

APPENDICES

APPENDIX A

SEQUENCE OF OPEN READING FRAMES 2 (*ORF2*) AND OPEN READING FRAMES 11 (*ORF11*) FROM T3SS-1 OF *B. PSEUDOMALLEI* K96243

ORF2

>AF074878.2: 25391-26344 *Burkholderia pseudomallei* putative type III secretion
system-associated gene cluster, complete sequence

ATGCCTGCACGAAAATCCTCCGAGGAGGCTTGCGTGAATCGCTCATTTCTTCCAATCATT
TGTCTCGCCGGCCCAGCTGCAACAGTCCGCAGATGCGCAGCAGCGCGCCGCCGACCGTCAGG
CGGCGCGCAACAGCGCGCTGACCGCGGCGCTGTATCGCGGCACGACGAATTTACCTACAGCA
AAGATCCTGCGCCACGCGGCAGGCTGCCGAGCGTCTCCAAGACGAATGCCCTTGCGCGAAAGC
GCAAGATCACGAGCCGTTGCGTCGAAACGCCGCCGGCAACGGCGATGGCGACGACGTTGACG
GCGCGCTCGTGCCCTCACTTCGAAGCCGAACCGGGCGGGGACCGCGATCGCGGTGGGCGTGGGG
GACGGGAGCAACCGCGCGACGATCCTATGGATATCGAAACTTGCAGGCGGCTTCCTCGCATGC
AGGCGGCCGAATCGCGCGCCGCGGCTGCCCCACGCGGCCGCTTCGATGCGGTGCGCGATCTGC
ATGCGGCCCCCGGTCAGGAAGGCGAGCGCGCCGACGCGATTGCCCGGGCCTGGGAGAGCGAGA
TGTTTCGGAATCCGAGTATTTTCCCCCAATGCGCCACGCACGGCGGAGATTCTCGAATTGTCGTT
GGACTTTCTTCTCATCCAGCGACGCATCGGGCCGATTCCCGTTAGTACGCTCGCCAGGCTGCGC
GAGTCTCCTGCTCCACACCGGTTGCTTGCGGCCGAGATCGCCTGCCTGGCGAGACGGCGCCG
GACGACAGACGGCGCTTCAATCTGCTCTTTCCGTTGCTGTGGTTGCAGGCGGGCATGCCGAGAA
CAGCCATCCGCCTCGATTGCGCGATCGCCAAGCTGCTCGCGATCCGCAACGCACTGCCTGACAA
TCCGCTAGCAACAGATGACGCGCAAGCGGAACCGACACCGGCACCTCACGCCTCATCGAGCGA
ATGA

ORF11

>AF074878.2:1064-4927 *Burkholderia pseudomallei* putative type III secretion system-associated gene cluster, complete sequence

TCACTCCCCGTCTTTACGGCACTTCCATGCGTGGAGCGGCCGCCAACGTCCGACGGTGCGTCC
 CCCCCGGCTTGAACCGATCCCACCGAGACGGGATGCCTCGCCAACGCCACGGGGTCGACATAC
 TCCGATGCGGCTTGATGAGCCAGCACCTGAACCGTGGAAGTACTAGACGATTTTTTCGCCTCCA
 CGCCGAATACGTTGTGCAGACCCACCGATTTTGCAGACGACGTCTCCACATAGTGCTGTATGGA
 ACTGGGCCGCCAACTCGCCGAGTTCTCGATGACTTCCGCATAGCGCCGCCGAAGTTCGCTGACG
 CGGTTTTTACTCGTACCAAGCATCTGCTCAAGATCGATTTGAGCGCGGTAGTGATTGAGCGCTG
 ACGCAGCTTCCTTTGTCATCCTATATGAAGCGACATAGGTTTCCGGCACCTCGGGACGCAGCGG
 GCGGTTTACGCCCCGGCCAGTTCTGAAGCGAAGAATCGGTTCAATGCCGCCTCGCCTTCAGCG
 TCGGTCTTACCCATTGCCTTGACCCATAGCGGATAGTCGCGCGCGATCAGCTCCACCGCACGCC
 GGCCGCGGTTGCGCGTTCGCTCCAGAATAGCCAATTCGGAATCGACCATGCCGTCACGCAAGC
 GGATAGCCACGGAAGCCCCCTCCTTCGCGGAGAAAAGCGTACCTCCGACGCTGAATGCCTGTG
 CGCTGGCGCTCGCCGACGTACTGAAATCCGGCGTCGGATTGAGTTGATAGGATTCGCTCGCGTT
 CAGGCCGGCGTTCCATGTCACGCTGGTTGACGACGATTGGTCAAACCTTGATTCCTCCATTTTA
 TTATCCAGCTCTCGAACCGCTTTATGCCGCCGCCATTCCCGCTTCACGCCGATGCTGGCACTTA
 CCCCCGCCCTCCGCGGAAATCGCCGCCGCTGCTGCTTGCTTCACCCCCGCCCAACGGCCG
 CCGAAGAGCTAATGACATTTTCCTCCTTCTCGGTATGCCGATTCCAATCTCACCAGCGTTCAC
 ATATTCTTCGCCAACCTTTCCAACAGGTCTGCGGAATCCGCTGAGTTACCGATGATCGACAGG
 CAATCTTGCGCCCAGCCGCGCCACGCCTCCCCGCTCCCAAAGATCCCGCCCTCGGCACAAACC
 TGACAATCGCGCCCTTTTCCCTGGTTTGCTCGGCGCTGACGAGTTCCAGGTTGCGGGAACCGCC
 GGCGCGCACGACATGGATGAATTGAGGCCCGGCGAACACCGATGCGCCGACTTTGAGTTTCTG
 GCTGTTGCCCCGTGTCACGGCGATCAATCCCCCCTGCCTGATGCGCCGCCGATCTCGACAGTG
 GCAACCCGCCCTCCGCCGCTCCAAGCGTAGGCGAAACACTGACAAGTGGCCCTATGGATTGC
 GAATCGCCCAGCGCCAATCCTCCGACTCCGCCACTGGAAAATTTCAAGGACTTCCCCGCCCGGT
 ATCGAGCCACGATCTCCGAGAACGCACTGAACACCGAGGTCCGATCGCCTAACTCCGGAATTTCC
 CCCATACCCGTTATTCGAAACCGATCGGAGATCATTCAATTGCCGCCGGAATTTCCGTCATCC
 AATCCCGCTCGGCCGCCCATCTTTCCAAGACGTGACAGAGAATCCATTATTCAGATCCCGAC
 GAATAGACTCGAACTCCAACTCCTTTTGACCGCATCGTTCTGCCACCTGCCACCGCGAGAGC
 CACCCACTTTTTTCAGGTCATCCGATTTTATTTCAATTTTAGATGAAAACCCGTCGATCTTTAGTT

TTTCGAAGACACGACTTACAACCTCCGATCTTGCCTTCGCCACGCCTGCGCCTGCGCCTGCGCC
TGCGCCTGCGCCTGCGCCTGCGCCTGCGCCTGCGCCTGCGCCTGCACCGGATTCTCGATATTTT
TCAATCAATGAATGGAATGCGGGCGCAATCTTTTCATTGAATACGTCTTCCCGCAGAAACCCGC
CCAGCAGCAACCCCTCCTCCAGCATCCCGACCGGGTTCTTCCTGTGACCGAAAGCGCCCGCTAT
ATCGTCCCCTATTGCGCTGAGCCTGCCAGTGAGATTTTTCCCTCTGGGAATCGCACGGTCGGCG
TACTTGTTAAGCTTATACAAGCGCTCGAAAACGAGACGGGCCTTCCTCTCGTCGGCGAGCCCAT
TTCGCCATTTGAAGTAAGCGATCTTCTGTTGCATTGGAATGGCCGCTCTTGCCGCCGCCCGAT
CGGAATATTGCCAATGTCGGGTGCGACAGCCAACGCGGCCCGCAACTTGCCGATCGCCTTTTG
GCGGACGCCTGCCCCATCGGATCGACACTGGAAACCGATGCACGTGCGCCCGGACCTTCGGCA
TTGGCATTCAAACCTTATCGAGATCCGCAACCGTTGCAATATCACGCCGAATTCCAAGCGGCA
GCAGTCTGACCAGCTCGCCGGCTTGTTCCAAAGCGTCCATTACATCAGCTTGTTGCTGCTCGA
CAGCATCGCAACCGCGCCTAGCGCCTGGCTCCATTATCACGATCTATCGATATCAGATTGGCG
GCGCATGCCAACGCATCGATCGACAGACGCTCTTCTTGATAAATCGAGCCAAGGTTATCCACTA
CTCTCGAAAAGTGGTACTCGCGCTCCGTCTGTGCCCCGAACCGCCAAGCGCCTCGGCCGAAAC
GATCTGCGCCGCGGCAAGCATATCCTCCAGAGAATCAGGCTTATATGCCGCATCGAGCTTTTCA
TGAAGATATTGCGCAATTGCAAGCGCATTTGATAAACCGATTTTTGCTCGGCATGATGCTCCC
GGCCGGTGATACTCGGCGGAATGCCTAAAATATCGAGCGACGTCCCGCGCGCGCTCACCAGAA
CGCGCTGCATCGAATAGGATTGGCGCTTCAGCTGAAGATCAGGGCGCTCGGTGACATGCCGC
AGTAGTTGCGGAGCAATCTAGCCGCGGCCCGGCCATTCTGTTTAAGAACCACGGCCGCCGCGTA
CATCAGCTGATGCATTGTTGAAGACGGGCGGCCCTCCCGGATCGAAATCGGCAATCCATCCC
GACAGCGCTTCGCGGCCTTCCTCGCCCGTGAAAAGGGACATCGCCAAATGCCGGGTTTCGGCG
TTTTCGAGCGCCGTTAATTTTCGCTCGGCGAACGCGATTTGATCGTTGCGGGATCGGTTACCC
CGAACATCACGATGGCCATTTGCCTTGCTTATCCGCACGGCTCAAGCCGTCAGGACGCGTCGC
GACGTGATTATTTGCTGTGCCCCGCGATCATAAATGCGTTGTCATCCGGCGTCCCGGCCAGT
GATTCGCCTGGCCTGCAATCCGACAAAAGCACGTCCGTCAAATGATCGGCATTGACGGCGCGG
CCTTTCCGGACACCCACCCGTGACGTTCAAAAATACATCCCTGCTGTTATGCCCAGACCCGGC
ATTACCCTGCACCAACGCGCGTGCGAACGGGGCCGTTTCGATCCGCTTCGGCAGTTTTCCGTCA
AGGCGGGGGAGCCCCTCGGCACGCGTGCTTTACGGCAAGCTCGCATGGTCCGGACGAACGGGT
TGCGCACAAACCTTGCCCTCTGATATAGCCCTAGCACCTGCAGGGAAAGCGGTATCGCGGGAG
TTCCGCCTTTGACCTTATCAGCCGTTTTGTTGAGCGGCGCCGGCTGAACGAAAGATCTCGATT
AGCGCCCCCACGCGATCTGGCTCGCGTGCCGGACCAGCCGCCGAGGATCGGACGCGTCGGC

TTCGGACCGCCTCGAACTCAAATCACTTTGATGAATGGATGCACCGCTTGATCGAATAATCACA
GGCATGATCTCTTTTAAAAAGAGCATCGACGTCAT

APPENDIX B

BLASTN RESULTS OF *B. PSEUDOMALLEI* K96243 AND PRIMERS BLAST OF ORF2 AND ORF11

An official website of the United States government. [Here's how you know.](#)

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BLAST® » blastn suite » results for RID-66PBVBJN016

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Job Title **AF074878:Burkholderia pseudomallei putative...**

RID **66PBVBJN016** [Search expires on 06-09 03:03 am](#) [Download All](#)

Program **BLASTN** [Citation](#)

Database **nt** [See details](#)

Query ID **AF074878.2**

Description **Burkholderia pseudomallei putative type III secretion syste ...**

Molecule type **nucleic acid**

Query Length **29814**

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Filter Results

Organism only top 20 will appear ☐ exclude

Type common name, binomial, taxid or group name

[+ Add organism](#)

Percent Identity to E value to Query Coverage to

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Sequences producing significant alignments [Download](#) [Select columns](#) [Show 100](#)

☒ select all 100 sequences selected

[GenBank](#) [Graphics](#) [Distance tree of results](#) [MSA Viewer](#)

	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
☒	Burkholderia pseudomallei putative type III secretion system-associated gene cluster, complete sequence	Burkholderia ps...	55057	57338	100%	0.0	100.00%	29814	AF074878.2
☒	Burkholderia pseudomallei KM376 DNA, chromosome 2, complete sequence	Burkholderia ps...	54743	63082	100%	0.0	99.81%	3143756	AP026084.1
☒	Burkholderia pseudomallei GTC3P0254T DNA, chromosome 2, complete sequence	Burkholderia ps...	54737	63077	100%	0.0	99.81%	3143428	AP026078.1
☒	Burkholderia pseudomallei KM391 DNA, chromosome 2, complete sequence	Burkholderia ps...	54704	61351	100%	0.0	99.79%	3144827	AP026089.1
☒	Burkholderia pseudomallei GTC3P0019 DNA, chromosome 2, complete sequence	Burkholderia ps...	54704	61351	100%	0.0	99.79%	3144273	AP026072.1
☒	Burkholderia pseudomallei GTC3P0050 DNA, chromosome 2, complete sequence	Burkholderia ps...	54665	61318	100%	0.0	99.77%	3138372	AP026074.1
☒	Burkholderia pseudomallei strain N2 chromosome 2, complete sequence	Burkholderia ps...	54665	63005	100%	0.0	99.77%	3142999	CP073726.1
☒	Burkholderia pseudomallei strain BD5 chromosome 2, complete sequence	Burkholderia ps...	54665	61312	100%	0.0	99.77%	3117866	CP073729.1
☒	Burkholderia pseudomallei strain AW17-22 chromosome 2, complete sequence	Burkholderia ps...	54626	61273	100%	0.0	99.75%	3139758	CP073738.1
☒	Burkholderia pseudomallei strain AW44 chromosome 2, complete sequence	Burkholderia ps...	54626	61273	100%	0.0	99.75%	3140230	CP073732.1
☒	Burkholderia pseudomallei 406e chromosome 2, complete sequence	Burkholderia ps...	52916	56193	96%	0.0	99.73%	3216446	CP009297.1
☒	Burkholderia pseudomallei strain BSR chromosome 2, complete sequence	Burkholderia ps...	52878	56154	96%	0.0	99.71%	3214293	CP009127.1
☒	Burkholderia pseudomallei Pasteur 52237 chromosome 2, complete sequence	Burkholderia ps...	52772	56032	96%	0.0	99.64%	3184745	CP009898.1
☒	Burkholderia pseudomallei strain 982 chromosome 2, complete sequence	Burkholderia ps...	52728	57694	100%	0.0	99.61%	3156645	CP012577.1
☒	Burkholderia pseudomallei strain BGK chromosome 2	Burkholderia ps...	52717	55993	96%	0.0	99.61%	3179106	CP008917.1
☒	Burkholderia pseudomallei 1710b chromosome II, complete sequence	Burkholderia ps...	52717	55993	96%	0.0	99.61%	3181762	CP000125.1
☒	Burkholderia pseudomallei strain 2002721100 chromosome 2	Burkholderia ps...	52623	59415	100%	0.0	99.52%	3233516	CP018367.1

ORF2 primers

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	CTCACTTCGAAGCCGAACC	Plus	19	25719	25737	58.55	57.89	8.00	0.00
Reverse primer	AGTCCGAACATCTCGCTCTC	Minus	20	25968	25949	59.26	55.00	4.00	0.00
Product length	250								

Products on intended targets

>AF074878.2 Burkholderia pseudomallei putative type III secretion system-associated gene cluster, complete sequence

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product length = 250
Forward primer 1   CTCACTTCGAAGCCGAACC  19
Template        25719 ..... 25737

Reverse primer 1   AGTCCGAACATCTCGCTCTC  20
Template        25968 ..... 25949

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ORF11 primers

Primer pair 1

	Sequence (5'->3')	Template strand	Length	Start	Stop	Tm	GC%	Self complementarity	Self 3' complementarity
Forward primer	AAGCGTAGGCGAAACACTGA	Plus	20	2426	2445	59.97	50.00	4.00	1.00
Reverse primer	ACGATGCGGTCAAAGGAG	Minus	19	2760	2742	58.16	52.63	2.00	0.00
Product length	335								

Products on intended targets

>AF074878.2 Burkholderia pseudomallei putative type III secretion system-associated gene cluster, complete sequence

```

product length = 335
Forward primer 1   AAGCGTAGGCGAAACACTGA  20
Template        2426 ..... 2445

Reverse primer 1   ACGATGCGGTCAAAGGAG  19
Template        2760 ..... 2742

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APPENDIX C

EQUIPMENTS AND INSTRUMENTS

Name	Source
Autoclave	Hirayama, Japan
Autopipettes (1, 20,100, 1000 ul.)	Satorius, Finland
Balance	Denver instrument, Germany
Electrophoresed	ELITE 300 plus,Wealtec corp.
Flask (250 ml)	Pirex, USA
Centrifuge	HERMLE, Germany
Class II Biohazard Safety Cabinet	Thermo scientific, USA
Incubator	SHEL LAB, Sheldon mfg. Inc.
96-well plate	Thermo scientific Nunc, Denmark
Heat block	Hangzhou Miu instruments
Fluorescence plate reader	Thermo variscanlux
Mixture	Scientific industries
Nanodrop	Thermo Fisher Scientific, DE,USA
Tips(10,200,1000ul)	Thermo scientific, Mexico
Thermocycler	BIO-RAD T100,Singapore