Prevalence and sequence of IS481 in *Bordetella bronchiseptica*: implications for IS481-based detection of *Bordetella pertussis*

Karen B. Register¹* and Gary N. Sanden²

¹Respiratory Diseases of Livestock Research Unit, USDA/ARS/National Animal Disease Center, P.O. Box 70, Ames, Iowa 50010 and ²U.S. Public Health Service Commissioned Corps (Ret.), Centers for Disease Control and Prevention, National Center for Infectious Diseases, Division of Bacterial and Mycotic Disease, Meningitis and Special Pathogens Branch, Epidemiologic Investigations Laboratory, Atlanta, Georgia 30333

Running Title: IS481 in *B. bronchiseptica*

*Send proofs and correspondence to:

Dr. Karen B. Register
Respiratory Diseases of Livestock Research Unit
USDA/ARS/National Animal Disease Center
P.O. Box 70, 2300 Dayton Road
Ames, Iowa 50010

Phone: (515) 663-7700
Fax: (515) 663-7458
E-mail: kregiste@nadc.ars.usda.gov
We report the prevalence in *Bordetella bronchiseptica* of IS481, a frequent target for diagnosis of *Bordetella pertussis*, as approximately 5%. However, PCR amplicons of the predicted size were detectable in 78% of IS481-negative strains. Our results suggest PCR targeting IS481 may not be sufficiently specific for reliable identification of *B. pertussis*.
*Bordetella pertussis* is the etiologic agent of whooping cough, an acute respiratory disease occurring exclusively in humans. Widespread vaccination provides a high level of protection in children, but unvaccinated populations and cohorts in whom vaccination-induced immunity has waned remain at risk. Recently a reemergence of the disease within highly vaccinated populations has occurred and transmission from adult reservoirs to unimmunized or partially immunized children is of increasing concern in many areas of the world (36). A sensitive and specific method for identification of *B. pertussis* is crucial for monitoring prevalence, accurately defining epidemiology, and optimizing intervention and prevention strategies, including management of outbreaks. Culture remains the diagnostic standard but suffers from poor sensitivity, slow turn-around time, and practical difficulties. Additionally, it is necessary to distinguish between *B. pertussis* and *Bordetella parapertussis*, which may cause a milder pertussis-like syndrome in humans. PCR assays that differentiate between these bacteria, either alone or combined with traditional culture, are increasingly being implemented in diagnostic laboratories.

A frequently used target for PCR detection of *B. pertussis* is the repetitive element IS481 (29, 30), which is absent in *B. parapertussis* (8, 48) but found in approximately 50 to a few hundred copies in *B. pertussis* (16, 27, 34, 35, 42). While generally regarded as specific for *B. pertussis*, few studies definitively examine the prevalence of IS481 in the closely related agent *Bordetella bronchiseptica*. Despite recent evidence that *B. pertussis* may have evolved from a human-associated lineage of *B. bronchiseptica* (8), the latter agent is primarily associated with disease in mammals other than humans and, therefore, has been considered to have little clinical significance. However, human illness and carriage associated with *B. bronchiseptica* is on the rise, particularly in infants or immunocompromised hosts with exposure to carrier animals (1, 4,
While rare, disease in immunocompetent adults may also occur (5, 22, 26, 54).

Analysis of a few isolates by Southern blotting suggested IS481 may not be found in *B. bronchiseptica* (19, 27, 34), but one study provided evidence for a single copy in an isolate of unstated origin (16). van der Zee et al. (50) reported a prevalence of approximately 1% based on a diverse group of 144 strains. Unfortunately, the data upon which this conclusion is based were not presented and specific methods used to identify IS481 are not detailed either in the original report nor in the supporting references provided (49, 51). A more recent investigation evaluating 91 *B. bronchiseptica* isolates from a variety of host species identified only 2 (both equine) positive by PCR for an amplicon derived from the central region of IS481 (8). From a clinical perspective, it is important to know whether amplicons can be generated from *B. bronchiseptica* strains using IS481-specific primers designed to detect *B. pertussis*, potentially leading to an erroneous diagnosis. A review of the literature encompassing PCR methods used to identify *B. pertussis* reveals that “diagnostic” amplicons were obtained from *B. bronchiseptica* isolates in at least 5 investigations using primers specific for the 5’ end of the repetitive element (7, 13, 16, 20, 21) but by none using primers that target the 3’ end (3, 9, 10, 45). No diagnostic PCRs targeting the central region of IS481 have been proposed. Only about 20 *B. bronchiseptica* isolates were evaluated overall in these studies (the exact number being unclear from the details provided) and only a few were obtained from humans. The goal of the present study was to establish the prevalence of IS481 based on a larger number of *B. bronchiseptica* isolates, including those of human origin, and to assess the potential for misidentification of IS481-containing strains of *B. bronchiseptica* as *B. pertussis* when using IS481-specific PCR primers.
PCR primers IS481-1 and IS481-2 (see Fig. 1) were used to screen for IS481 in B. bronchiseptica. This primer set is used by the Pertussis Program Laboratory of the Centers for Disease Control for IS481-based identification of B. pertussis and encompasses a 252 bp region of the insertion sequence frequently selected as a target in B. pertussis diagnostic PCRs (3, 9, 10, 45, 53). Reactions included 0.4µM primers, 1U AmpliTaq polymerase (Applied Biosystems, Foster City, CA), 2.5µl 10X Buffer II (100mM Tris-HCl, pH 8.3, 500mM KCl), 2.5mM MgCl₂, 200µM dNTPs, and either 100ng of purified DNA (n=17), 0.5µl of a cell lysate (n=9; 41), or, in separate reactions, both (n=123), in a final volume of 25µl. PCR was carried out in an Applied Biosystems (Foster City, CA, USA) 9700 thermal cycler. Cycling conditions were 3min at 95°C, 35 cycles of 95°C for 15sec, 58°C for 30sec, and 72°C for 30sec, and a final extension step of 72°C for 10min. A total of 149 B. bronchiseptica isolates was evaluated (Table S1, supplemental material). These were obtained primarily from humans and companion or farm animal species; some have previously been examined for IS481 by other methods (8, 50). B. pertussis (Tohama) was used as a positive control. The integrity of templates and reagents was established by parallel testing with a 16S rRNA-specific PCR (41). Five µl of each PCR was analyzed by agarose gel electrophoresis.

Unexpectedly, an amplicon of the predicted size was detectable from 118 B. bronchiseptica isolates, including 22/24 of human origin, following PCR with IS481-1/IS481-2. Strain RB50, included as one of the negative controls since it does not contain IS481 (35), was falsely positive. One or two additional bands were present in reactions from 12 isolates. No amplification products were visualized from negative controls containing water in place of template, included with every set of PCRs. Representative results are shown in Fig. 2A and 2B. Increasing the annealing temperature to 60°C (primer Tₘ is 62-63°C) reduced the intensity of
some weakly fluorescent amplicons, but did not completely eliminate them. Significant differences in band intensity apparent in reactions with a standardized amount of purified DNA (Fig. 2A) suggest some isolates may contain one or more copies of IS481, although it seems likely that weaker amplicons of the expected size represent false positive results.

The strongly fluorescent amplicons of ~250 bp generated with purified DNA from 3 *B. bronchiseptica* isolates, representing 3 different species of origin, were each purified with spin columns (Qiagen, Valencia, CA) and sequenced directly (minimum of 3 reactions, at least 1 from each strand) as described (39). Sequence alignment showed 98.8-100% identity with the sequence originally reported for this region of *B. pertussis* IS481 (27, K.B. Register, unpublished data). The specificity of the IS481-1/IS481-2 PCR products from the 118 positive isolates was evaluated by Southern blotting, as described (40), using as a probe one of the sequenced *B. bronchiseptica* amplicons labeled with digoxigenin. Following exposures of 30 min or less an intense signal was evident only from the ~250 bp product of 7 isolates (Table 1, Table S1), all of which displayed strongly fluorescent amplicons of this size in ethidium bromide-stained gels. Representative results are shown in Fig. 2C.

The 7 confirmed IS481-positive isolates, as well as 48 PCR-positive isolates whose amplicons failed to hybridize with the IS481 probe, were further evaluated by Southern blotting with genomic DNA. Following digestion of 3ug of purified DNA with *Pst* I, for which no recognition sites are present in *B. pertussis* IS481 (27, 28), fragments were separated on 0.6% agarose gels and blots were prepared and hybridized with the *B. bronchiseptica* IS481 probe as described above. Signals were detected only from the 7 isolates already identified as containing IS481 and at least some appeared to contain more than a single copy (K.B. Register, unpublished data). Additional Southern blot analysis using alternative restriction enzymes clearly demonstrated all 7
isolates possess multiple copies of IS481. Results obtained with NarI, which has the most frequently occurring recognition sequence of the enzymes tested, are shown in Fig. 3.

These data suggest that moderately to weakly fluorescent IS481-1/IS481-2 amplicons, although of the predicted size, are not derived from IS481. Amplicons obtained from six IS481-negative B. bronchiseptica isolates, including RB50, were sequenced in an attempt to determine their origin. All sequences shared 100% identity with one another as well as with the 5’ and flanking region of an RB50 ORF predicted to encode an extracellular solute-binding protein (locus tag BB5004), identified from a BLASTN search of the genome database (http://www.sanger.ac.uk/Projects/B_bronchiseptica). Alignment of the IS481 primers with this segment of the RB50 genome revealed likely primer-binding locations which would result in a product of 252 bp (Fig. 4), consistent with our observations. No other regions of significant identity with the IS481-1/IS481-2 amplicon were noted within the sequence of the extracellular solute-binding protein gene spanned by the primers.

Because of this unexpected finding, additional analysis was carried out to assess the potential for unintended primer binding to DNA from other genera that might contaminate clinical samples or be inadvertently introduced from the environment. A BLAST search of the GenBank RefSeq database revealed regions of 100% identity with the thirteen to fifteen consecutive 3’ terminal bases of IS481-1 in sequences derived from Aspergillus oryzae, Candida albicans, and Streptomyces avermitilis and of IS481-2 in sequences from Saccharomyces cerevisiae, Homo sapiens, and multiple strains of both Streptococcus pyogenes and Escherichia coli. However, when the database was queried with a single sequence comprised of the concatenated primers and an N22 spacer no alignments with the potential to result in amplification of a product were identified. These results suggest that while there may be sequences in human DNA and some
bacterial and fungal species capable of weakly binding IS481-1 or IS481-2, a PCR amplicon is not likely to be generated.

Since some *B. bronchiseptica* strains possess at least a portion of IS481, misidentification of such strains as *B. pertussis* could occur when IS481-based PCR assays are used as the basis for a diagnosis of whooping cough. To more precisely evaluate the potential for misidentification with particular primer sets, we determined the DNA sequence of the *B. bronchiseptica* insertion element. Primers specific for the 5’ and 3’ ends of *B. pertussis* Tohama IS481 (IS481-5 and IS481-12; see Fig. 1), were used to generate amplicons comprising the entire insertion element from the 7 IS481-containing *B. bronchiseptica* isolates. Amplicons were purified and directly sequenced as described above. Sequence alignment revealed 2 variants of IS481, neither of which is identical to any of the 3 variants reported for *B. pertussis* (Fig. 1). It should be noted that, since all positive isolates have multiple copies of IS481 and the DNA sequences were obtained from purified PCR products, it is not possible to rule out the existence of additional, less frequently occurring variants. *B. bronchiseptica* variant 1 was found only in the isolate MBORD731 and shares the greatest degree of identity with *B. pertussis* variants (1 to 3 base substitutions depending on the variant used for comparison). The IS481 sequences from the 6 remaining *B. bronchiseptica* isolates are identical to one another (*B. bronchiseptica* variant 2) and have 25 to 27 base substitutions as compared to the *B. pertussis* variants. No base substitutions are shared between *B. pertussis* IS481 variants and those of *B. bronchiseptica*. Interestingly, one substitution in *B. bronchiseptica* variant 2 eliminates a purported start codon for ORF 3 (28), suggesting the insertion sequence may not be functional in most IS481-containing *B. bronchiseptica* isolates and perhaps explaining both its low prevalence and copy number. The single *B. bronchiseptica* isolate with an intact ORF3 start codon contains many
more copies of IS481 than the other positive isolates. Alternatively, if IS481 were relatively recently acquired, insufficient time may have elapsed for generating additional copies.

Based on a comparison of the *B. bronchiseptica* IS481 sequences with those of IS481 primers used for conventional PCR identification of *B. pertussis* (2, 3, 9, 10, 12, 13, 16, 18, 20, 21, 23, 25, 37, 45, 46, 47, 53), misidentification of IS481-containing strains of *B. bronchiseptica* seems likely to occur. Many of the suggested primers are 100% identical to the *B. bronchiseptica* sequence reported here while others have a few base substitutions which may still permit amplification. A subset of the most commonly used primer sets, including IS481-5/IS481-6 (18, 23, 46, 47), IS481-7/IS481-8 (21), IS481-5/IS481-8 (25), IS481-5/IS481-10 (13, 16), IS481-9/IS481-12 (9), IS481-11/IS481-14 (53), and IS481-13/IS481-16 (45; see Fig. 1 for primer sequences and locations), was tested with genomic DNA from the 7 *B. bronchiseptica* isolates containing IS481. Selection of an appropriate melting temperature during cycling was problematic, since the temperature used by different investigators for the same primer set sometimes varied greatly (e.g., a range of 55°C to 66°C for IS481-5/IS481-6) and many primer pairs are poorly matched (some differing by 10°C or more). In keeping with the conditions reported most frequently, primer pairs were tested at both 55°C and 58°C. PCR components and other cycling parameters were as described above. Amplicons were obtained from all isolates at both melting temperatures with all primer pairs tested, including those having one or more mismatched bases in comparison with *B. bronchiseptica* IS481 sequence. Representative results are shown in Fig. 5. Although no false positive results were noted with RB50, faint bands ~100-150 bp larger and/or smaller than the IS481-specific amplicons were evident for a few of the *B. bronchiseptica* isolates using 2 of the 3 primer pairs directed against the 3’ portion of the insertion element (K.B. Register, unpublished data).
Our data indicate an IS481 prevalence of roughly 5% in the group of *B. bronchiseptica* isolates examined (Table 1); others have reported a prevalence of roughly 1 to 2% (8, 50). The isolates evaluated by Diavatopoulos et al. (8) include 35 also evaluated here. Our results are concordant with the exception of strain MBORD669, previously reported to be IS481-negative but found here to be positive. Comparison of the sequence of MBORD669 IS481 with the primers used by Diavatopoulos et al. reveals the sequences are identical except for a single, internal base mismatch in the reverse primer, which seems unlikely to explain the discrepancy. Accidental cross-contamination of cultures in our laboratory also seems unlikely since the PvuII ribotype of MBORD669 is unique among our IS481-positive isolates (40, K.B. Register, unpublished data). A comparative evaluation of MBORD669 DNA obtained from each laboratory could perhaps assist in resolving the discrepancy. The remaining study (50), in which the method used to identify IS481 is not detailed, included 76 strains examined here. Our results are in agreement with theirs, except for 5 isolates reported here as positive for IS481 which were previously reported as IS481-negative. In the absence of additional experimental details the reason for this discrepancy is unclear. It could be postulated that our results are confounded by the presence of IS1002, an insertion sequence closely related to IS481 found in multiple *Bordetella* species including, rarely, *B. bronchiseptica* (8, 49). However, the degree of sequence identity between IS1002 (49) and our IS481 probe is insufficient to withstand the stringency of the conditions used for hybridizations. Detection of many identically sized restriction fragments with the IS481 probe in all strains positive by Southern blot (Fig. 3A, K.B. Register, unpublished data) also argues against cross-hybridization of the probe with IS1002.

The prevalence and sequences of IS481 in *B. bronchiseptica* reported here suggest the specificity of this target for identification of *B. pertussis* is less than presently perceived.
Although the insertion sequence was not detected in any of the 26 human isolates tested, its occurrence in strains obtained from farm and companion animals suggests transmission of IS481-containing *B. bronchiseptica* strains to humans remains a possibility. Also of concern is the finding that secondary amplicons not derived from IS481 but of the predicted size, as well as some with slightly different mobilities, were often apparent when using some primers derived from the 3’ region of *B. pertussis* IS481. Utilization of amplicon-specific probes in realtime PCR formats may alleviate this problem, but careful evaluation of IS481-based diagnostic assays for *B. pertussis* is essential, including an analysis of proven IS481-positive and -negative strains of *B. bronchiseptica*. It is unclear whether *B. bronchiseptica* isolates reported as PCR positive in assays targeting the 5’ region of IS481 are, in fact, IS481 positive (7, 13, 16, 20, 21). The details available suggest at least some amplicons may have been derived from secondary primer binding sites (16, 21) as observed for most isolates in this study with IS481-1/IS481-2.

Our observations, together with reports identifying numerous IS481-positive *Bordetella holmesii* strains (21, 24, 42, 45, 46, 47), raise significant concerns as to the likelihood of a false diagnosis of *B. pertussis* if IS481 PCR is the only test carried out, as observed in a recent quality assessment proficiency panel (33). All laboratories utilizing IS481 PCR mistakenly identified as *B. pertussis* both *B. bronchiseptica* and *B. holmesii*. The suggestion that IS481 assays are sufficiently specific for diagnosis of *B. pertussis* (43) may be premature. Consequently, promising alternative and/or confirmatory PCR targets should continue to be evaluated and clinical sensitivity and specificity of IS481-based PCR for pertussis need to be more rigorously defined.
The authors gratefully acknowledge the technical assistance of Michael Mullins. We are indebted to David Alt and the National Animal Disease Center Genomics Unit for DNA sequence data and to B. Rath, G. Foster, R. Welsh, and H.-J. Riising for providing *B. bronchiseptica* isolates.

GenBank accession numbers for the DNA sequences determined in this study are EF043395-EF043401.
Figure Legends

Fig. 1. Sequence and location of IS481 primers used in this study and comparison of B. bronchiseptica IS481 sequence variants with those reported for B. pertussis IS481. The top line shows the sequence originally reported for IS481 from B. pertussis Tohama (27). Sequence of Bp IS481v1 was also derived from the Tohama strain but from a different copy of IS481 (28). Sequence of Bp IS481v2 was derived from the Wellcome 28 strain (28). Dashes represent conserved bases; substitutions are indicated by the appropriate letter. Primer location and direction are indicated by shaded areas and arrows. Bases 601-612 are more darkly shaded to represent a region of overlap between the 3' end of IS481-1 and the 5' end of IS481-9. The proposed start codon and direction of transcription for ORF 3 (27) are specified by bolded, underlined bases and an arrow, respectively. The terminal inverted repeats are underlined. Nucleotide positions are numbered according to the sequence of McLafferty et al. (27).

Fig. 2. Amplicons from IS481-1/IS481-2 PCRs with B. bronchiseptica purified DNA (A) or cell lysates (B) detected by ethidium bromide staining. Lanes, A): 1) rabbit isolate (M116919/02/1); 2) dog isolate (MBORD595); 3) guinea pig isolate (MBORD673); 4) cat isolate (MBORD733); 5) human isolate (W48661); 6) turkey isolate (4448); 7) rabbit isolate (RB50); 8) negative control; 9) dog isolate (MBORD599); 10) B. pertussis. Relative positions of DNA molecular mass markers are indicated to the left of the panel. Lanes B): 1) DNA molecular mass marker; 2) B. pertussis; 3, 4) guinea pig isolates (MBORD762, MBORD665); 5) dog isolate (MBORD839); 6) pig isolate (5238); 7) human isolate (MO289); 8) rabbit isolate (MBORD835); 9) pig isolate (SG8); 10, 11) rabbit isolates (RB50, M602871/00/1); 12) negative control. C) Southern blot of the gel in panel
B hybridized with an IS481-specific probe as described in the text.

Fig. 3. Southern blots with NarI digests of *B. bronchiseptica* or, for comparison, *B. pertussis* genomic DNA hybridized to an IS481-specific probe. Lanes, panel A: 1) dog isolate (MBORD595), 2-6) guinea pig isolates (MBORD665, MBORD666, MBORD668, MBORD669 and MBORD678, respectively), 7) horse isolate (MBORD731). Lanes, panel B: 1) *B. pertussis*; 2) *B. bronchiseptica* RB50. Relative positions of DNA molecular mass markers are indicated at the side of each panel.

Fig. 4. Potential binding sites for IS481-1 and IS481-2 (shown as the reverse complement) within and flanking the BB5004 locus of the RB50 genome. Base matches are indicated in bold. Coordinates shown are from the genome database sequence. The RB50 sequence in the intervening segment is 100% identical to the sequence derived from false positive amplicons, as described in the text.

Fig. 5. Amplicons obtained from PCR with primers targeting the 5’ end (IS481-5/IS481-6, panel A) or 3’ end (IS481-13/IS481-16, panel B) of *B. pertussis* IS481 using *B. bronchiseptica* genomic DNA and a melting temperature of 58°C. Lanes: 1) DNA molecular mass marker; 2) MBORD595; 3) MBORD665; 4) MBORD666; 5) MBORD668; 6) MBORD669; 7) MBORD678; 8) MBORD731; 9) *B. pertussis*; 10) RB50.
Table 1. Prevalence and host species distribution of IS481 in the *B. bronchiseptica* isolates included in this study.

<table>
<thead>
<tr>
<th></th>
<th>Guinea Pig</th>
<th>Pig</th>
<th>Turkey</th>
<th>Seal</th>
<th>Sea Otter</th>
<th>Leopard</th>
<th>Unknown</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Dog</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>Cat</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Horse</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rabbit</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Pig</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Turkey</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Seal</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sea Otter</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Leopard</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Unknown</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>24</strong></td>
<td><strong>30</strong></td>
<td><strong>8</strong></td>
<td><strong>7</strong></td>
<td><strong>20</strong></td>
<td><strong>11</strong></td>
<td><strong>27</strong></td>
<td><strong>13</strong></td>
</tr>
</tbody>
</table>

1. Number of isolates in which an amplicon of the predicted size could be detected in agarose gels and which hybridized with an IS481-specific probe.
2. Number of isolates from which no amplicon could be detected in agarose gels.
3. Number of isolates from which an amplicon of the predicted size was present which failed to hybridize with an IS481-specific probe (false positives).


clinical manifestations of respiratory infections due to *Bordetella pertussis* and other

characterization of a repetitive DNA element from the genome of *Bordetella pertussis*


*pertussis* carry a repeated DNA sequence not found in other *Bordetella* species. FEMS
Microbiol. Letts. **41**:357-360.


sequencing for identification of "Pseudomonas-like" isolates from sputum of patients

33. Muyldermans, G., O. Soetens, M. Antoine, S. Bruisten, B. Vincart, F. Doucet-
Populaire, N. K. Fry, P. Olcén, J. M. Scheftel, J. M. Senterre, A. van der Zee, M.
43:30-35.

repeated DNA sequence specific for Bordetella pertussis. FEMS Microbiol. Letts.

35. Parkhill, J., M. Sebaihia, A. Preston, L. D. Murphy, N. Thomson, D. E. Harris, M.
T. Holden, C. M. Churcher, S. D. Bentley, K. L. Mungall, A. M. Cerdeño-Tárraga,
L. Temple, K. James, B. Harris, M. A. Quail, M. Achtman, R. Atkin, S. Baker, D.
Basham, N. Bason, I. Cherevach, T. Chillingworth, M. Collins, A. Cronin, P. Davis,
J. Doggett, T. Feltwell, A. Goble, N. Hamlin, H. Hauser, S. Holroyd, K. Jagels, S.
Rabbinowitsch, S. Rutter, M. Sanders, D. Saunders, K. Seeger, S. Sharp, M.
Simmonds, J. Skelton, R. Squares, S. Squares, K. Stevens, L. Unwin, S. Whitehead,
B. G. Barrell, and D. J. Maskell. 2003. Comparative analysis of the genome sequences
of Bordetella pertussis, Bordetella parapertussis and Bordetella bronchiseptica. Nat.
Genet. 35:32-40.


O. Stapp, and P. M. Abe. 2002. Bordetella pertussis PCR: simultaneous targeting of


901
\(\rightarrow IS481-16\)

Bp IS481
CACACCTACC AAGAACTCCCA ACACCGAGCC GATGCCATGA AATCCTGGCT ACACCACTAC

Bp IS481v1
---------- ---------- ---------- ---------- ---------- ----------

Bp IS481v2
---------- ---------- ---------- ---------- ---------- ----------

Bb IS481v1
---------- ---------- ---------- ---------- ---------- ----------

Bb IS481v2
---------- A---------- C----------

Bb IS481v2

961
\(\leftarrow IS481-14\)

Bp IS481
AACTGGCATC GACCCCACCA AGGCATCGGG CGCGCTGTAC CCATCTCCAG ACTCAACCTG

Bp IS481v1
---------- ---------- ---------- ---------- ---------- ----------

Bp IS481v2
---------- ---------- ---------- ---------- ---------- ----------

Bb IS481v1
---------- ---------- ---------- ---------- ---------- ----------

Bb IS481v2
---------- G---------- T----------

Bb IS481v2

1021
\(\leftarrow IS481-12\)

Bp IS481
GACGAATACA ACCTATTGAA TCTTCACAGC TAG

Bp IS481v1
---------- C AG----------

Bp IS481v2
---------- C AG----------

Bb IS481v1
-----

Bb IS481v2
-----
Bb 5004 ...ATCGAAGCTGGCTCCGCTTTCAACGCCACC....CTTACCTTTACGCAGATGCTTGCCATGTCCCTGTA...
IS481-1 GCCGGCTGGGACTTCGTCTTC
rcIS481-2 CTTACC G CCTTACC G CCCACAG