFUB_002040	kinesin family protein	EDP55509.1
A06	AFUB_004840	regulator of gluconeogenesis Rmd5, putative	EDP55788.1
A07	AFUB_004930	nitrogen permease regulator Npr2, putative	EDP55797.1
A09	AFUB_005170	transcription factor AbaA	EDP55821.1
A11	AFUB_006730	pathogenesis associated protein Cap20, putative	EDP55971.1
B01	AFUB_008080	SWIRM domain protein	EDP56107.1
B02	AFUB_010360	MAP kinase kinase kinase SskB, putative	EDP56327.1
B03	AFUB_010850	cell wall biogenesis protein phosphatase Ssd1, putative	EDP56375.1
B04	AFUB_013560	chromosome segregation protein BIR1, putative	EDP56644.1
B05	AFUB_014080	Mn superoxide dismutase MnSOD	EDP56690.1
B06	AFUB_014820	heat shock protein Hsp98/Hsp104/CipA, putative	EDP56763.1
B07	AFUB_015440	C6 transcription factor RosA-like, putative	EDP56824.1
B08	AFUB_015480	MAP kinase kinase (Pbs2), putative	EDP56828.1
B09	AFUB_016740	heat shock protein Awh11/Hsp9, putative	EDP56952.1
B12	AFUB_017500	conserved hypothetical protein	EDP53708.1
C01	AFUB_018660	non-classical export protein Nce2, putative	EDP53822.1
C02	AFUB_020120	cactin, putative	EDP53966.1
C03	AFUB_022340	C6 transcription factor, putative	EDP54182.1
C04	AFUB_024520	4'-phosphopantetheinyl transferase NpgA	EDP54396.1
C05	AFUB_026040	NAD binding Rossmann fold oxidoreductase, putative	EDP54546.1
C06	AFUB_028280	allergen Asp F13	EDP54768.1
C08	AFUB_030400	BCS1-like ATPase, putative	EDP54979.1
C09	AFUB_033090	Pfs and NB-ARC domain protein	EDP55245.1
C10	AFUB_033930	C6 transcription factor, putative	EDP52229.1
C11	AFUB_034230	DUF895 domain membrane protein	EDP52259.1
D01	AFUB_034520	polyketide synthase, putative	EDP52288.1
D02	AFUB_035460	nonribosomal peptide synthase, putative	EDP52383.1
D03	AFUB_036250	C6 transcription factor, putative	EDP52459.1
D04	AFUB_036300	C6 transcription factor GliZ-like, putative	EDP52464.1

Well Position	Locus ID	Description	Accession Number
D05	AFUB_038060	MAP kinase kinase kinase (Bck1), putative	EDP52640.1
D06	AFUB_038100	SNARE-dependent exocytosis protein (Sro7), putative	EDP52644.1
D09	AFUB_039370	class II aldolase/adducin domain protein	EDP52768.1
D10	AFUB_039530	camp independent regulatory protein	EDP52784.1
D11	AFUB_043130	MAP kinase kinase Ste7	EDP53143.1
E01	AFUB_046050	alpha,alpha-trehalose glucohydrolase TreA/Ath1	EDP53429.1
E02	AFUB_046990	polyketide synthase, putative	EDP53518.1
E03	AFUB_047090	AMID-like mitochondrial oxidoreductase, putative	EDP53528.1
E04	AFUB_047470	C6 transcription factor, putative	EDP53565.1
E05	AFUB_050190	bZIP transcription factor JIbA/IDI-4	EDP51017.1
E10	AFUB_057500	nitroreductase family protein, putative	EDP51734.1
E11	AFUB_058890	thioredoxin TrxA	EDP51868.1
F01	AFUB_061540	transcriptional regulator, putative	EDP52118.1
F02	AFUB_064810	pyruvate carboxylase, putative	EDP50149.1
F03	AFUB_066580	NACHT domain protein	EDP50323.1
F05	AFUB_069770	methyalmalonate-semialdehyde dehydrogenase, putative	EDP50640.1
F06	AFUB_070380	SNF2 family helicase/ATPase, putative	EDP50698.1
F08	AFUB_071780	C6 transcription factor (AifR), putative	EDP50838.1
F09	AFUB_071800	polyketide synthase, putative	EDP50840.1
F10	AFUB_071830	C6 transcription factor, putative	EDP50843.1
G01	AFUB_072940	SAM and PH domain protein	EDP49268.1
G04	AFUB_078040	nonribosomal peptide synthase, putative	EDP49773.1
G05	AFUB_078070	nonribosomal peptide synthase, putative	EDP49776.1
G07	AFUB_078180	conserved hypothetical protein	EDP49786.1
G08	AFUB_078810	MAP kinase MpkB/Fus3	EDP49848.1
G09	AFUB_080600	C6 transcription factor, putative	EDP48624.1
G10	AFUB_083460	N-acetylglucosamine-6-phosphate deacetylase (NagA), putative	EDP48897.1
G11	AFUB_083510	NDT80_Phog domain protein PcaG	EDP48902.1
H01	AFUB_086200	polyketide synthase, putative	EDP49172.1
H02	AFUB_086680	C6 transcription factor, putative	EDP47962.1
H03	AFUB_087140	NACHT and WD domain protein	EDP48003.1
H04	AFUB_088630	AIF-like mitochondrial oxidoreductase (Nfri), putative	EDP48153.1
H05	AFUB_089970	mitochondrial ribosomal protein DAP3, putative	EDP48282.1
H07	AFUB_094810	nonribosomal peptide synthase, putative	EDP47634.1
H08	AFUB_094860	C6 finger domain protein, putative	EDP47639.1
H10	AFUB_100730	polyketide synthase, putative	EDP47078.1
H11	AFUB_101990	C6 transcription factor, putative	EDP47206.1

¹All information in this table was provided by J. Craig Venter Institute at the time of deposition.