Supporting Information

Legends of figures

Figure 1. Workflow of the MGparC-AsyHRM method
WT: Wild-type

Figure 2. Results of assays 1 and 2 of the MGparC-AsyHRM method

Figure 3. Flexibility of the MGparC-AsyHRM method. (a) Effect of a different probe; (b) performance of a probe harboring double mutations (S83I+D87Y); (c) compatibility of the probe with other genes; (d) generalizability of the MGparC-AsyHRM model; (e) adjustability of the MGparC-AsyHRM model.

Supplementary information

Table S1 Primers and probes of real-time PCR used in this method.

Table S2 Primers of common PCR used in this study.

Table S3 Limit of detection of each target variant.

Figure S1 Illustration of sequence structure from 83 to 87 position in parC gene.
Table S1. Primers and probes of real-time PCR used in this method.

| Target gene | Direction | Primers | Reference |
|-------------|-----------|---------|-----------|
| MGpa        | Forward   | GAGAAATACCTTGATGGTCAGCAA | (1)       |
|             | Reverse   | GTTAATATCATATAAAAGCTCTACCGTTGTTATC |           |
|             | probe     | FAM-ACTTTGCAATCAGAAGGT-MGB |           |

standard curve method:
We prepared plasmid containing porA and was diluted into a series of gradients (range from 1 copy/μL to 1 x 10^4 copy/μL) for making standard curve. The procedure of quantification is the same as described previously (2).
| Target gene | Direction | Primers | Size (bp) | Reference |
|-------------|-----------|---------|-----------|-----------|
| parC        | in-Forward-119F1 | 5’GGTTAAAAACCAGTACAAAGACGGA3’ | 226 | this study |
|             | in-Reverse-385R1  | 5’ACACAGAAACCGCTTAAGCT3’ | 27  |           |
|             | out- Forward -97F2 | 5’GCTTACCTGATCTAAAGATGGGT3’ | 427 |           |
|             | out- Reverse--524R2 | 5’GCTATCCACTCGACCATT3’ | 427 |           |
Table S3. Limit of detection of each target variant.

| Target | No. of replicates (%) for positive standards, copies/reaction |
|--------|-------------------------------------------------------------|
|        | 1000 500 200 100 50 20 10 2                                |
|        | **probe**, **parC**, **parC**-amplicon; **parC**-amplicon   |
| Assay2 | WT                             10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 |
|        | S83C                           10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 |
|        | S83N                           10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 |
|        | S83R                           10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 |
|        | S83I                           10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 |
|        | D87H                           10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 |
|        | D87Y                           10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 |
|        | D87N                           10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 |
|        | D87G                           10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 |
| Assay1 | D87MT                          10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 9/10 |
|        | D87WT                          10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 |
|        | mgpa                           10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 |
|        | HBB                            10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 10/10 |

1. Jensen JS, Björnelius E, Dohn B, Lidbrink P. Use of TaqMan 5' nuclease real-time PCR for quantitative detection of Mycoplasma genitalium DNA in males with and without urethritis who were attendees at a sexually transmitted disease clinic. J Clin Microbiol. 2004 Feb;42(2):683-92. doi: 10.1128/JCM.42.2.683-692.2004. PMID: 14766837; PMCID: PMC344445.

2. Dupin N, Bijaoui G, Schwarzinger M et al. Detection and quantification of Mycoplasma genitalium in male patients with urethritis. Clin Infect Dis. 2003 Aug 15;37(4):602-5. doi: 10.1086/376990. Epub 2003 Jul 30.
Figure S1. Illustration of sequence structure from 83 to 87 position in parC gene.