Supplemental Methods

Bacterial DNA preparation

To evaluate the specificity of Lepto-*rrs* LAMP, genomic DNA was extracted from bacterial cultures using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The concentration of the extracted DNA was determined using an ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). Genomic DNA was serially diluted for the sensitivity test, and the number of genome copies in the Lepto-*rrs* LAMP reaction mixture was calculated based on a molecular size of 5.1 Mbp (*L. interrogans* Fiocruz L1-130) (1).

Primer design

The *rrs* sequences from pathogenic, intermediate, and non-pathogenic *Leptospira* spp. (accession nos. AB279549, AE016823, AY631876, AY631877, AY631878, AY631879, AY631880, AY631881, AY631883, AY631884, AY631885, AY631886, AY631895, AY796065, EF025496, EF612284, and Z21634) and other bacteria belonging to the family Leptospiraceae, e.g., *Leptonema illini* (AY714984) and *Turneriella parva* (AY293856), were aligned and compared using GENETYX software ver. 9 (Genetyx Cooperation, Tokyo, Japan). Lepto-*rrs* LAMP primers were designed using PrimerExplorer V4 software (available online at https://primerexplorer.jp/lamp4.0.0/index.html) and manually modified. The location of the

primers in *rrs* is shown in Fig. S1. The primer set was designed to amplify a region of *rrs* from both pathogenic and intermediate *Leptospira* spp but not from non-pathogenic ones. Nucleotides at the 5′ terminus of FIP and 3′ terminus of BIP primers were mismatched for *rrs* sequences of non-pathogenic spp., thereby allowing amplification of only pathogenic and intermediate *Leptospira* spp. (Fig. S1).

Reference

1. **Nascimento, A. L. T. O., S. Verjovski-Almeida, M. A. Van Sluys, C. B. Monteiro-Vitorello, L. E. Camargo, L. A. Digiampietri, R. A. Harstkeerl, P. L. Ho, M. V. Marques, M. C. Oliveira, J. C. Setubal, D. A. Haake, and E. A. L. Martins.** 2004. Genome features of *Leptospira interrogans* serovar Copenhageni. Braz. J. Med. Biol. Res. **37:**459–477.

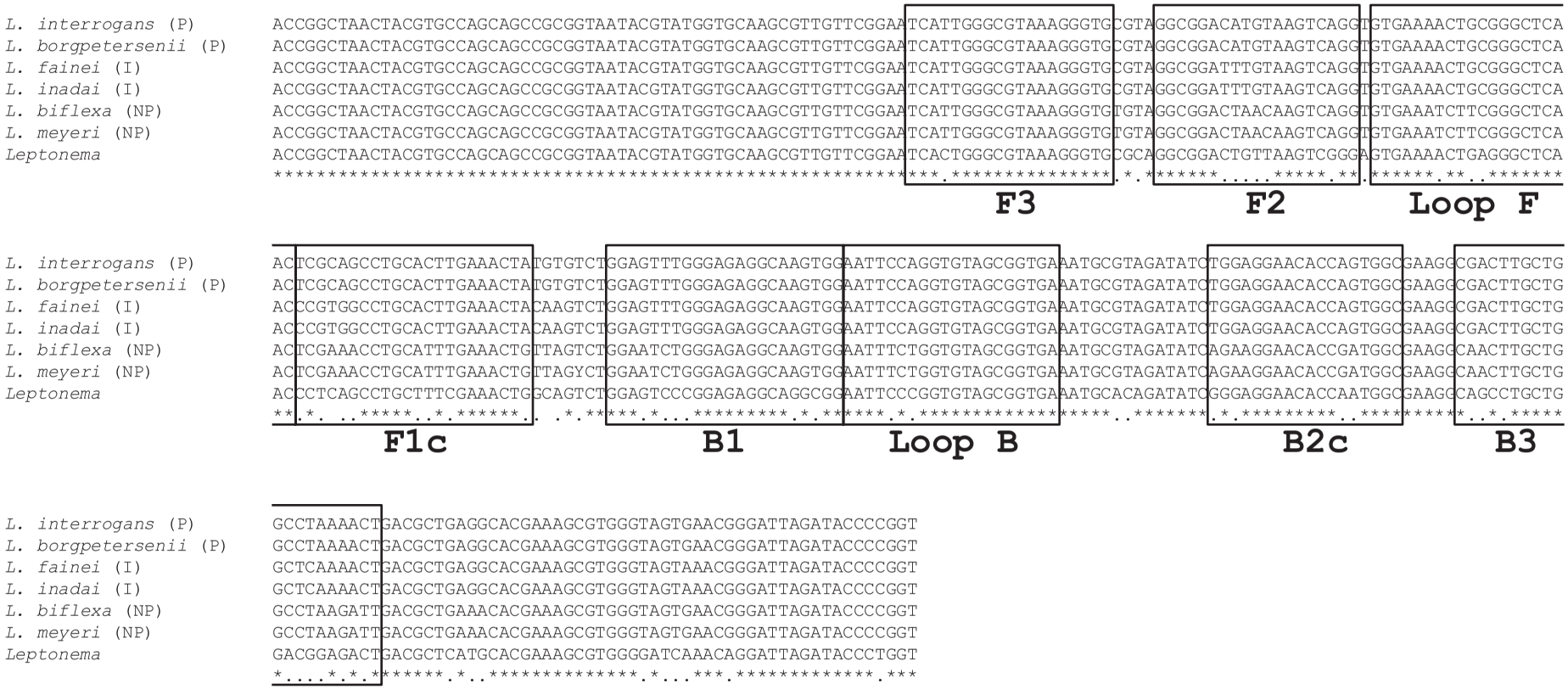

Fig. 1. Location of LAMP primers in *Leptospira rrs*. Accession numbers of the *rrs* sequences used in this figure are AE016823 (*L. interrogans*), AY631876 (*L. biflexa*), AY631878 (*L. meyeri*), AY631884 (*L. borgpetersenii*), AY631885 (*L. fainei*), AY714984 (*Leptonema illini*), and Z21634 (*L. inadai*). P, pathogenic species; I, intermediate species; NP, nonpathogenic species as per 16S rRNA gene sequence analysis.

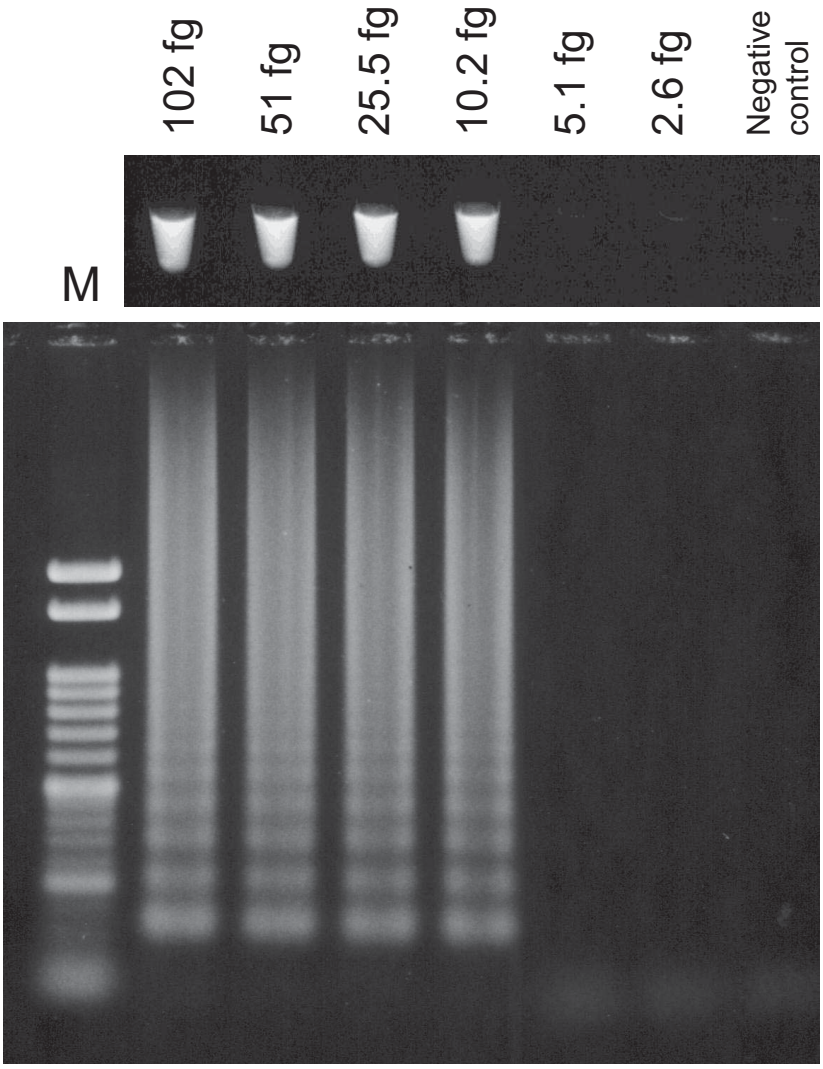

Fig. S2. Sensitivity test using purified genomic DNA from *L. interrogans*. Lepto-*rrs* LAMP reactions were detected by UV fluorescence (upper panel) or by confirming a ladder-like pattern on 2% agarose gel (lower panel). M, DNA marker (OneSTEP Ladder 50; Nippon Gene, Tokyo, Japan).

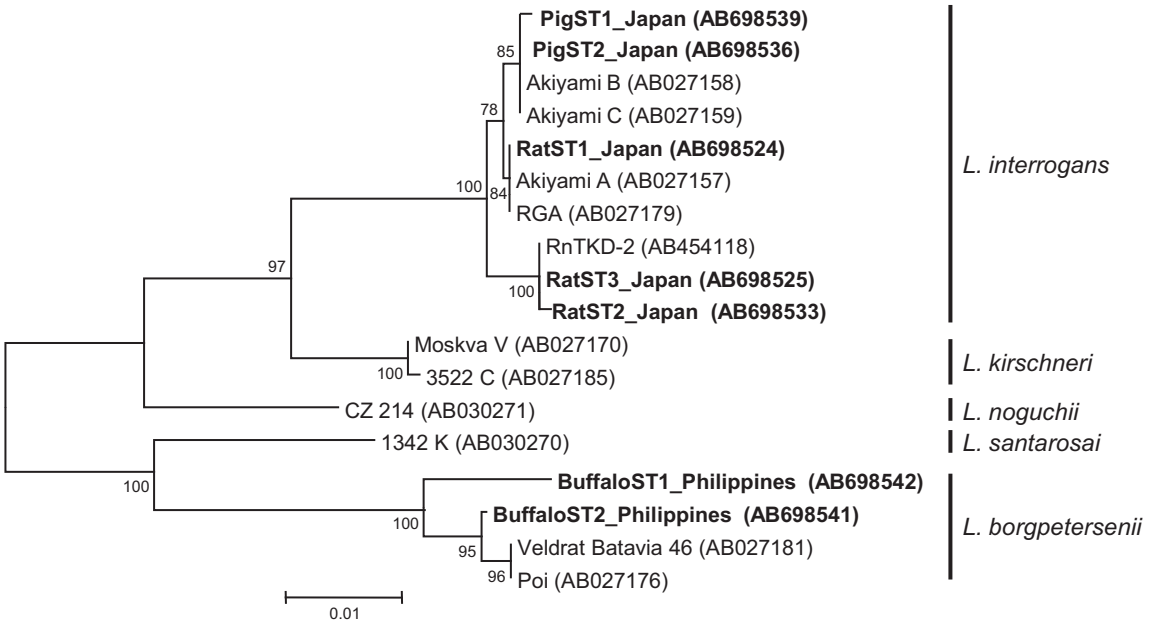

Fig. S3. Phylogenetic tree based on the *Leptospira flaB* gene sequence. The sequences obtained in this study were indicated in bold type. An accession number of each sequence is indicated in parentheses. The sequences were aligned in MEGA4 (1) using CLUSTALW, and phylogenetic distances were calculated in MEGA4 using the neighbor-joining method. Numbers on nodes are bootstrap support after 1000 replicates.

Reference

1. **Tamura, K., J. Dudley, M. Nei, and S. Kumar.** 2007. MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. **24:**1596–1599.