Supplementary file: Validation of bisulphite multiplex real-time PCR assays using synthetic constructs

Method:

Dilute 10ng/uL synthetic constructs in molecular grade water to 2x10^9c/uL.

Bisulphite convert 10^8 copies using SP009 (Genetic Signatures Ltd.) according to manufacturer’s instructions to a final concentration of 10^6c/uL.

Dilute from 10^6c/uL to 1c/uL in molecular grade water.

Synthetic constructs were assayed according to methods described in manuscript.

Synthetic construct sequence data:

*L. tropica*, partial sequence derived from accession number KF633255.1

TGCGTCGCGCGGGGCGCCGCCACCCGGTGCGTTTGGAGCGCGGCGCGCGGCGGTCGGCGTGACACGCGGTCGCGTGCGCGGAGAACATCCACCAACCGGCGCCGGGCTGTGTGGGTGGTG

*L. infantum*, partial sequence derived from accession number [AF097655.1](https://www.ncbi.nlm.nih.gov/nucleotide/AF097655.1?report=genbank&log$=nuclalign&blast_rank=1&RID=B7FAH2C801R)

GCCCGCCGCGGTGTGTGCCAGGGCGCGGCGCCCCGCACCGCCCGCGCGAGGCGAGCCCGGTGCGCGGCCATGGTGGTGACGCGCGGGCCCGTGCGCGGAGAACATCCGCCCCGCGAATGC

*L. major*, sequence derived from accession number XR\_002460056.1 AACTAACGCTATATAAGTATCAGTTTCTGTACTTTATTGGTATGCGAAACTTCCGGAACCTGTCTTCCGGCAAGATTTTGGAAGCGCGCAAGCGCTATTTTTTTTTGTGTGCGTGCGTGTGGTGGTGGCGCCCCCCTGCTGTCCGGCGGGTGGCGCGGCTCCCGCGGCGGTGGCCACGCCGCGCTGCAAGGGTGCGCCCGCGCCGCTGGGGGCCACGGGGCCGCGGAGCAGGCGTCCCCCGAGCGCCGCGGCCCAGGAGCAACGCGGCCGCGGTGTGCGTGCGCGCGGCCGCCTCCGTCCCGCGGGCGCTGCCGGCGAGTGTGTGTCGGGGGCGCGGCACCGCCTGGCCCGTGGGGAGCGCGGTGCGCGGCAGTGTGCATGCCACGCGGCGGCGTGCGCGGGGAACGGCCACCCCCCAGCGCCGGGCTGCGGTGGTGATGGCTTTT

*L. mexicana*, partial sequence derived from accession number [AY155508.1](https://www.ncbi.nlm.nih.gov/nucleotide/AY155508.1?report=genbank&log$=nucltop&blast_rank=1&RID=B7G3PTGN013)

GTGGTGGCCGCCGTGGCGGTGGTGGTGCGCCGGCGCCGGGCACGGGGTGTGCGCGCGCGGCCGCTCCCCGCGCGACGCGGCGCTGCCCCGGGCCGTGGGGAACGCCGAGGGCGCCGGCGG

*L. amazonensis,* partial sequence derived from accession number [L05395.1](https://www.ncbi.nlm.nih.gov/nucleotide/L05395.1?report=genbank&log$=nucltop&blast_rank=1&RID=B7G61YET016)

GCGCTGCCCCGGGCCGTGGGGNGTGGGCGCCGGCAGCCGTGACACGTGGCCCGGTGCGGTGCGGCGGCCCCCCCGGCGCCGTGCTGTGGGTGAGCGGCTTTTAACTAACGCTATATAAGT

*L. panamensis*, sequence derived from accession number MG010486.1 AACTAACGCTATATAAGTATCAGTTTCTGTACTTTATTGGTATGCGAAACTTCCGGGACCCGTCTTCCGGCAACATTTTGGGTGCGCCATGGCGCTTTTTTTTTTTCTTTTTTGTGTGTGGCGGGGGTGTGTCCTGGAGGTGGCGCCCAGGACCGCCGCCCATGACACGGGGTTCGGCACGCCCCAAAACGGCACCCCCCTCACAACGACCTGGGCACCCTTGGGCTTCTAACTAACGCTATATAAGTATCAGTTTCTGTACTTTATTG

Results:

Figure S1. Results obtained using the differentiation assays on synthetic constructs (Cycle threshold (Ct) averaged from 3x PCR replicates). The standard curve was plotted using log10 concentration versus Ct value.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Ave. log c/uL | *L. tropica* | *L. infantum* | *L. major* | *L. mexicana* | *L. amazonensis* | *L. panamensis* |
| 6.00 | N/A | N/A | 14.58 | N/A | N/A | N/A |
| 5.00 | 25.37 | 24.18 | 18.04 | 24.47 | 24.09 | 22.62 |
| 4.00 | 27.79 | 26.26 | 21.48 | 27.77 | 27.15 | 27.93 |
| 3.00 | 30.87 | 29.41 | 24.71 | 31.39 | 30.90 | 30.92 |
| 2.00 | 33.70 | 31.32 | 28.12 | 36.55 | 34.44 | 34.54 |
| 1.00 | 36.38 | 37.08 | 33.49 | 39.85 | N/A | 36.74 |
| - | N/A | 39.84 | 36.11 | N/A | N/A | N/A |

Table S1. Results obtained using the differentiation assays on synthetic constructs (Cycle threshold (Ct) averaged from 3x PCR replicates). N/A- not recorded as data points were excluded if they did not follow the one-in-ten series of approximately 3.3Ct values between points (due to inhibition by purification artefacts at high copy numbers or poor linearity at low copy numbers).

The following synthetic constructs were additionally evaluated during early development and assessment. Early development and assessment and results not reported here.

Synthetic construct sequence data:

*L. braziliensis*, partial sequence derived from accession number [MG010484.1](https://www.ncbi.nlm.nih.gov/nucleotide/MG010484.1?report=genbank&log$=nucltop&blast_rank=1&RID=BJ81WCPT013)

AACATTTTGGGTGCGCCATGGCGCTTTTTTTTTTTTTTGTGTGTGTGGTGGCGGGTGTGTGTCCTGGAGGTGGCGCCCAAGGCCGCCGCCCATGACACGGGGTTCGGGACGCCCCAAAAC

*L. colombiensis*, partial sequence derived from accession number [AY155502.1](https://www.ncbi.nlm.nih.gov/nucleotide/AY155502.1?report=genbank&log$=nucltop&blast_rank=1&RID=BJ80BM4P016)

TGCCGGCGCGATTCCGGCACCCCATGCCCAGTGGCCGGGACCCCCCCCTCTGGTCCCCCCCCATGATCCGGCGCACGCCTGACACGCGGTTAGTGCATGCCAAATGAGCCACCCCCCTCA

*L. garnhami,* sequence derived from accession number [KF633213.1](https://www.ncbi.nlm.nih.gov/nucleotide/KF633213.1?report=genbank&log$=nucltop&blast_rank=1&RID=BJ7W4EMR013) GAACCCGTCTTCCGGCAAGATTTTGGAAGCGCGCAGGCGCTATTTTTTTTGTGTGCGGCAGCGCCCCGCCCGCCATTGCGCGGGTGTGCGCCGGGCACGGGGTGTGCGTGCGCGCGGCCGTTCCCCGCACGGCGCGGCGCTGCCCCGGGCCGTGGGGAGCGCCGAGGGCGCCGGCAGCCG

*L. killicki,* partial sequence derived from accession number [AY155504.1](https://www.ncbi.nlm.nih.gov/nucleotide/AY155504.1?report=genbank&log$=nucltop&blast_rank=2&RID=BJ7TZ6WE016)

TGCGTCGCGCGGGGCGCCGCCACCCGGTGCGTTTGGAGCGCGGCGCGCGGCGGTCGGCGTGACACGCGGTCGCGTGCGCGGAGAACATCCACCAACCGGCGCCGGGCTGTGTGGGTGGTG

*L. aethiopica,* partial sequence derived from accession number [KF633188.1](https://www.ncbi.nlm.nih.gov/nucleotide/KF633188.1?report=genbank&log$=nucltop&blast_rank=1&RID=BJ7RG4ZJ013)  
TATTTTTTTTGTGTGCGTGTGGTGGTGGCGGCGCCCCCCGTGCTGTCCGGCGGGGTGGCGCGGCTCCCGTGGCGGTGGCTCGGCCGCGCTGCGAGGGTGCGCCCGCGCCCCTGGGGGCCACAGGGCCGCGGAACCGGCGTCCGCCAGAGCGCGGCGGCCCGGGAGCGCCGGGCGCGGGGT

*L. guyanensis,* sequence derived from accession number MG010485.1 AACTAACGCTATATAAGTATCAGTTTCTGTACTTTATTGGTATGCGAAACTTCCGGGACCCGTCTTCCGGCAACATTTTGGGTGCGCCATGGCGCTTTTTTTTTTTCTTTTTTGTGTGTGGCGGGTGTGTGTCCTGGAGGTGGCGCCCAAGACCGCCGCCCATGACACGGGGTTCGGCACGCCCCAAAACGGCACCCCCCTCACAACGACCTGGGCACCCTTGGGCTTCTAACTAACGCTATATAAGTATCAGTTTCTGTACTTTATTG

*L. naiffi,* sequence derived from accession number AY155505.1 ACTTTATTGGTATGCAAAACTTCCGGGACCCGTCTTCCGGCAACATTTTGGGAGCGCGATGGCGCTGTTTTTTTTCTTTTTTTTTTTTGGCCTGTGTCCTGGTGGTGGCGCCCAAGGCCGCCGCCCATGACACGAGGTTCGGTACGCCCAAAAATGGCACCCCCCTCACAACGACCTGGGCACCCCAGGGCTTCTAACTAACGCTATATAACTATCAGTTTCTGT

*L. peruviana,* sequence derived from accession number AY155506.1 ACTTTATTGGTATGCGAAACTTCCGGGACCCGTCTTCCGGCAACATTTTGGGTGCGCCATGGCGCTGTTTTTTTTTTTTGTGTGTGTGGTGGCGGGTGTGTGTCCTGGAGGTGGCGCCCAAGGCCGCCGCCCATGACACGGGGTTCGGGACGCCCCAAAACGGCACCCCCCTCACAACGACCTGGGCACCCTTGGGCTTCCAACTAACGCTATATAAGTATCAGTTTCTGT

*L. lainsoni,* sequence derived from accession number X69448.1 ACTTTATTGGTATGCGAAACTTCCGGGACCCGTCTTCCGGCAACATTTTGGGAGCGCGATGGCGTTTCTTTTTTTTTGTCGTTTGGTGTGTGTTTCTTCTTTTTTTTTGGTGGGGATATAAGTATCAGTTTCTGT

*L. venezueliensis*, partial sequence derived from accession number [AY155507.1](https://www.ncbi.nlm.nih.gov/nucleotide/AY155507.1?report=genbank&log$=nucltop&blast_rank=18&RID=BJ8D22WJ013)

GTCGGGGGCGCGGCACCGCCTGGCCCGTGGGGAGCGCGGTGCGCGGCGGTGTGCATGACACGCGGCGGCGTGCGCGGGGAACGGCCACCCCCCAGCGCCGGGCTGCGGTGGTGATGGCT