Yersinia pestis Multiplex PCR Primers for Plasmid Detection

Catalog No. NR-14686

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

For research use only. Not for human use.

Contributor:

NIH Biodefense and Emerging Infectious Research Resources Repository, NIAID, NIH

Product Description:

Yersinia pestis organisms may carry three plasmids that contribute to pathogenicity in addition to chromosomal virulence factors on the *pgm* locus.[1,2] The pPCP1 (pPla) plasmid contains genes that encode the pesticin immunity protein and the plasminogen activator, which promote bacterial dissemination.[3,4] The pMT1 (pFra1) plasmid, encodes capsular fraction 1 protein which allows evasion of phagocytosis.[5] The pCD1 (pYV) plasmid encodes the low-calcium response V-antigen, which is 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, together with the *pgm* locus, classifies *Yersinia pestis* as a select agent.

NR-14686 consists of a mixture of forward and reverse primers that are designed to detect the pPCP1, pMT1 and pCD1 plasmids using standard polymerase chain reactions, resulting in amplicons of approximately 400, 1200, and 1900 base pairs, respectively. The primer mixture also contains a positive control primer set that amplifies an approximately 800 base pair chromosomal marker unique to *Yersinia pestis*.[7] Each primer set is available individually by requesting the indicated BEI Resources NR number (Table 1). Additionally, a positive control (BEI Resources NR-2715, Genomic DNA from *Yersinia pestis*, Strain ZE94-2122) that contains all three plasmids, and a negative control (BEI Resources NR-2646, Genomic DNA from *Yersinia enterocolitica* subsp. *enterocolitica*, Strain 33114) that contains none of the plasmids are available.

Table 1

Cat. No.	Primer Target	Expected Amplicon (bp)
NR-9686	pPCP1	398
NR-9687	pMT1	1178
NR-9688	pCD1	1864
NR-9689	Chromosomal	799

Please see Appendix I for assay information.

Material Provided:

NR-14686 contains approximately 300 µL of a mixture of forward and reverse primers in TE buffer (pH 7.0).

Packaging/Storage:

NR-14686 was packaged aseptically in screw-capped plastic cryovials. The product is provided frozen on dry ice and should be stored at -20°C upon arrival. Freeze-thaw cycles should be minimized.

Citation:

Acknowledgment for publications should read "The following reagent was obtained through the NIH Biodefense and Emerging Infections Research Resources Repository, NIAID, NIH: *Yersinia pestis* Multiplex PCR Primers for Plasmid Detection, NR-14686."

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, 2007; see www.cdc.gov/od/ohs/biosfty/bmbl5/bmbl5toc.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 make 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. This material may be subject to third party patent rights.

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.

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

Support Provided by NIAID

APPENDIX I

Yersinia pestis Multiplex PCR Plasmid Detection Assay

Recommended Reagents/Equipment

Reagent/Equipment	Source	Catalog #
Yersinia pestis Multiplex PCR Primers	BEI Resources	NR-14686
Positive Control Template (Genomic DNA from *Yersinia pestis*, Strain ZE94-2122)	BEI Resources	NR-2715
Negative Control Template (Genomic DNA from *Yersinia enterocolitica*, Strain 33114)	BEI Resources	NR-2646
PCR Optimized™ Buffer B Kit (or equivalent)	Invitrogen™	K1220-02B
Platinum® Taq DNA Polymerase (or equivalent)	Invitrogen™	10966-034

Reaction Mix[1]

Reagent	Stock Concentration	Volume per Reaction (µL)
Molecular Biology Grade Water	---	2.8
$MgCl_2$	50 mM	2.5
Buffer B	5X	5
dNTP Mix	2.5 mM each	2.5
Platinum® Taq DNA Polymerase	5 units per µL	0.2
Primer mix	---	10
Template	1.0 ng per µL	2
		Total – 25 µL

[1]Reaction mix should be kept on bench-top cooler until ready for use.

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	63.0 °C for 1 minute
		3	72.0 °C for 1 minute 30 seconds
3	1	1	72.0 °C for 2 minute
4	Indefinite	1	Hold at 4.0 °C