Yersinia pestis Plasmid Detection Kit

Catalog No. NR-9562

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Contributor:

BEI Resources

Manufacturer:

BEI Resources (NR-9562 and NR-9551) and Integrated DNA Technologies, Inc. (NR-9686 to NR-9689)

Product Description:

In addition to chromosomal virulence factors, *Yersinia pestis (Y. pestis)* can carry three plasmids that contribute to pathogenicity.[1,2] pPCP1 (pPla) is a 9.5 kb monomer (or ~ 19 kb dimer) encoding the pesticin immunity protein and plasminogen activator, which promote bacterial dissemination.[3,4] The 110 kb plasmid, pMT1 (pFra1), encodes capsular fraction 1 protein which allows evasion of phagocytosis.[5] pCD1 (pYV) is a 70 kb plasmid encoding the low-calcium response V-antigen, part of the type III secretion system involved in directing bacterial proteins to the host cell cytosol.[6] The presence of pCD1 is required for full virulence and classifies *Yersinia pestis* as a select agent.

The *Yersinia pestis* Plasmid Detection Kit (the Kit) is designed to detect the presence of virulence plasmids pPCP1, pMT1 and pCD1. The Kit consists of the following components:

BEI Resources	Component	Description
NR-9686	pPCP1 Primer Set	Designed to detect the pPCP1, pMT1, and pCD1 plasmids using standard PCR, resulting in amplicons of approximately 400, 1200 and 1900 base pairs, respectively
NR-9687	pMT1 Primer Set	
NR-9688	pCD1 Primer Set	
NR-9689	*Yersinia pestis* Primer Set	Positive control primer set that amplifies an approximately 800 base pair chromosomal marker unique to *Yersinia pestis*[7]
NR-9551	Internal DNA Control	Linearized plasmid internal control DNA; amplification using each of the primer sets will result in a band of approximately 130 base pairs

BEI Resources NR-2715 (Genomic DNA from *Y. pestis*, strain ZE94-2122) contains all three plasmids and may be used as an additional positive control. The successful amplification of an approximately 1900 base pair band from this control DNA is essential when reporting results for the presence or absence of the pCD1 plasmid. BEI Resources NR-2646 (Genomic DNA from *Y. enterocolitica* subsp. *enterocolitica*, strain 33114) contains none of the plasmids and may be used as a negative control.

The specificity of each primer set has been confirmed using a genomic DNA panel from eight *Yersinia pestis* organisms with known plasmid profiles (BEI Resources NR-2715, NR-2716, NR-2717, NR-2718, NR-2719, NR-2720, NR-2644, and NR- 2645). Genomic DNA from twenty-five *Yersinia* organisms that contain no plasmids, two *Francisella* organisms and one *Escherichia coli* organism were used as negative controls for specificity. The identities of the amplicons obtained from NR-2715 with these primer sets have been verified by nucleotide sequencing.

See Appendix I for assay information. Successful use of this kit necessarily depends on third party reagents. Please be advised that BEI Resources has observed considerable variation in the performance of polymerases and buffers obtained from different suppliers, and even among different lots of these reagents obtained from the same supplier. It is therefore critically important that users confirm effective assay performance in their own laboratories using the appropriate control templates.

Material Provided:

NR-9686, NR-9687, NR-9688 and NR-9689 contain approximately 400 µL of combined forward and reverse primers in TE buffer (10 mM Tris-HCl, 1 mM EDTA,) (pH 7.0). NR-9551 contains approximately 40 ng of plasmid DNA in TE buffer (pH 7.0). Primer and plasmid concentrations are shown on the Certificate of Analysis of each component.

Packaging/Storage:

Primers and plasmid DNA were packaged aseptically in screw-capped plastic cryovials. The product is provided frozen on dry ice and should be stored at -60°C upon arrival. Freeze-thaw cycles should be minimized.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through BEI Resources, NIAID, NIH: *Yersinia pestis* Plasmid Detection Kit, NR-9562."

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/bmbl5/index.htm.

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Disclaimers:

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References:

1. Parkhill, J., et al. "Genome Sequence of *Yersinia pestis*, the Causative Agent of Plague." Nature 413 (2001): 523-527. PubMed: 11586360.
2. Ferber, D. M. and R. R. Brubaker. "Plasmids in *Yersinia pestis*." Infect. Immun. 31 (1981): 839-841. PubMed: 7216478.
3. Lähteenmäki, K., et al. "Expression of Plasminogen Activator Pla of *Yersinia pestis* Enhances Bacterial Attachment to the Mammalian Extracellular Matrix." Infect. Immun. 66 (1998): 5755-5762. PubMed: 9826351.
4. Chu, M. C., X. Q. Dong, X. Zhou, and C. F. Garon. "A Cryptic 19-Kilobase Plasmid Associated with U.S. Isolates of *Yersinia pestis*: A Dimer of the 9.5-Kilobase Plasmid." Am. J. Trop. Med. Hyg. 59 (1998): 679-686. PubMed: 9840581.
5. Du, Y., Rosqvist, R., Forsberg, A. "Role of Fraction 1 Antigen of *Yersinia pestis* in Inhibition of Phagocytosis." Infect. Immun. 70 (2002): 1453-1460. PubMed: 11854232.
6. Fields, K. A., et al. "Virulence Role of V Antigen of *Yersinia pestis* at the Bacterial Surface." Infect. Immun. 67 (1999): 5395-5408. PubMed: 10496922.
7. Chain, P. S. G., et al. "Insights into the Evolution of *Yersinia pestis* through Whole-Genome Comparison with *Yersinia pseudotuberculosis*." Proc. Natl. Acad. Sci. USA 101 (2004): 13826-13831. PubMed: 15358858.

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Support Provided by NIAID

Appendix I

Yersinia pestis Plasmid Detection Kit

Reagents/Equipment

Reagent/Equipment	Source	Catalog Number
Y. pestis pPCP1 Primer Set	BEI Resources	NR-9686
Y. pestis pMT1 Primer Set	BEI Resources	NR-9687
Y. pestis pCD1 Primer Set	BEI Resources	NR-9688
Y. pestis Species Primer Set	BEI Resources	NR-9689
Internal DNA Control	BEI Resources	NR-9551
Positive Control Template[1,2] (Genomic DNA from *Y. pestis*, strain ZE94-2122)	BEI Resources	NR-2715
Negative Control Template[2] (Genomic DNA from *Y. enterocolitica*, strain 33114)	BEI Resources	NR-2646
PCR Optimized Buffer B[3]	Invitrogen™	K1220-02B
TaKaRa Ex Taq™[3]	TaKaRa	RR001A

[1]The use of NR-2715 as a positive control is strongly recommended, especially for the detection of the pCD-1 plasmid by amplification of a 1900 base pair band.
[2]This reagent is not supplied with the kit and may be ordered separately from BEI Resources using the listed catalog number.
[3]This polymerase/buffer combination has been successfully employed at BEI Resources to detect the presence of the pPCP1, pMT1 and pCD1 plasmids and the *Y. pestis*-specific chromosomal marker. Users must confirm effective assay performance in their own laboratories using appropriate control templates, whether using the reagents listed here or enzymes and buffers obtained from other sources.

Reaction Mix[1]

Reagent	Stock Concentration	Volume per Reaction (µL)
PCR Water	N/A	5.3
Buffer	5×	5
dNTP Mix	2.5 mM each	2.5
Thermostable DNA Polymerase	5 units per µL	0.2
Primer Set	5 or 10 µM (each primer)	10
Template	0.1 pg to 10.0 ng per µL	2
		Total – 25 µL

[1]Reaction mix should be kept on bench-top cooler until ready for use.
[2]Primers are supplied at working stock concentrations.

Cycling Protocol

Cycle	# of Repeats	Step	Conditions
1	1	1	94.0°C for 7 minutes
2	30	1	94.0°C for 1 minute
		2	60.0°C for 1 minute
		3	72.0°C for 1 minute 10 seconds
3	1	1	72.0°C for 1 minute
4	Indefinite	1	Hold at 4.0°C

Appendix II

Y. pestis Plasmid Primer Sets

TEST	EXPECTED RESULTS					
	NR-9686 (pPCP1)		NR-9687 (pMT1)		NR-9688 (pCD1)	
	Forward	Reverse	Forward	Reverse	Forward	Reverse
PCR Amplification and Sequencing[1] Amplicon size (Figure 1)[2] NCBI blast of sequence	~ 400 base pairs pPCP1		~ 1200 base pairs pMT1		~ 1900 base pairs pCD1	
Specificity[3]	Specific for pPCP1		Specific for pMT1		Specific for pCD1	
Concentration (µM)	10 µM	5 µM	5 µM	5 µM	5 µM	5 µM

[1]Genomic DNA from *Y. pestis*, Strain ZE94-2122 (BEI Resources NR-2715) was used as template.
[2]The gel image provided is representative of expected results.
[3]Confirmed using a genomic DNA panel from eight *Y. pestis* with known plasmid profiles (BEI Resources NR-2715, NR- 2716, NR-2717, NR-2718, NR-2719, NR-2720, NR-2644 and NR-2645). Genomic DNA from twenty-five *Yersinia* organisms that contain no plasmids, two *Francisella* organisms and one *Escherichia coli* organism were also used to establish specificity.

Y. pestis Chromosomal Primer Set

TEST	EXPECTED RESULTS	
	NR-9689 (*Y. pestis*)	
	Forward	Reverse
PCR Amplification and Sequencing[1] Amplicon size NCBI blast of sequence	~ 800 base pairs *Y. pestis*	
Specificity	Specific for *Y. pestis*	
Concentration (µM)	5 µM	5 µM

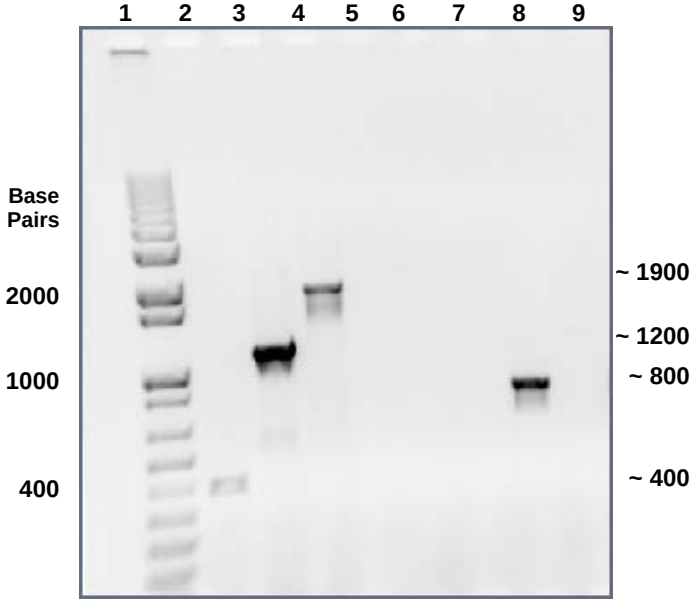
[1]Genomic DNA from *Yersinia pestis*, Strain ZE94-2122 (BEI Resources NR-2715) was used as template.

Internal DNA Control

TEST	EXPECTED RESULTS
	NR-9551 (Internal Control DNA)
Agarose Gel Electrophoresis of Linearized Plasmid DNA[1] (Figure 2)[2]	~ 3,500 base pairs
PCR Amplification Using Primer Sets (Figure 3)[2] pPCP1 (NR-9686) pMT1 (NR-9687) pCD1 (NR-9688) *Yersinia pestis* (NR-9689)	 ~ 130 base pairs ~ 130 base pairs ~ 130 base pairs ~ 130 base pairs
DNA Concentration by PicoGreen® Measurement	~ 1 µg/mL

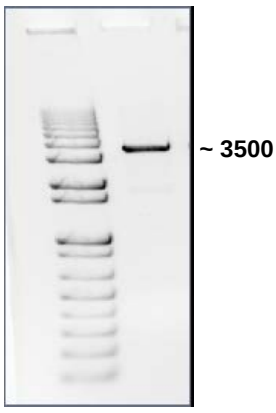
[1]Plasmid DNA was extracted using a HiSpeed Plasmid Midi Kit (QIAGEN 12643). Purified plasmid DNA was linearized with *Bgl* II (New England BioLabs, Inc. R0144S).
[2]The gel image provided is representative of expected results.

Figure 1: PCR Amplification and Sequencing Amplicon Size



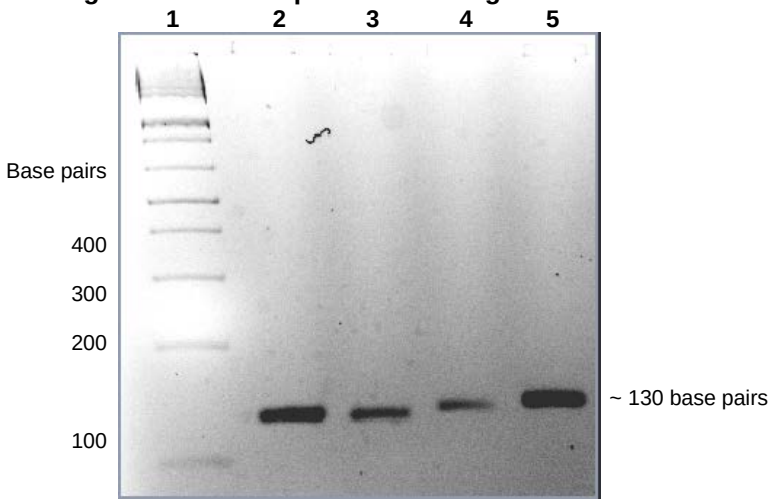
Lane 1: Invitrogen™ 1 kb plus DNA ladder
Lane 2: pPCP1 primers with Genomic DNA from *Y. pestis*, Strain ZE94-2122 (BEI Resources NR-2715)
Lane 3: pMT1 primers with NR-2715
Lane 4: pCD1 primers with NR-2715
Lane 5: pPCP1 primers with Genomic DNA from *Y. enterocolitica*, Strain 33114 (BEI Resources NR-2646)
Lane 6: pMT1 primers with NR-2646
Lane 7: pCD1 primers with NR-2646
Lane 8: *Y. pestis* primers with NR-2715
Lane 9: *Y. pestis* primers with NR-2646

Figure 2: Agarose Gel Electrophoresis of Linearized Plasmid DNA



Lane 1: Invitrogen™ 1 kb plus DNA ladder
Lane 2: Internal DNA Control (BEI Resources NR-9551)

Figure 3: PCR Amplification Using Primer Sets



Lane 1: Invitrogen™ 1 kb plus DNA ladder
Lane 2: *Y. pestis* pMT1 Primer Set (BEI Resources NR-9687)
Lane 3: *Y. pestis* pCD1 Primer Set (BEI Resources NR-9688)
Lane 4: *Y. pestis* pPCP1 Primer Set (BEI Resources NR-9686)
Lane 5: *Y. pestis* Species Primer Set (BEI Resources NR-9689)