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Sequencing of *Mycoplasma genitalium* genomes using a tiling amplicon method on the Nanopore MinION

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Abstract

Mycoplasma genitalium is challenging to work with and new methods are needed to study this bacterium directly in clinical samples. This study designed and validated a proof-of-concept polymerase chain reaction (PCR)-based 'tiling' methodology to sequence *M. genitalium* genomes. Primers were designed to produce 2.5 kb amplicons covering the 580 kb genome with a minimum overlap of 100 bp. Analysis was performed using the laboratory strain G37 and a clinical isolate. Amplicons were sequenced on the Oxford Nanopore MinION using ligation sequencing. Reads were mapped to a reference to produce a consensus genome. A total of 262 primer pairs were designed and amplification was successful for 99.5% (261/262) of 2.5 kb amplicons, with G37 genome coverage of 99.5% (mean read depth, 1973X). Using larger 5kb amplicons, amplification was successful for 92.4% (121/131) of primer pairs, with a coverage of 92.2% (mean read depth, 223X). When validated on a clinical isolate, 98.3% coverage was achieved (read depth, 443X). In conclusion, this study developed a PCR-based tiling approach to whole genome sequencing of *M. genitalium* by designing and validating a set of 262 primer pairs.

Impact Statement

The challenge of isolating and propagating *Mycoplasma genitalium* from clinical samples for laboratory analysis (such as antibiotic susceptibility testing and whole genome sequencing) has hindered research into antimicrobial resistance and pathogenesis. This study capitalizes on the small genome of *M. genitalium* to develop a method for genome sequencing from clinical samples. PCR primers validated here can be used for other applications, e.g. molecular typing or antimicrobial resistance determination.

Keywords: *Mycoplasma genitalium*; amplicon sequencing; genomics

Introduction

Mycoplasma genitalium is a sexually transmitted pathogen of global health importance due to a limited number of available treatment options combined with increasing levels of antimicrobial resistance. Azithromycin and moxifloxacin are the two main antibiotic treatments; however, in 2018–21 global resistance levels were 33% and 13%, respectively, an increase from 2012 levels of 13% and 7%, respectively (Chua et al. 2025). Azithromycin resistance is conferred by single nucleotide polymorphisms (SNPs) at positions 2058 and 2059 of the 23S rRNA gene (*E. coli* numbering) (Jensen et al. 2008), and fluoroquinolone resistance is associated with amino acid changes in the topoisomerase enzyme (subunit ParC) and DNA gyrase (subunit GyrA) (Deguchi et al. 2001, Couldwell et al. 2013, Kikuchi et al. 2014, Murray et al. 2017, Chambers et al. 2019, Conway et al. 2020, Murray et al. 2020, Murray et al. 2023). Molecular typing suggests that resistance is likely arising from independent sporadic mutations, which are generated during treatment, rather than the spread of resistant strains (Twin et al. 2012, Anagrius et al. 2013, Kikuchi et al. 2014, Pond et al. 2014, Bissessor et al. 2015, Fookes et al. 2017, Read et al. 2017, Fernández-Huerta et al. 2020).

There is a need for surveillance to monitor the levels of antimicrobial-resistant *M. genitalium* to inform public health and treatment guidelines (CDC 2019). While some commercial diagnostic assays can detect resistance-conferring mutations, they typically do not report individual mutations so are unable to provide suitable data for surveillance of resistance mutations. Molecular typing of the *mgpB* and MG309 genes has also been performed by select laboratories around the world for retrospective molecular epidemiological analyses but sequencing of a single gene can be too discriminatory and group similar strains into separate groups (Piñeiro et al. 2019, Fernández-Huerta et al. 2020, Guiraud et al. 2020, Sweeney et al. 2020, Chua et al. 2021, Seo et al. 2021, Dumke 2022). To address these issues, and the limitations associated with these approaches, whole genome sequencing can be utilized.

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Isolation of *M. genitalium* remains difficult and its routine application is impractical in clinical settings, rendering traditional genomic sequencing a challenge. Direct shotgun sequencing of *M. genitalium* from a clinical sample is a potential solution; however, low bacterial load and an abundance of host DNA present barriers. To overcome such limitations, this proof-of-concept study investigates the feasibility of a tiling amplicon sequencing method for whole genome sequencing of the relatively small 580 kb *M. genitalium* genome, using an approach described previously for viruses (Quick et al. 2017).

Materials and methods

Primer design and preparation

Primers were designed to generate 'tiling' amplicons spanning the entire *M. genitalium* genome. The web-based primer design tool, Primal Scheme (https://primalscheme.com) (Quick et al. 2017) was used to produce amplicons of an average length of 2.5 kb with a 100 bp overlap and annealing temperature of 60°C. All five *M. genitalium* genomes from GenBank (accession numbers NC_000 908, NC_018 495, NC_018 496, NC_018 497, and NC_018 498) were used as a template to design the primers. Primer pairs were manually checked on Geneious Prime (version 2021.2; Biomatters Ltd, Auckland, New Zealand) and redesigned where required. The final primer panel contained 262 primer pairs (Supplementary File S1). Each primer was ordered at 100 μM, totalling six 96-well plates. Primers constituting a pair were mixed in a separate 96-well plate to achieve a final concentration of 10 μM each.

Mycoplasma genitalium DNA for primer validation

For primer validation, a *M. genitalium* strain G37 co-cultured with Vero cells, described previously (Jensen et al. 1996, 2004), was used. For specificity testing, a previously-isolated *M. genitalium* clinical isolate (MGA20) propagated in Vero cells was used (Huaman et al. 2025). Cultures were centrifuged at 20 000 *g* for 30 min and DNA was extracted on the MagNA Pure 96 instrument using the MagNA Pure 96 DNA and Viral NA Small Volume Kit (Roche, Basel, Switzerland).

To validate the primer pairs, the G37 DNA extract was initially quantified using the Nanodrop (Thermo Fisher Scientific, Massachusetts, USA) and diluted to 2 ng/μl. To determine the minimum concentration of *M. genitalium* DNA required for successful amplification, a ten-fold limiting dilution of the G37 DNA extract was performed and quantified using digital droplet PCR targeting the *mgpB* gene (Peh et al. 2023). Three randomly selected primer pairs were used to amplify the dilution series. PCR reactions with ≥5000 genome equivalents of *M. genitalium* DNA were amplified successfully. The MGA20 DNA extract was diluted to the minimum number of genome equivalents for subsequent validation of primer pair specificity.

Amplification of the complete *M. genitalium* genome by PCR

Polymerase chain reactions (PCRs) were set up in 96-well plates with a 2.5 kb primer pair in each well. Each PCR reaction consisted of 1X Q5® Hot Start High-Fidelity 2X Master Mix (New England Biolabs, Massachusetts, USA; amplicon length up to 20 kb), nuclease-free water, 10 ng DNA, and a primer mix containing 0.5 μM of each respective forward and reverse primer. Cycling conditions were as follows: initial denaturation at 98°C for 40 s, 30 cycles of 98°C for 10 s, 60°C for 15 s, and 72°C for 2 min, and final extension at 72°C for 2 min. Successful amplification was determined visually on an agarose gel. Primers which did not successfully amplify were repeated once. To produce 5 kb amplicons, the odd numbered forward primers were matched with the even numbered reverse primers (e.g. forward primer mg_1a and reverse primer mg_2b; Supplementary File S1). Cycling conditions were as above, except the extension time was increased to 2 min and 30 s. To produce 10 kb amplicons 12 primer pairs, covering the first 107 576 bp of the genome, were used and the extension time was increased to 7 min (Supplementary File S1).

Sequencing of amplicons on the Oxford Nanopore MinION

Amplicons were purified using the Mag-Bind® Total Pure NGS Kit (Omega Bio-tek, Georgia, USA) then quantified using the Qubit dsDNA HS Assay Kit (Thermo Fisher Scientific, Massachusetts, USA). Amplicons of 2.5 kb were normalized to 10 ng/μl using elution buffer prior to pooling. For samples with less than 10 ng/μl, the whole volume was used (the median amplicon concentration was 20.2 ng/μl and ranged from 0.53–49 ng/μl). For the 5 kb amplicons, equal volumes of each amplicon were pooled based on the band intensity visualized using gel electrophoresis. The whole volume of amplicon was added if the corresponding band intensity showed a faint band.

The pooled amplicons were prepared for sequencing using the SQK-LSK114 Ligation Sequencing Kit (Oxford Nanopore Technologies, Oxford, UK) and sequenced on the MinION using a FLOW-MIN114 R10.4.1 Flow Cell (Oxford Nanopore Technologies, Oxford, UK) until a minimum of 100 reads per amplicon was generated to achieve a mean read depth of at least 100X.

Whole genome sequencing of the laboratory G37 and MGA20 isolate

To evaluate the amplicons generated for the clinical *M. genitalium* isolate propagated in Vero cells, whole genome sequencing of a laboratory G37 strain and the MGA20 isolate was performed to act as reference sequences. The laboratory G37 was cultured in Hayflicks medium until stationary phase, as described previously (Peh et al. 2023) and the MGA20 isolate was co-cultured with Vero cells as described previously (Jensen et al. 1996, 2004) until stationary phase. To extract DNA for sequencing, cultures were centrifuged at 20 000 *g* for 30 min. Pellets were resuspended in 1X DNA/RNA Shield (Zymo Research, California, USA) and DNA extracted using the Microbial Lysis Protocol of the Quick-DNA™ HMW MagBead Kit (Zymo Research, California, USA). DNA was prepared for sequencing using a ligation sequencing kit (SQK-NBD114.96; Oxford Nanopore Technologies, Oxford, UK) and sequenced on the MinION using a R10.4.1 flow cell (FLO-MIX114; Oxford Nanopore Technologies, Oxford, UK).

Basecalling was performed with the Super Accurate (SUP) model version 5.0.0 and adaptor trimming using Dorado version 0.7.2 (https://github.com/nanoporetech/dorado). Reads were filtered based on quality (quality score of 9 or above) using nanoq version 0.10.0 (https://github.com/esteinig/nanoq). Filtered reads were assembled *de novo* into contigs using Flye

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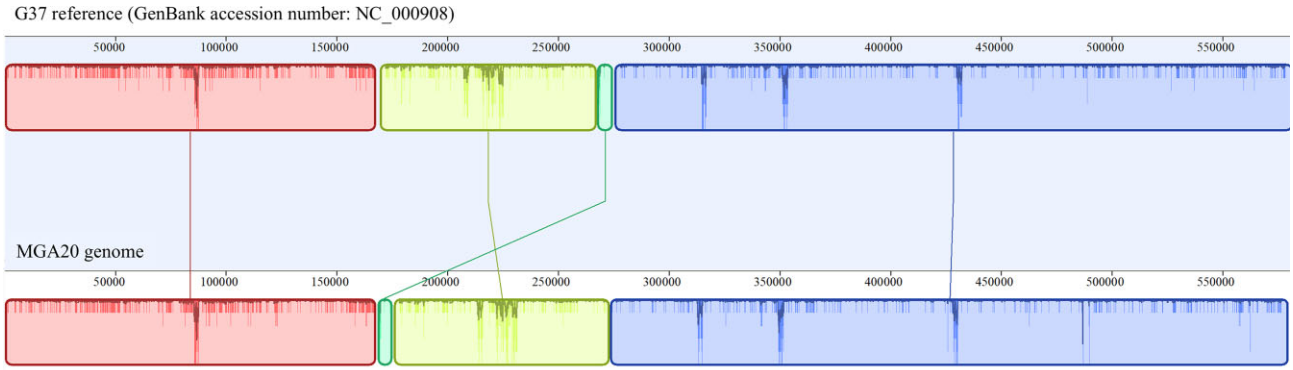


Figure 1. Rearrangement of the rRNA operon in the MGA20 *Mycoplasma genitalium* clinical isolate (bottom) compared to the *M. genitalium* G37 reference genome (top). Numbers indicate position on genome. Coloured blocks indicate regions of the genome; the green block is the portion of the genome that has moved from between the yellow and blue block in the G37 reference to between the red and yellow block in the MGA20 genome. This green region corresponds to a portion of the *mgpC* gene and the rRNA operon (16S rRNA, 23S rRNA, and rrf rRNA genes). Visualized using Mauve genome alignment version 1.1.3 (Darling et al. 2010) through Geneious version 2025.02 (Biomatters Ltd, Auckland, New Zealand).

version 2.9.2 (https://github.com/fenderglass/Flye) and contigs were polished using Medaka version 1.9.1 (https://github.com/nanoporetech/medaka).

The laboratory G37 and MGA20 genomes were successfully sequenced with 100% coverage and a read depth of 206X for the former and a coverage of 98.8% and read depth of 27.9X for the latter. Genome sequences can be found in Supplementary File S2 and the MGA20 genome sequence is available at GenBank (accession: CP199926). Both genomes were compared to the GenBank G37 genome sequence (accession number NC_000 908) with the progressive Mauve algorithm (Darling et al. 2010) using Geneious Prime version 2025.0.2 (Biomatters Ltd, Auckland, New Zealand) to identify any variations. Alignment of the laboratory G37 genome identified several SNPs, particularly within adhesin genes. Alignment of the MGA20 genome identified a rearrangement of the rRNA operon (Fig. 1) in which a portion of the genome (6173 bp in length corresponding to a majority of the *mgpC* cytoadherence gene, and 16S, 23S, and *rrf* rRNA genes) was transferred to a position downstream of the G37 genome.

Data analysis and visualization of whole genomes generated by tiling amplicon sequencing

Dorado version 0.7.2 (https://github.com/nanoporetech/dorado) was used for basecalling raw reads with the high accuracy protocol (version 5.0.0) and for trimming adaptors. Reads were filtered based on size (1200 to 4000 bp for 2.5 kb amplicons and 3500 to 6100 bp for 5 kb amplicons) and quality (quality score of 9 or above) using nanoq version 0.10.0 (https://github.com/esteinig/nanoq). Mapping of filtered reads was performed using Minimap2 version 2.26 (Li 2018, 2021). SAMtools version 1.16.1 (Danecek et al. 2021) was used to filter mapped reads by mapping quality score (quality score of 60 or above) and to generate alignment statistics.

To generate a consensus genome, consensus sequences were generated for each amplified region using Flye version 2.9.2 (https://github.com/federglass/Flye) and polished with Medaka version 1.9.1 (https://github.com/nanoporetech/medaka). These were then mapped to the respective reference genome with Minimap2 version 2.26 (Li 2018, 2021) and a consensus sequence covering the complete genome was extracted. The consensus genome was mapped to the reference using the progressive Mauve algorithm (Darling et al. 2010) as a plugin in Geneious Prime version 2025.0.2 (Biomatters Ltd, Auckland, New Zealand) to determine the % identity.

Results and discussion

Evaluation of primer pairs and amplicon size using G37 DNA

A total of 262 primer pairs, covering the entire *M. genitalium* genome at 2.5 kb intervals, were generated using the tool Primal Scheme (Quick et al. 2017) (Supplementary File S1). In brief, primers ranged from 19–30 bp with an average GC content of 43% (range 30%–60%) and an average melting temperature of 60.3°C (range 52.8–65°C). The primers produced on average 2.5 kb amplicons (range 1399–3780 bp) with an average overlap of 283 bp (range 108–1773 bp). Primers were validated using DNA from the *M. genitalium* laboratory strain G37. All 2.5 kb primer pairs produced an amplicon except for one set spanning the *ori* region of the genome (i.e. primer pair 262). This was unexpected as this region of the genome is highly conserved. Several additional primers were designed around this region with no success. PCR failure across the *ori* could be due to a tendency for the region to form secondary structures, or the very low GC content (~16%, compared to genome average of ~32%).

To reduce the number of PCR reactions and DNA requirement, forward and reverse primers were mixed to produce larger amplicons of 5 kb and 10 kb. This approach also reduced mispriming and, if multiplexing PCR reactions, primer dimer formation. Of the 131 primer pairs required to produce 5 kb amplicons (sizes ranging between 3635 bp and 6033 bp with an overlap between 108 bp and 1689 bp), 121 (92.4%) successfully amplified. The primer pairs which did not amplify were not localized to one section of the genome but dispersed throughout. Lack of amplification was most likely due to DNA fragmentation. Again, primers covering the *ori* region failed to amplify. To evaluate the feasibility of generating amplicons with an average size of 10 kb, the first 12 primer pairs were tested; amplifica-

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tion was unsuccessful, suggesting fragmentation of the DNA template.

For amplicon sequencing, both the DNA for G37 and MGA20 were extracted using the MagNA Pure 96, a standard diagnostic extraction platform which is not specifically designed for high molecular weight extraction. In contrast, for the direct whole genome sequencing, DNA was extracted using the Quick-DNA™ HMW MagBead Kit (Zymo Research, California, USA) which can extract DNA up to 150 kb: however, the average fragment size of the reads was only 2 kb. This indicates that there is difficulty retaining the structural integrity of whole *M. genitalium* genomes, regardless of whether a high molecular weight extraction method is used. This could be due to the fragile cell envelope of *M. genitalium* making its genome prone to fragmentation. Failure to produce 10 kb amplicons and a reduction in the proportion of 5 kb amplicons that successfully amplified, compared to 2.5 kb amplicons (92.4% and 99.6%, respectively), suggests an average fragment size between 2.5 and 5 kb after DNA extraction. This constraint on amplicon length can be overcome by using 2.5 kb primer pairs in regions where 5 kb primers fail to amplify.

Assembly of G37 genome from tiled amplicon sequences

Mapping of the 2.5 kb amplicons to the laboratory G37 genome reference covered 99.5% (577.2 out of 580.1 kb) of the genome with a mean read depth of 1973X (Table 1; Fig. 2). A consensus sequence was generated for 228 out of the 261 2.5 kb amplicons; the number of consensus sequences generated was less than the number of amplicons due to two reasons: (i) some abutting amplicons were assembled together to form one consensus, and (ii) some amplicons did not reach the threshold for read depth. Of these, 223 mapped to the laboratory G37 genome sequence to generate a consensus whole genome sequence while the remaining five sequences either did not align to anything, or were sequences from bacteria commonly found as trace contaminants in reagents (e.g. *Acinetobacter*) and Vero cells. The percentage identity between this consensus genome and the laboratory G37 sequence was 95.8%; this discrepancy was due to gaps within the genome as well as random variations between the two G37 strains used (amplicon template DNA from G37 co-cultured with Vero cells and whole genome sequencing DNA from G37 grown in Hayflick medium). Mapping of the 5 kb amplicons to the laboratory G37 genome reference covered 91.6% (531.4 out of 580.1 kb) of the genome with a mean depth of 223X (Table 1; Fig. 2). A total of 133 consensus sequences were generated for each sequenced amplicon region, of which 130 aligned to the laboratory G37 genome sequence to generate a consensus whole genome sequence. The percentage identity between the consensus genome and the reference was 88.0%; this was lower than for the 2.5 kb genome because amplification success was lower for the 5 kb primers.

The coverage for the genome generated using 5 kb amplicons was less than that generated using 2.5 kb amplicons; 10 primer pairs failed to amplify, resulting in up to 10% (~50 kb) of the genome missing. The majority of reads (over 50%) were <3500 bp, indicating fragmented DNA resulting in higher levels of incomplete extension for the larger amplicons. Enrichment of longer fragments (i.e. >3 kb) could be included during the ligation step, however,

Table 1. Summaryof sequencing results for both 2.5 kb and 5 kb amplicons from *Mycoplasma genitalium* laboratory G37 strain and the MGA20 clinical isolate.

Sample (amplicon size)	Total number of reads	Number of reads after filtering* (n; %)	Average read quality	Mean read length (bp)	% coverage	Mean read depth (X; range)^	Number of consensus sequences mapped	Percent identity to reference (%)
G37 (2.5 kb only)	642 356	475 518 (74.0)	16.9	2 486	99.5	1 973 (0–10 379)	223/228	95.8
G37 (5 kb only)	64 121	28 158 (43.9)	16.8	4 656	91.6	223 (0–1 636)	130/133	88.0
MGA20 (2.5 and 5 kb)	183 677	72 717 (39.6)	15.3	3 474	98.4	448 (0–3 388)	135/135	89.4

*Filtered by quality score (>9) and length (1200 bp to 4000 bp for 2.5 kb reads; 3500 bp to 6100 bp for 5 kb reads).
^Note this is the mean read depth of the whole genome, including regions where no amplicon was present.

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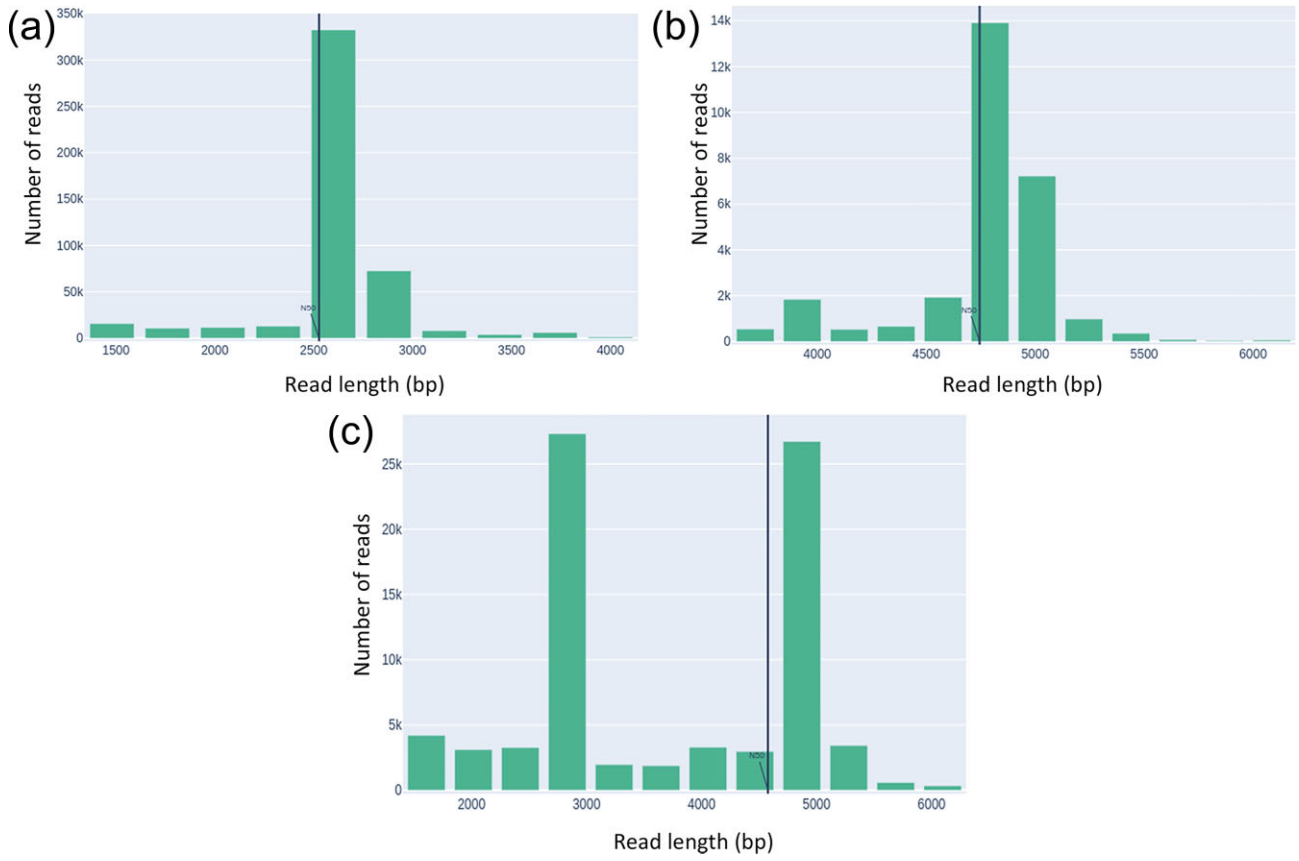


Figure 2. Distribution of read lengths after filtering for (a) G37 (2.5 kb amplicon size), (b) G37 (5 kb amplicon size), and (c) MGA20 (2.5 and 5 kb amplicon sizes). Black line represents N50.

a more useful strategy could be to use the 2.5 kb primer pairs (amplicon sizes 1399 bp to 3780 bp) to amplify the failed 5 kb regions, increasing the chance of amplifying regions where DNA is fragmented. In the case of insufficient DNA for repeat PCR reactions, the 5 kb amplicons alone can still generate a large portion of the genome, saving cost and DNA.

Amplification and genome assembly of the MGA20 clinical isolate

Based on the G37 results, the 5 kb primer pairs were chosen to amplify the MGA20 clinical isolate. Of the 131 primer pairs required to amplify the whole gnome, 114 (87%) successfully produced an amplicon. To recover more of the MGA20 genome, regions that failed to amplify with 5 kb primer pairs were amplified with the corresponding 2.5 kb primer pairs; of the 17 regions missing an amplicon, 15 (88%) were successfully amplified. Reads were mapped to the GenBank G37 reference genome (GenBank accession: NC000908) with a coverage of 98.4% (570.8 out of 580.1 kb) and mean read depth of 448X (Table 1; Fig. 2). A total of 135 consensus sequences were generated for each amplicon of which all aligned to the GenBank G37 reference genome to generate a consensus whole genome. The pairwise identity between the consensus genome and the MGA20 reference genome was 89.4%. Most importantly, the rRNA rearrangement in the genome was not identified as the G37 reference was used. To overcome this *de novo* assembly of the consensus sequences can be used, however, the number of consensus sequences makes this computationally difficult. Regardless, these initial results indicate that the tiling amplicon method is a feasible approach to sequencing *M. genitalium* genomes, although, regions of the genome where rearrangements have occurred will require further investigation.

Alternative applications: targeted sequence analysis of key genomic regions

Amplicon-based sequencing for *M. genitalium* has previously been performed for a small number of key genes for molecular typing and antimicrobial resistance determination (Plummer et al. 2020, Zhou et al. 2022, Chiribau et al. 2024). The primer pairs designed in this study can be adapted for these applications. Analysis of three key resistance genes (23S rRNA, *parC*, and *gyrA*) and two genes commonly used for strain typing (*mgpB* and MG309) was performed for the two amplicon-generated genomes.

Sequencing of the 23S rRNA gene of G37 using the 2.5 kb and 5 kb amplicons, identified no mutations at the locations conferring macrolide resistance in 23S rRNA gene, indicating this is a macrolide susceptible strain. Analysis of the MGA20 sample found an A2059G mutation (*M. genitalium* numbering) in the 23S rRNA gene, conferring macrolide resistance. This mutation was confirmed by the MGA20 whole genome reference sequence. Similarly, for the *parC* and *gyrA* genes, no mutations were identified in the G37 sample generated using either 2.5 or 5 kb amplicons, although amplification was

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only successful in the *gyrA* region for the 2.5 kb amplicons. For the MGA20 sample, a G248T mutation in *parC*, corresponding to an S83I amino acid change, and a G285A mutation in *gyrA*, corresponding to an M95I change, were found, consistent with the independently generated whole genome reference sequence.

In addition to analysing key resistance genes, sequences for the *mgpB* and MG309 genes used for strain typing were assessed for reproducibility to the sample reference genomes. A small portion of the *mgpB* gene of 281 bp is used for strain typing *M. genitalium* (Hjorth et al. 2006). For G37, analysis of this portion of the gene did not identify any SNPs in the region when amplified with either the 2.5 kb or 5 kb primer sets, indicating no variation from the G37 reference (GenBank accession number NC_000 908). For the MGA20 sample, sequencing identified that it belonged to sequence type 161 (PubMLST numbering) (Jolley et al. 2018). Analysis of the MG309 lipoprotein gene in both G37 genomes generated with the 2.5 kb and 5 kb amplicons had the same number of repeats as the laboratory G37 reference and the GenBank G37 reference (GenBank accession number NC_000 908), indicating no variation from the wild type reference strain. Likewise, the same number of repeats were identified for MGA20 as the independently generated MGA20 reference genome.

Typing of *M. genitalium* has often been limited to single- or dual-locus typing. Whole genome sequences generated using the primer panel presented in this study provides the opportunity to amplify a large portion of the genome, facilitating phylogenetic analysis at a higher resolution than single- or dual-locus typing. Alternatively, current strain typing methods used for *M. genitalium* can be expanded using the primer panel presented here, although, more genomes are required to determine additional regions of the genome suitable for inclusion in an extended typing scheme.

Limitations

The study has limitations. First, primers were designed using the limited set of complete *M. genitalium* genomes on GenBank; therefore, they may be impacted by genomic rearrangements such as that seen in the MGA20 sample. Moreover, the analysis method presented in this study relies on a reference genome, thus, genomic sequencing of more *M. genitalium* samples is required to understand the genetic structure of these bacteria. Second, DNA fragmentation could limit the utility of this approach, but this can be overcome by using the smaller 2.5 kb primers. Third, this approach requires over 100 PCR reactions; while the reaction volume was reduced to minimize cost, multiplexing primers is an alternative which reduces both the number of PCR reactions and the amount of DNA required. This is especially important for clinical samples, in which DNA is often a limiting constraint (Murray et al. 2019). Finally, validation of this method directly on clinical samples is still required. While this tiling amplification method may not always produce a full genome, generating a substantial portion of the genome remains beneficial to our understanding of this bacterium.

There are advantages to this method, mainly that specific regions of the genome can be selectively sequenced, enabling 'patching' of regions which failed to be sequenced or had low coverage, and confirmation of mutations or recombinant regions identified using alternative whole genome sequencing approaches.

Conclusions

This study presents a proof-of-concept tiling amplicon approach to sequencing the *M. genitalium* genome by (i) designing a set of primer pairs which produce overlapping amplicons that cover the whole genome, and (ii) validating these primers using the G37 laboratory strain and a clinical isolate. A large proportion of a genome was generated from a clinical isolate, despite the presence of genomic alterations. In addition to genomic sequencing, the primers can be adapted for other applications such as resistance surveillance and molecular typing. Increasing antimicrobial resistance in *M. genitalium* necessitates routine surveillance, however, this has been hindered by the fastidious nature of *M. genitalium* and the low bacterial load of clinical samples. The tiling amplicon method enables the amplification of *M. genitalium* DNA without the need for host DNA depletion or hybridization baits for DNA enrichment, providing an alternative and inexpensive method for whole genome sequencing of *M. genitalium*.

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Author contributions

Teck-Phui Chua (Data curation [equal], Formal Analysis [equal], Investigation [equal], Methodology [equal], Writing – original draft [equal], Writing – review & editing [equal]), Jennifer Danielewski (Conceptualization [equal], Data curation [equal], Formal Analysis [equal], Supervision [equal], Writing – review & editing [equal]), Jose L. Huaman (Formal Analysis [equal], Supervision [equal], Writing – review & editing [equal]), Erica Plummer (Formal Analysis [equal], Supervision [equal], Writing – review & editing [equal]), Catriona Bradshaw (Funding acquisition [equal], Supervision [equal], Writing – review & editing [equal]), Lenka Vodstrcil (Resources [equal], Supervision [equal], Writing – review & editing [equal]), Dorothy Machalek (Supervision [equal], Writing – review & editing [equal]), Suzanne Garland (Funding acquisition [equal], Supervision [equal], Writing – review & editing [equal]), and Gerald L. Murray (Conceptualization [equal], Formal Analysis [equal], Funding acquisition [equal], Methodology [equal], Project administration [equal], Supervision [equal], Writing – review & editing [equal])

Supplementary data

Supplementary data is available at *LAMBIO Journal* online.

Conflict of interest: The authors have no conflicts to declare.

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tralian Government Research Training Program Scholarship (T.P.C.).

Data availability

The data underlying this article are available in the article and in its online supplementary material. The MGA20 genome sequence is available at GenBank (accession: CP199926).

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