

Supplementary Table 1 Measurement of melting temperatures of L/I/L2 variants by LNA real-time PCR

| Sample [copies, number of samples (n)] | Target sequence* | Measured T_m (°C) at Channel | | | | | | | | Cy5 | | | |
|---|-------------------------|--------------------------------|-----|-------------------|---|-----|-----|-------------------|---|------|-----|-------------------|---|
| | | FAM | | | | Hex | | | | | | | |
| | | Mi | Ma | Mean | ± | Mi | Ma | Mean | ± | Min | Ma | Mean | ± |
| | | n | x | SD | | n | x | SD | | | x | SD | |
| L + I positive control mixture (4.0E+01 ~ 4.0E+08, n= 64) | AAACTCAAGCAATG | 59. | 63. | 62.0 ± 0.5 | | 58. | 60. | 59.4 ± 0.5 | | 54.6 | 55. | 55.1 ± 0.4 | |
| | TT + | 7 | 8 | (63, 98.4%) | | 8 | 9 | (64, 100%) | | 8 | | (27, | |
| | AAAATCAAGCAATG | | | | | | | | | | | 42.2%) | |
| I + L2 positive control mixture (4.0E+01 ~ 4.0E+08, n= 64) | AAAATCAAGCAATG | - | - | - | | 58. | 60. | 59.5 ± 0.4 | | 64.4 | 64. | 64.6 ± 0.1 | |
| | TT + | | | (0, 0%) | | 8 | 7 | (63, 98.4%) | | 8 | | (64, | |
| | AAACTAAAGCAATG | | | | | | | | | | | 100%) | |
| L + I' positive control mixture (4.0E+01 ~ 4.0E+08, n= 64) | AAACTCAAGCAATG | 61. | 62. | 62.0 ± 0.3 | | 54. | 57. | 56.7 ± 0.6 | | 54.8 | 55. | 55.3 ± 0.3 | |
| | TT + | 3 | 8 | (64, 100%) | | 4 | 5 | (64, 100%) | | 9 | | (18, | |
| | AAAATCAA <u>A</u> CAATG | | | | | | | | | | | 52.9%) | |

| | | | | | | | | | | |
|------------------------------|-------------------------|-----|-----|-------------------|-----|-----|-------------------|---|---|---------|
| | TT | | | | | | | | | |
| L' + I positive control DNA | AAACTCAA <u>A</u> CAATG | 57. | 58. | 57.9 ± 0.3 | 58. | 61. | 59.5 ± 0.5 | - | - | - |
| mixture | TT + | 3 | 7 | (64, 100%) | 8 | 6 | (64, 100%) | | | (0, 0%) |
| (4.0E+01 ~ 4.0E+08, n= 64) | AAAATCAA <u>A</u> CAATG | | | | | | | | | |
| | TT | | | | | | | | | |
| L' + I' positive control DNA | AAACTCAA <u>A</u> CAATG | 57. | 61. | 58.1 ± 0.5 | 56. | 57. | 56.7 ± 0.3 | - | - | - |
| mixture | TT + | 5 | 0 | (63, 98.4%) | 3 | 5 | (64, 100%) | | | (0, 0%) |
| (4.0E+01 ~ 4.0E+08, n= 64) | AAAATCAA <u>A</u> CAATG | | | | | | | | | |
| | TT | | | | | | | | | |

T_m , melting temperature; -, no significant T_m

^a SD, standard deviation

^b by direct sequencing of PCR products