Letters to the Editor

A Specific Polymerase Chain Reaction Based on the *gyrB* Gene Sequence and Subsequent Restriction Fragment Length Polymorphism Analysis of *Pasteurella pneumotropica* Isolates from Laboratory Mice

Dear Editor,

During a routine check of published polymerase chain reaction (PCR) conditions for detection of laboratory animal pathogens prior to using the assay, I discovered an error of omission in the forward primer sequence proposed by Hayashimoto, et al, 2007.[1] Specifically, the CZO-1 primer contains a deletion of 4 nucleotides found in all *P. pneumotropica* gyrB gene sequences deposited in the GenBank as of this date. Below is a Clustal-X DNA sequence alignment using the published CZO-1 primer sequence in relationship to the GenBank accessions.

	1	25
AB213398	AATTACAGCT CACTATTCGC	CGTCA
AB213399	AATTACAGCT CACTATTCGT	CGTCA
AB269888	AATTACAGCT CACTATTCGC	CGTCA
AB269889	AATTACAGCT CACTATTCGC	CGTCA
AY258262	AATTACAGCT TACTATCCGC	CGTCA
CZO-1	AATTACAG.....	CTATTCGY CGTCA

The authors submitted the first three accessions to GenBank and accession AY258262 was submitted by another laboratory. As you can see, 4 centrally located nucleotides are not contained in the CZO-1 primer. A BLAST search of the GenBank using the published CZO-1 primer discloses no matches to any deposited *P. pneumotropica* sequences. Central mismatch of nucleotide sequence in primers leads to a substantial reduction in annealing temperature, leading me to believe that this primer sequence would not reliably detect *P. pneumotropica*. In addition, the CZO-1 primer has a nucleotide mismatch at position 12 in relation to accession AY258262 that would further diminish the specificity of this primer for *P. pneumotropica.* I conclude that either the GenBank accessions are incorrect (however, this conclusion is unlikely in that the sequences were obtained by 2 different laboratories) or the published CZO-1 primer sequence is inappropriate for reliable detection of *P. pneumotropica*.

Individuals planning to use published amplification primer sequences should consider checking the sequence specificity using National Center for Biotechnology Information (NCBI) Basic Alignment Search Tool (BLAST) at URL http://blast.ncbi.nlm.nih.gov/Blast.cgi To use this tool, select "nucleotide blast," changing the database to "nucleotide collection", enter the primer nucleotide sequence into the FASTA textbox, and click the BLAST button. The NCBI website will by default return the first 100 best-match GenBank sequences aligned to the input primer sequence regardless of whether the match is sense or anti-sense. This list and the associated alignments allow rapid determination of the specificity of the sequence for its intended gene target.

Sincerely,
Sanford H Feldman, DVM, PhD, DACLAM
Professor and Director, Center for Comparative Medicine
University of Virginia

Reference

1. **Hayashimoto N, Masami U, Takakura A, Itoh T.** 2007. A specific polymerase chain reaction based on the *gyrB* gene sequence and subsequent restriction fragment length polymorphism analysis of *Pasteurella pneumotropica* isolates from laboratory mice. J Am Assoc Lab Anim Sci **46:**54-58.

Response to Dr Feldman's Letter to the Editor:

We thank Dr Feldman for his comments regarding our recently published article.[2] As pointed out by Dr Feldman, the published sequence of primer "CZO-1" lacks 4 nucleotides at positions 11-14 in Figure 1.[2] The corrected primer sequence for CZO-1 was submitted and appeared in the *Journal of the American Association for Laboratory Animal Science*.[3] The sequence and PCR data in this article was obtained by the appropriate methods and we are confident that it is correct.

Dr Feldman compared the sequence data AY258262 with our sequence data. In the NCBI website (http://www.ncbi.nlm.nih.gov/nuccore/32364163), the sequence data AY258262 originates from the strain Bisgaard Taxon 17 CCUG 17206 that was regarded as a closely related different species (taxon) with *P. pneumotropic*a.[1] Although it is currently indicated as *P. pneumotropica* in CCUG (Culture Collection University of Göteborg), it is still classified as Bisgaard Taxon 17 in the literature.[1] In this study, we considered Bisgaard Taxon 17 not to be *P. pneumotropica* and never used strain CCUG 17206 or the sequence data AY258262 in Genbank.

Sincerely,
Nobuhito Hayashimoto, DVM
Central Institute for Experimental Animals, Japan

References

1. **Olsen I, Dewhirst FE, Paster BJ, Busse HJ.** 2005. Family *I. Pasteurellaceae*. In: Gerrity GM, editor. Bergey's manual of systematic bacteriology, 2nd ed, vol 2. New York (NY): Springer-Verlag. p 851-866.
2. **Hayashimoto N, Masami U, Takakura A, Itoh T.** 2007. A specific polymerase chain reaction based on the *gyrB* gene sequence and subsequent restriction fragment length polymorphism analysis of *Pasteurella pneumotropica* isolates from laboratory mice. J Am Assoc Lab Anim Sci **46:**54-58.
3. **Hayashimoto N, Ueno M, Takakura A, Itoh T.** 2009. Erratum: a specific polymerase chain reaction based on the *gyrB* gene sequence and subsequent restriction fragment length polymorphism analysis of *Pasteurella pneumotropica* isolates from laboratory mice. J Am Assoc Lab Anim Sci **48:**137.

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