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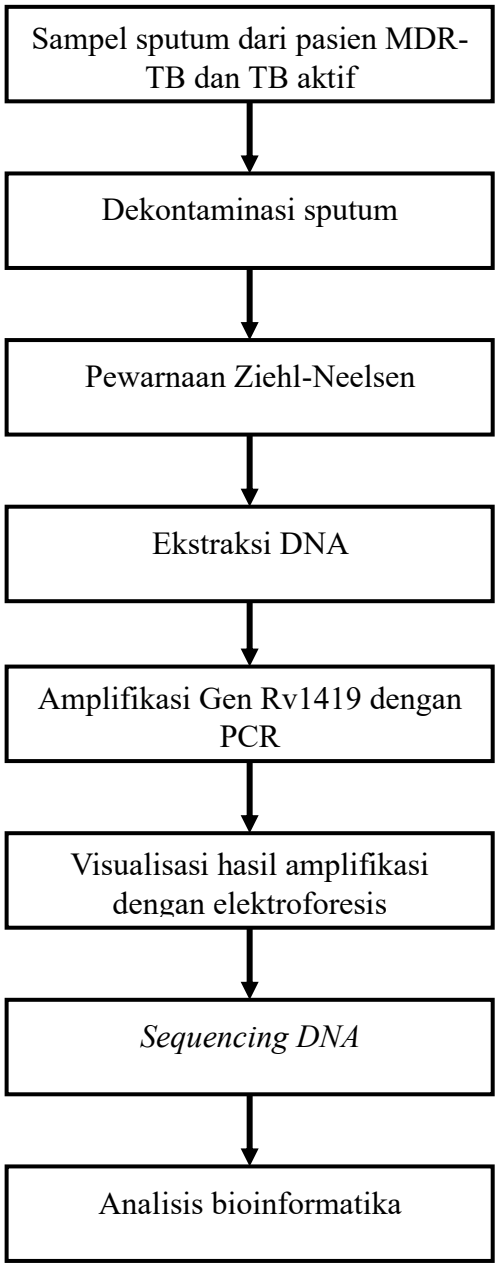
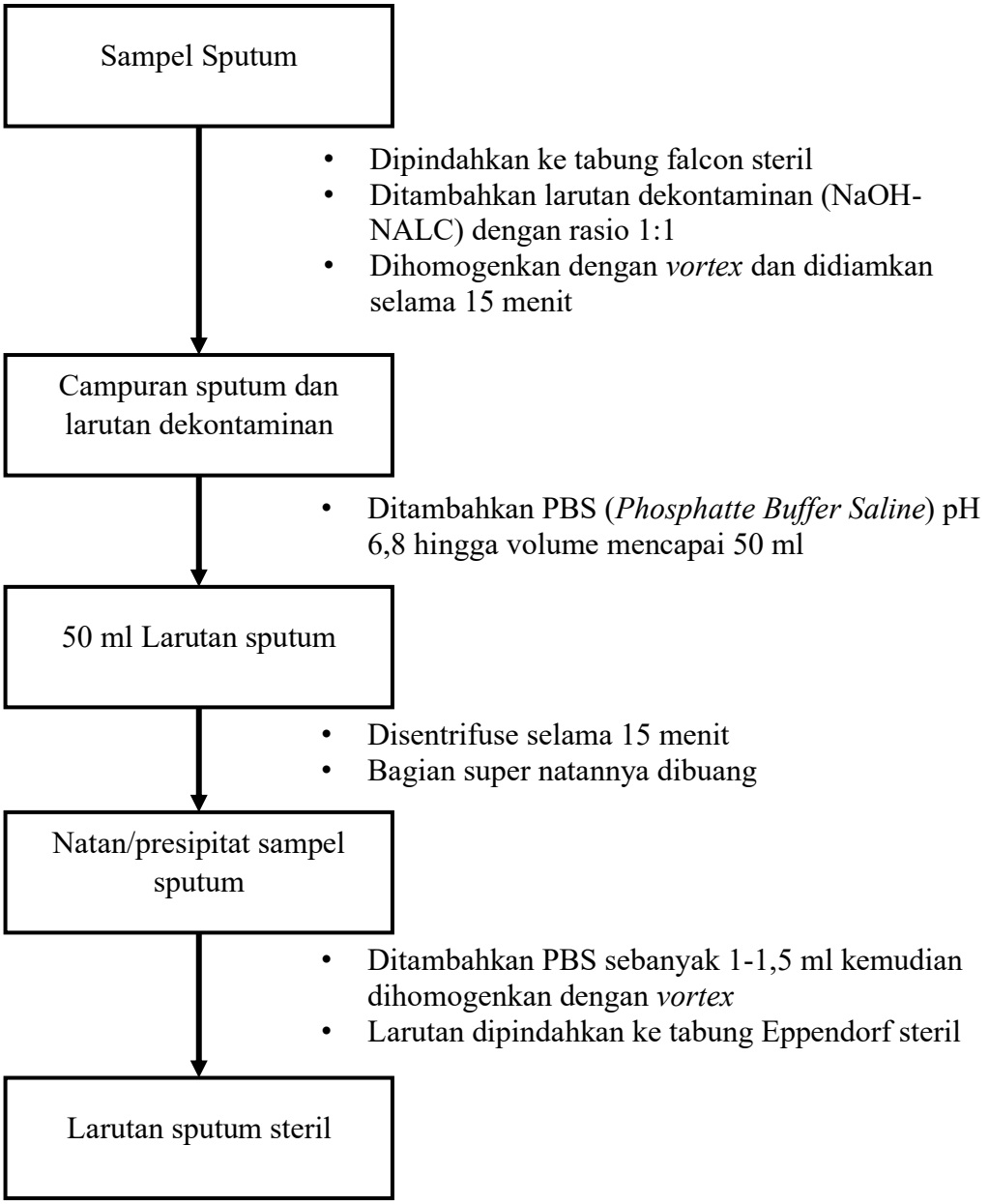
LAMPIRAN

Lampiran 1. Skema Kerja

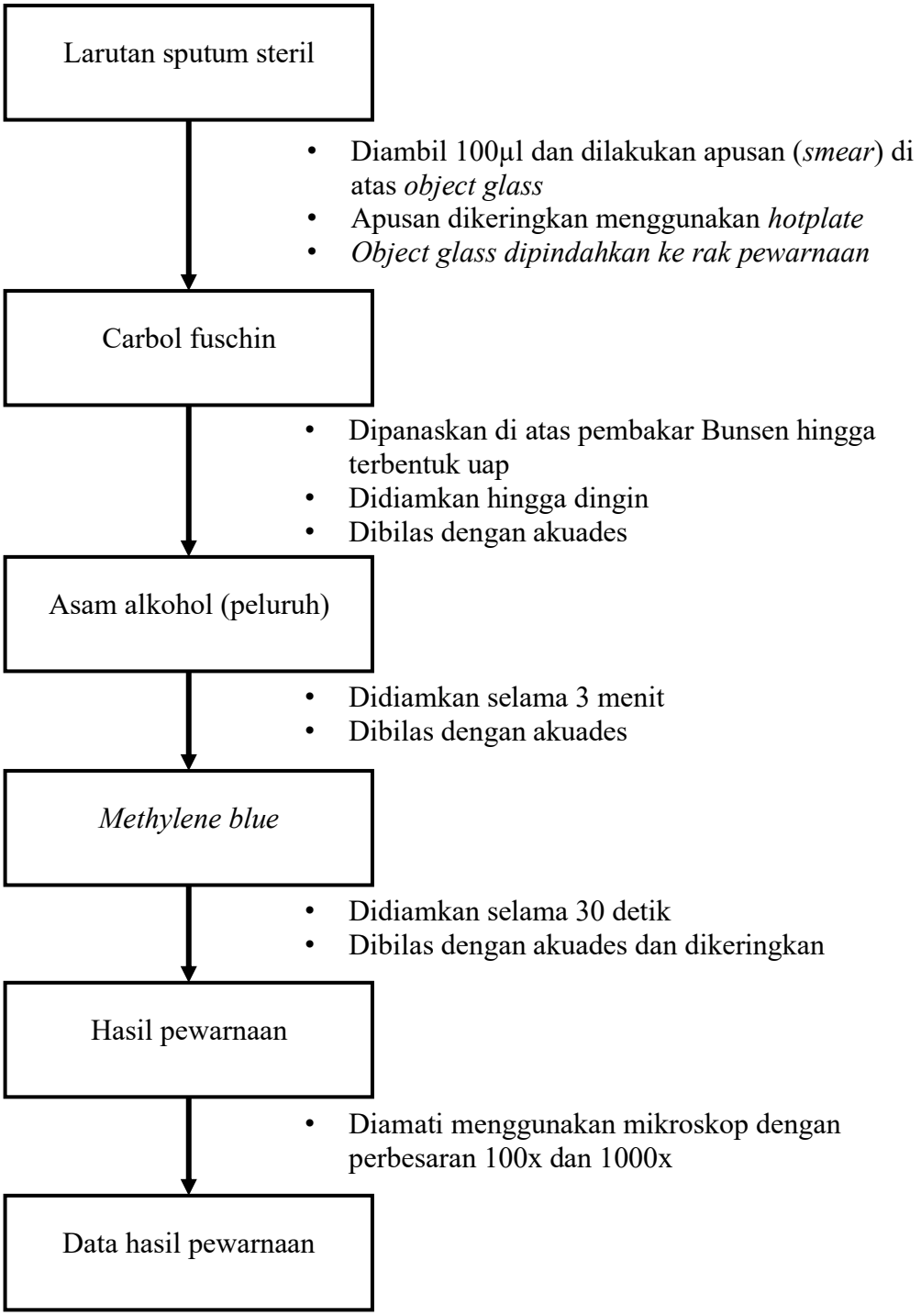
A. Skema Kerja Penelitian



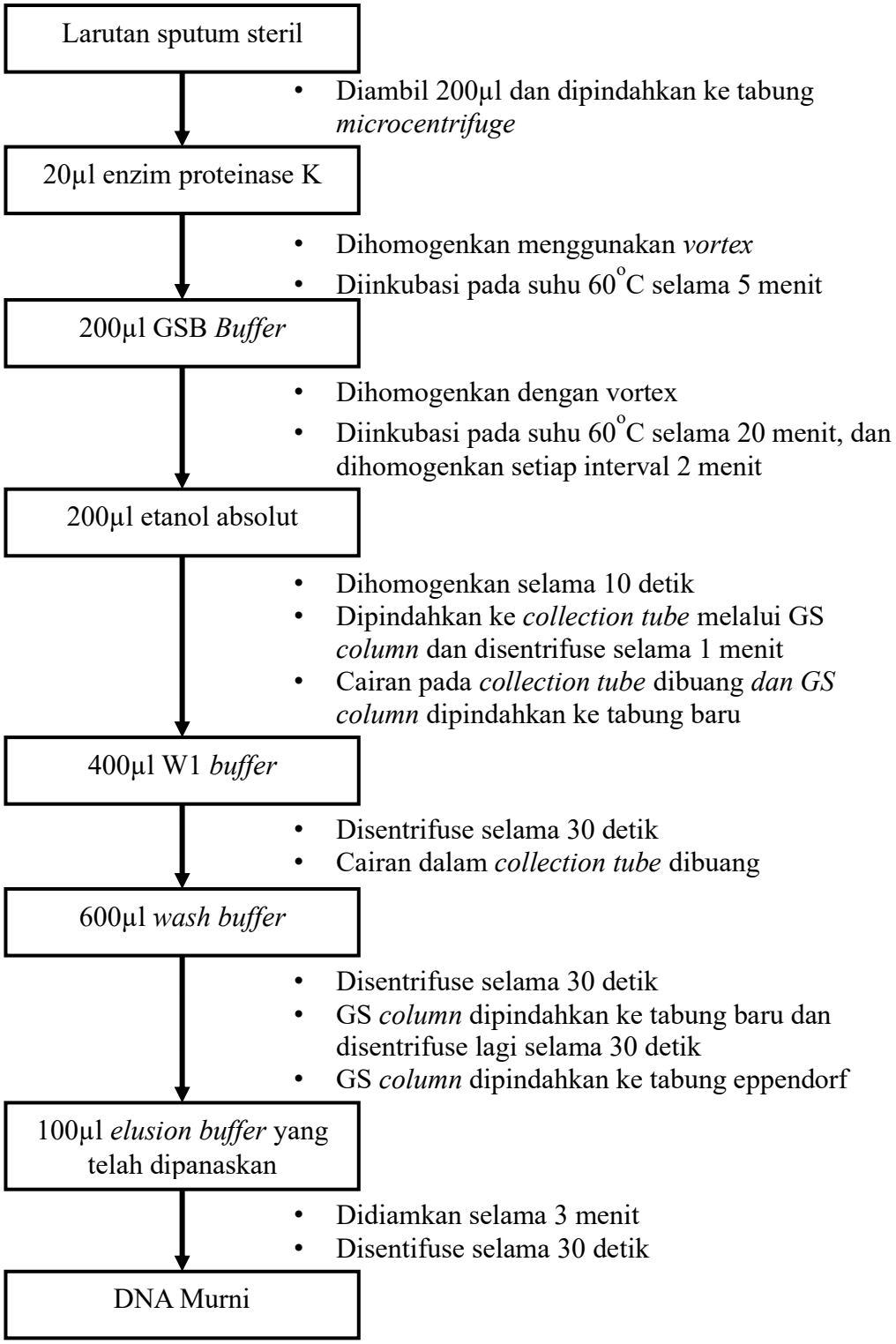
B. Skema Kerja Dekontaminasi Sputum



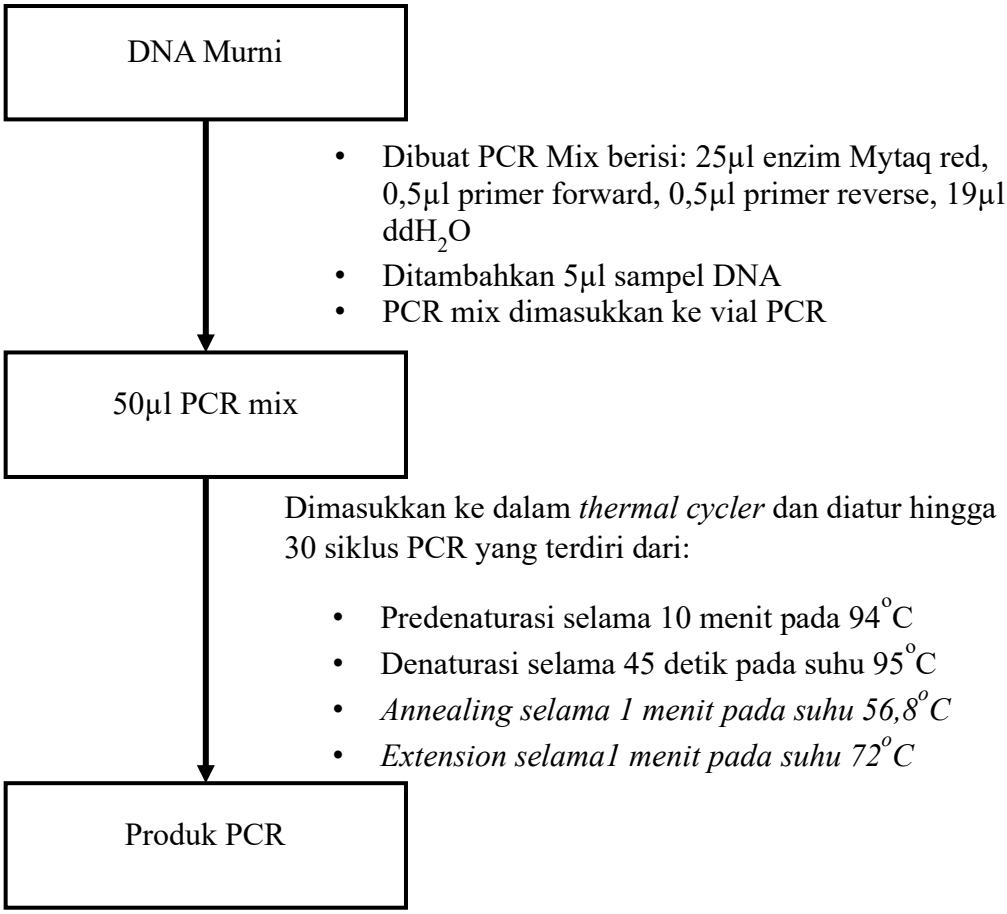
C. Skema Kerja Pewarnaan Ziehl-Neelsen



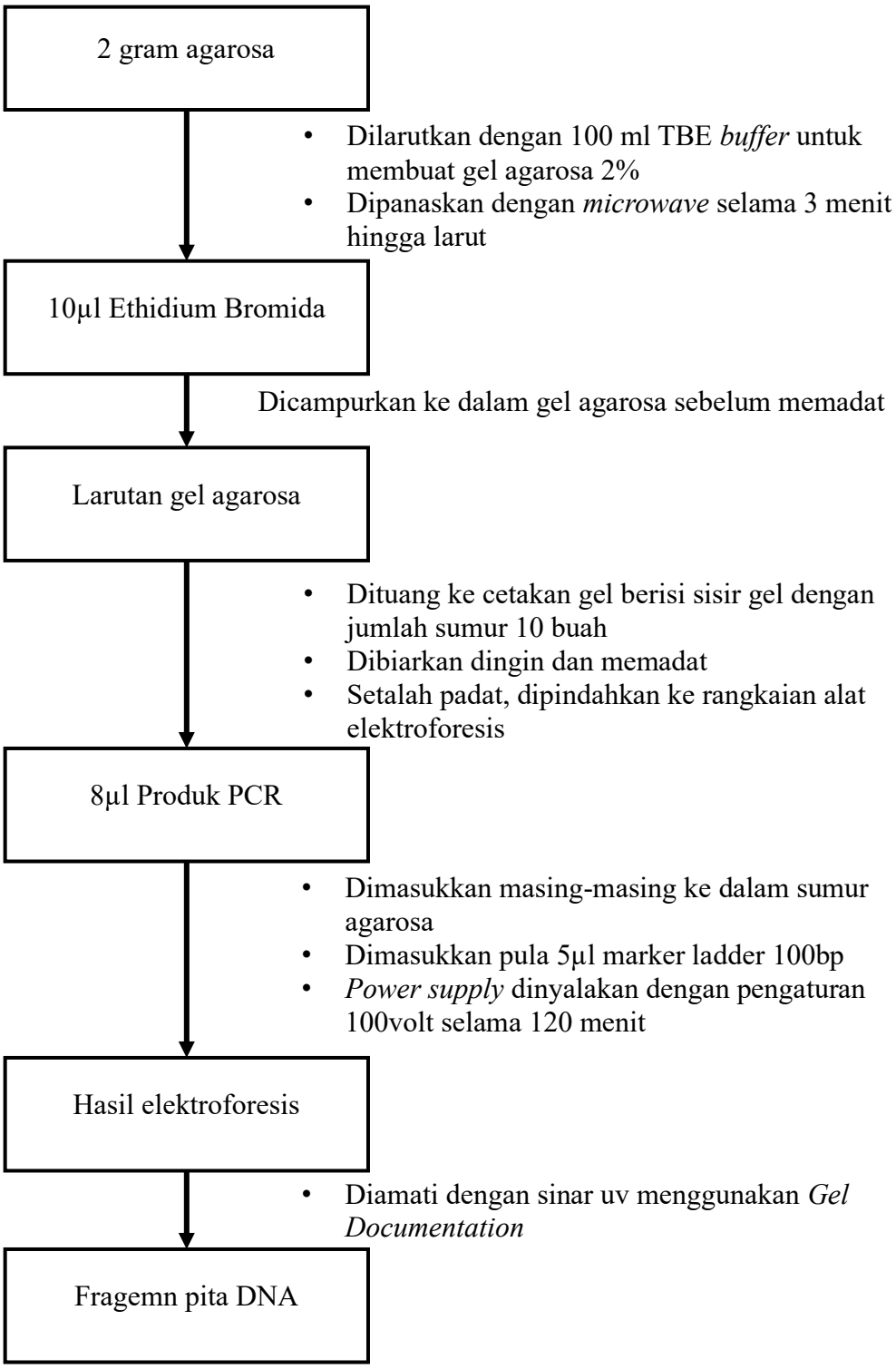
D. Skema Kerja Ekstraksi DNA



E. Skema Kerja Amplifikasi Gen Rv1419 dengan PCR

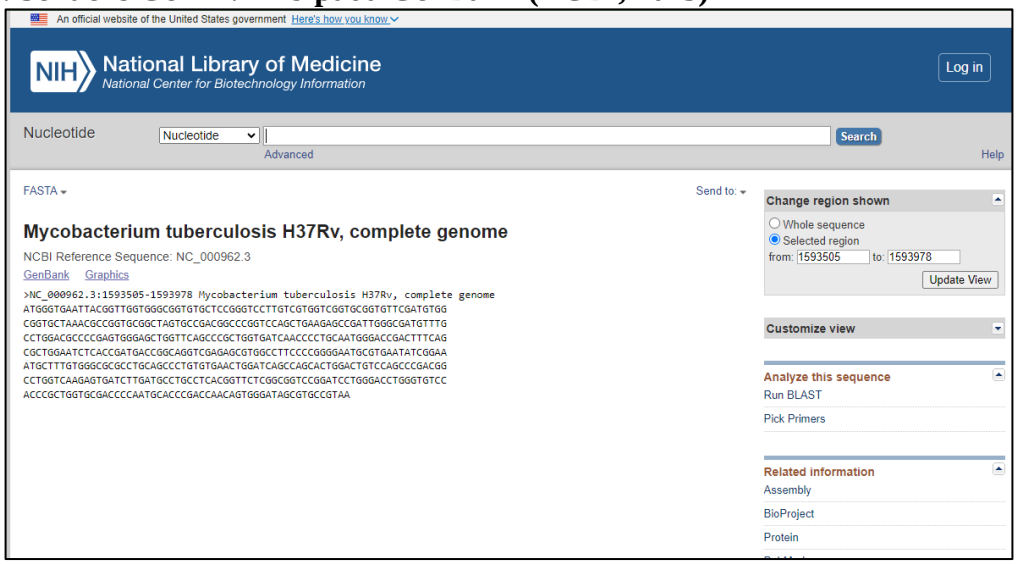


F. Skema Kerja Elektroforesis



Lampiran 2. Peta Origin Antara Primer dan Gen Rv1419 dari DataBank

A. Sekuens Gen Rv1419 pada GenBank (NCBI, 2023)



B Hasil Pemetaan Origin Primer pada Gen Rv1419

```
forward primer

5'-GATCGCTAGCATGGGTGAATTACGGTTG-3'
               ATGGGTGAATTACGGTTGGTGGGCGGTGTGCTCCGGGTCCTTGTCGTGGTCGGTGCGGTGTTCGATGTGG

               CGGTGCTAAACGCCGGTGCGGCTAGTGCCGACGGCCCGGTCCAGCTGAAGAGCCGATTGGGCGATGTTTG

               CCTGGACGCCCCGAGTGGGAGCTGGTTCAGCCCGCTGGTGATCAACCCCTGCAATGGGACCGACTTTCAG

               CGCTGGAATCTCACCGATGACCGGCAGGTCGAGAGCGTGGCCTTCCCCGGGGAATGCGTGAATATCGGAA

               ATGCTTTGTGGGCGCGCCTGCAGCCCTGTGTGAACTGGATCAGCCAGCACTGGACTGTCCAGCCCGACGG

               CCTGGTCAAGAGTGATCTTGATGCCTGCCTCACGGTTCTCGGCGGTCCGGATCCTGGGACCTGGGTGTCC

               ACCCGCTGGTGCGACCCCAATGCACCCGACCAACAGTGGGATAGCGTGCCG
                                                  3'-TGGGATAGCGTGCCGCTCGAGATA -'5

                                                  Reverse primer


forward primer

5'-GATCGCTAGCATGGGTGAATTACGGTTG-3'

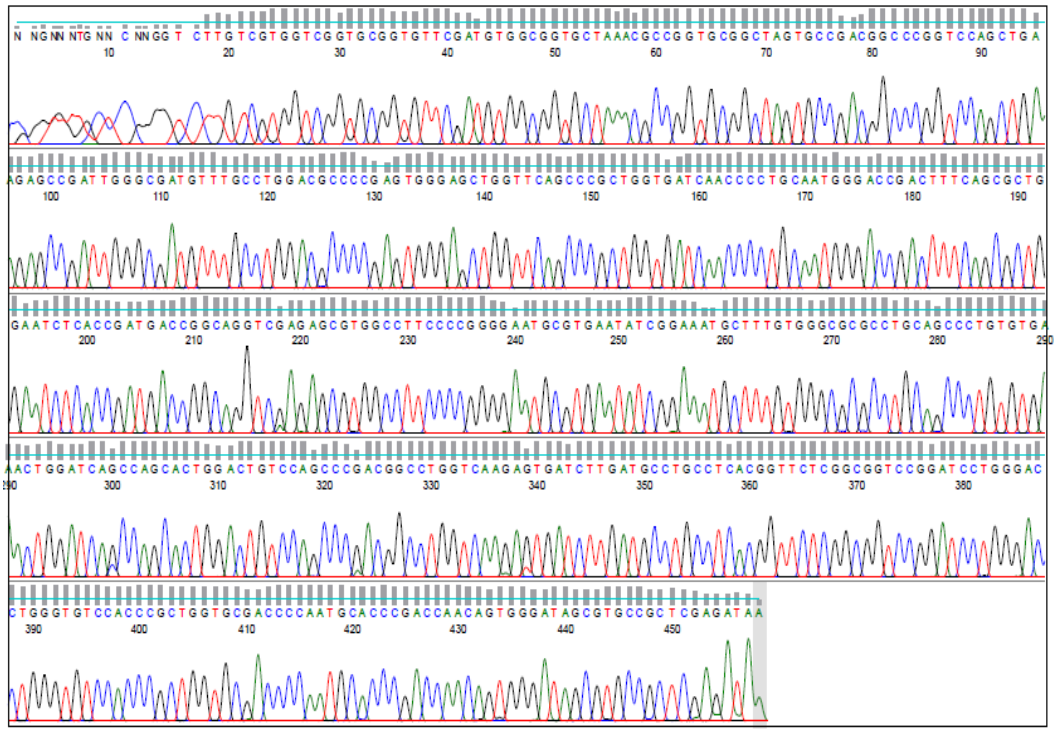

reverse primer

5'-TATCTCGAGCGGCACGCTATCCCA-3'

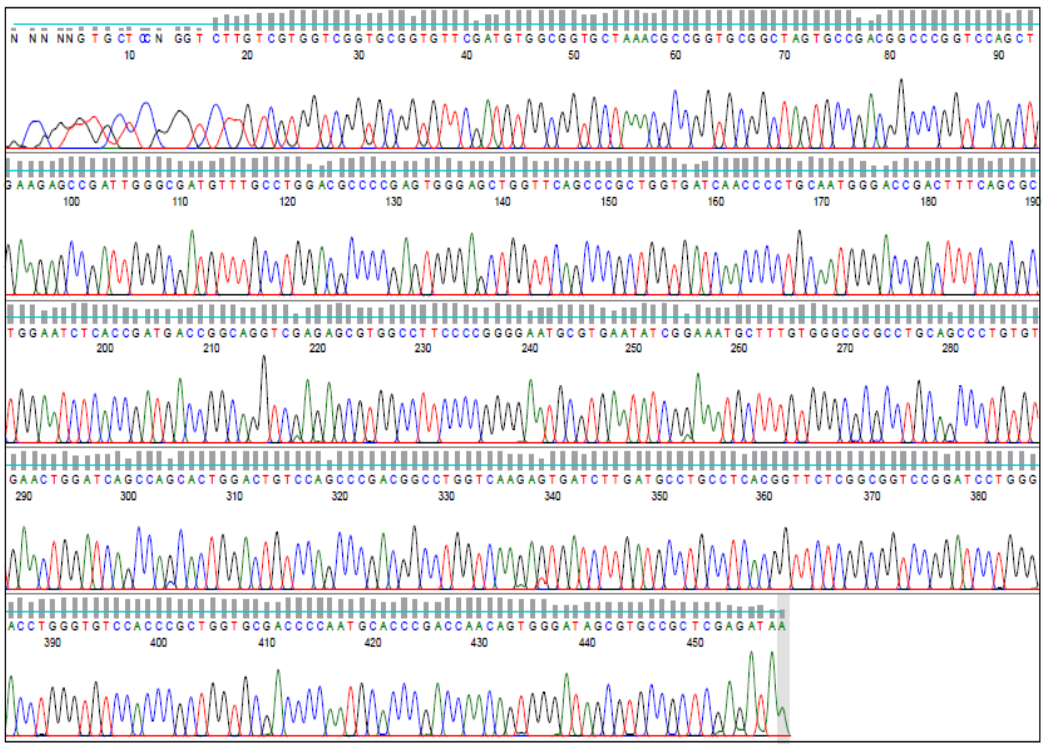
3'-TGGGATAGCGTGCCGCTCGAGATA-5'(Reverse Compliment)
```

Lampiran 3. Hasil Sekuensing Sampel

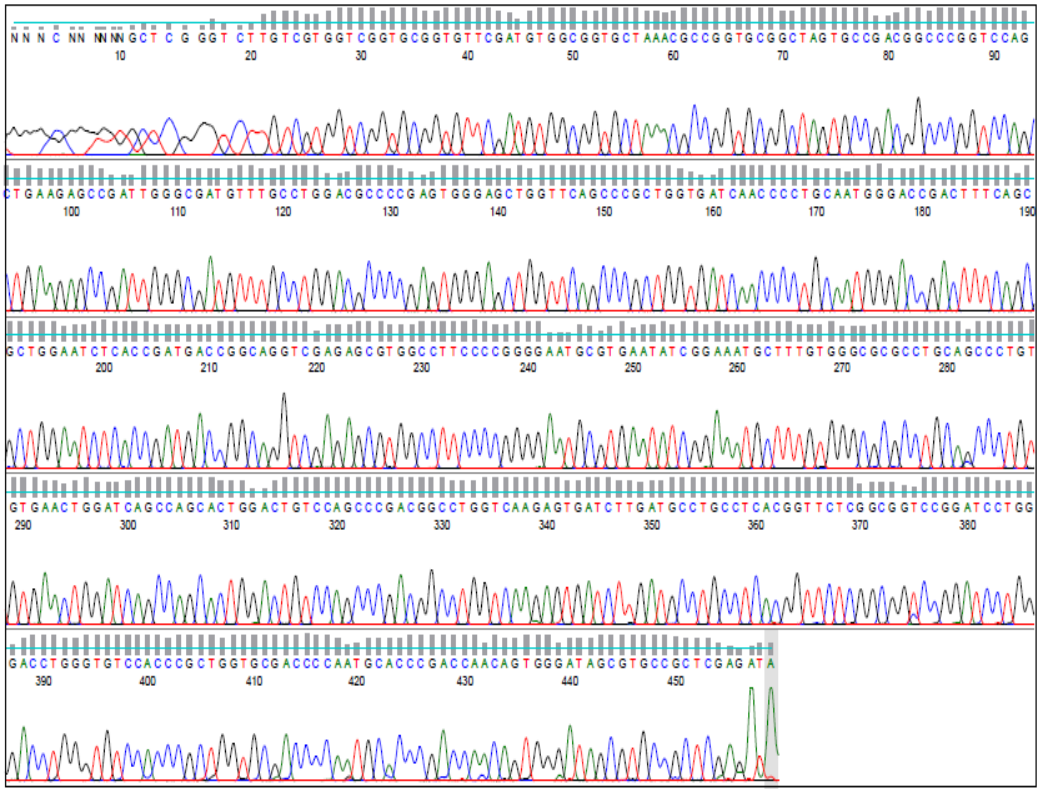
A. Visualisasi Sekuens DNA Sampel R1



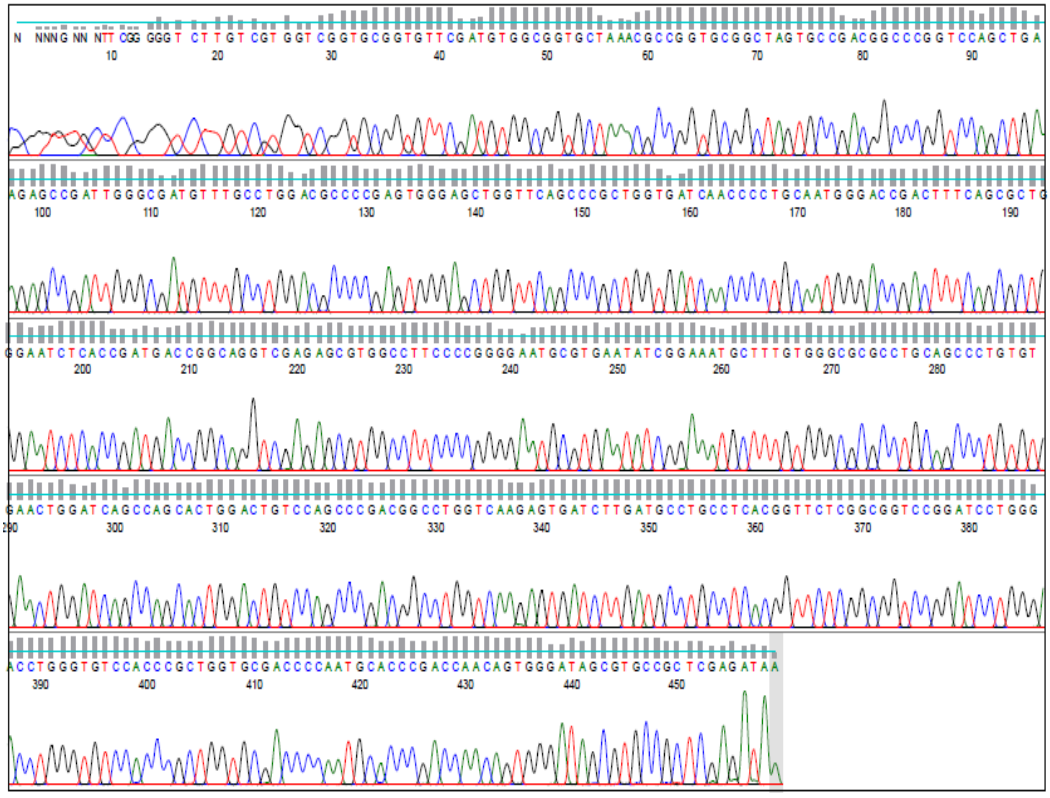
B. Visualisasi Sekuens DNA Sampel R2



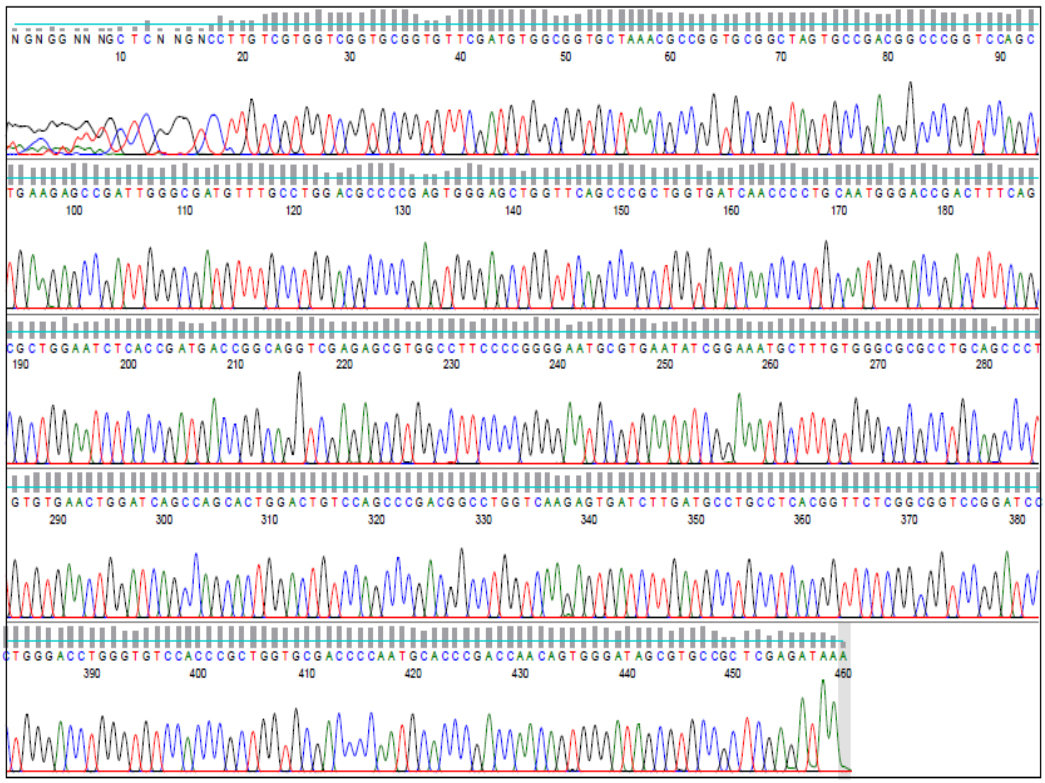
C. Visualisasi Sekuens DNA Sampel R3



D. Visualisasi Sekuens DNA Sampel R4



E. Visualisasi Sekuens DNA Sampel R5



Lampiran 4. Komposisi Bahan

A. Komposisi Larutan Stok Dekontaminasi

Stok larutan NaOH-Sodium Sitrat:

NaOH 1 N : 20 g dalam 500 ml akuades

Sodium Sitrat : 14,5 g dalam 500 ml akuades

Stok larutan NaOH-NALC:

NaOH-Sodium sitrat : 100 ml

Massa NALC : 1 g

Larutan stok PBS *buffer*:

Na_2HPO_4 : 300 ml (0,947%)

KH_2PO_4 : 200 ml (0,947%)

PBS *buffer* : 500 ml (0,067 M, pH 6,8)

B. Komposisi Reagen Ekstraksi DNA

Sampel sputum : 200 µl

Enzim Proteinase K : 20 µl

GSB *buffer* : 200 µl

Etanol absolut : 200 µl

W1 *buffer* : 400 µl

Wash buffer : 600 µl

Elusion buffer : 100 µl

C. Komposisi Larutan Stok Primer

Massa primer kering : 0,23 mg (26,1 nmol)

Volume ddH_2O : 261µl

Konsentrasi stok : 100 mol (261 µl)

Pengenceran larutan stok:

$V_1N_1 = V_1N_1$

$V_1 \cdot 100 = 100\ \mu l \cdot 20$

$V_1 = 20\ \mu l \rightarrow$ **Volume ddH_2O = 80µl**

D. Komposisi PCR *Mix*

Enzim *mytaq red* : 25 µl

Primer *forward* : 0,5 µl

Primer *reverse* : 0,5 µl

ddH_2O : 19 µl

Sampel DNA : 5 µl

Volume Total : 25 µl

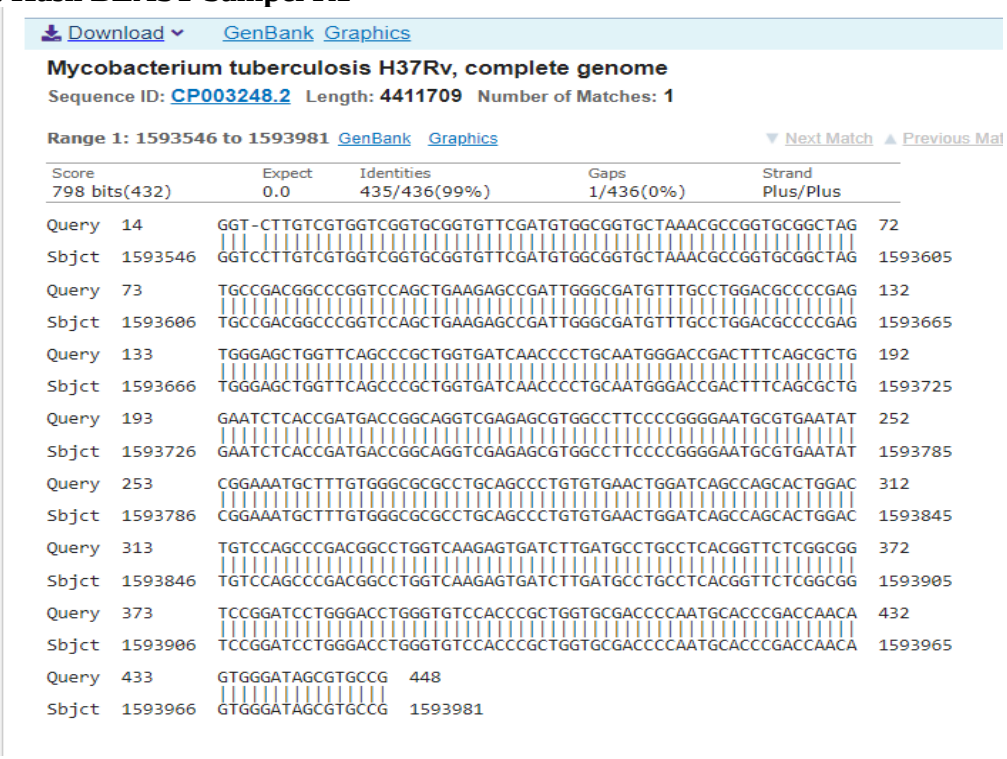
E. Komposisi Gel Agarosa

Berat agarosa : 2 g

Volume TBE *buffer* : 100 ml

Lampiran 5. Hasil Analisis BLAST

A. Hasil BLAST Sampel R1



Download GenBank Graphics

Mycobacterium tuberculosis H37Rv, complete genome

Sequence ID: CP003248.2 Length: 4411709 Number of Matches: 1

Range 1: 1593546 to 1593981 GenBank Graphics Next Match Previous Mat

Score	Expect	Identities	Gaps	Strand
798 bits(432)	0.0	435/436(99%)	1/436(0%)	Plus/Plus

```
Query  14       GGT-CTTGTCGTGGTCGGTGCGGTGTTCGATGTGGCGGTGCTAAACGCCGGTGCGGCTAG  72
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Query  73       TGCCGACGGCCCGGTCCAGCTGAAGAGCCGATTGGGCGATGTTTGCCTGGACGCCCCGAG  132
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Sbjct  1593606  TGCCGACGGCCCGGTCCAGCTGAAGAGCCGATTGGGCGATGTTTGCCTGGACGCCCCGAG  1593665

Query  133      TGGGAGCTGGTTCAGCCCGCTGGTGATCAACCCCTGCAATGGGACCGACTTTCAGCGCTG  192
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Sbjct  1593666  TGGGAGCTGGTTCAGCCCGCTGGTGATCAACCCCTGCAATGGGACCGACTTTCAGCGCTG  1593725

Query  193      GAATCTCACCGATGACCGGCAGGTCGAGAGCGTGGCCTTCCCCGGGGAATGCGTGAATAT  252
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Sbjct  1593726  GAATCTCACCGATGACCGGCAGGTCGAGAGCGTGGCCTTCCCCGGGGAATGCGTGAATAT  1593785

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Sbjct  1593846  TGTCCAGCCCGACGGCCTGGTCAAGAGTGATCTTGATGCCTGCCTCACGGTTCTCGGCGG  1593905

Query  373      TCCGGATCCTGGGACCTGGGTGTCCACCCGCTGGTGCGACCCCAATGCACCCGACCAACA  432
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Sbjct  1593906  TCCGGATCCTGGGACCTGGGTGTCCACCCGCTGGTGCGACCCCAATGCACCCGACCAACA  1593965

Query  433      GTGGGATAGCGTGCCG  448
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Sbjct  1593966  GTGGGATAGCGTGCCG  1593981
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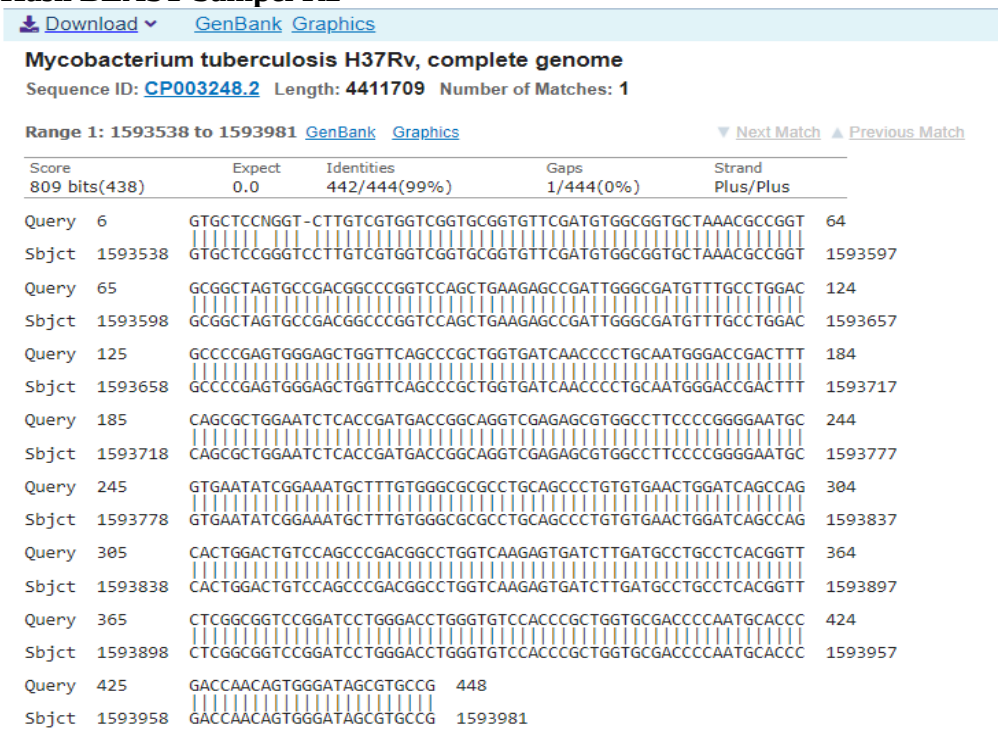
B. Hasil BLAST Sampel R2



Download GenBank Graphics

Mycobacterium tuberculosis H37Rv, complete genome

Sequence ID: CP003248.2 Length: 4411709 Number of Matches: 1

Range 1: 1593538 to 1593981 GenBank Graphics Next Match Previous Match

Score	Expect	Identities	Gaps	Strand
809 bits(438)	0.0	442/444(99%)	1/444(0%)	Plus/Plus

```
Query  6        GTGCTCCNGGT-CTTGTCGTGGTCGGTGCGGTGTTCGATGTGGCGGTGCTAAACGCCGGT  64
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Sbjct  1593778  GTGAATATCGGAAATGCTTTGTGGGCGCGCCTGCAGCCCTGTGTGAACTGGATCAGCCAG  1593837

Query  305      CACTGGACTGTCCAGCCCGACGGCCTGGTCAAGAGTGATCTTGATGCCTGCCTCACGGTT  364
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593838  CACTGGACTGTCCAGCCCGACGGCCTGGTCAAGAGTGATCTTGATGCCTGCCTCACGGTT  1593897

Query  365      CTCGGCGGTCCGGATCCTGGGACCTGGGTGTCCACCCGCTGGTGCGACCCCAATGCACCC  424
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593898  CTCGGCGGTCCGGATCCTGGGACCTGGGTGTCCACCCGCTGGTGCGACCCCAATGCACCC  1593957

Query  425      GACCAACAGTGGGATAGCGTGCCG  448
               ||||||||||||||||||||||||
Sbjct  1593958  GACCAACAGTGGGATAGCGTGCCG  1593981
```

C. Hasil BLAST Sampel R3

Download GenBank Graphics

Mycobacterium tuberculosis H37Rv, complete genome

Sequence ID: CP003248.2 Length: 4411709 Number of Matches: 1

Range 1: 1593540 to 1593981 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
804 bits(435)	0.0	440/442(99%)	2/442(0%)	Plus/Plus

```
Query  11       GCT-CGGGT-CTTGTCGTGGTCGGTGCGGTGTTCGATGTGGCGGTGCTAAACGCCGGTGC  68
               ||| ||||| ||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593540  GCTCCGGGTCCTTGTCGTGGTCGGTGCGGTGTTCGATGTGGCGGTGCTAAACGCCGGTGC  1593599

Query  69       GGCTAGTGCCGACGGCCCGGTCCAGCTGAAGAGCCGATTGGGCGATGTTTGCCTGGACGC  128
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593600  GGCTAGTGCCGACGGCCCGGTCCAGCTGAAGAGCCGATTGGGCGATGTTTGCCTGGACGC  1593659

Query  129      CCCGAGTGGGAGCTGGTTCAGCCCGCTGGTGATCAACCCCTGCAATGGGACCGACTTTCA  188
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593660  CCCGAGTGGGAGCTGGTTCAGCCCGCTGGTGATCAACCCCTGCAATGGGACCGACTTTCA  1593719

Query  189      GCGCTGGAATCTCACCGATGACCGGCAGGTCGAGAGCGTGGCCTTCCCCGGGGAATGCGT  248
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593720  GCGCTGGAATCTCACCGATGACCGGCAGGTCGAGAGCGTGGCCTTCCCCGGGGAATGCGT  1593779

Query  249      GAATATCGGAAATGCTTTGTGGGCGCGCCTGCAGCCCTGTGTGAACTGGATCAGCCAGCA  308
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593780  GAATATCGGAAATGCTTTGTGGGCGCGCCTGCAGCCCTGTGTGAACTGGATCAGCCAGCA  1593839

Query  309      CTGGACTGTCCAGCCCGACGGCCTGGTCAAGAGTGATCTTGATGCCTGCCTCACGGTTCT  368
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593840  CTGGACTGTCCAGCCCGACGGCCTGGTCAAGAGTGATCTTGATGCCTGCCTCACGGTTCT  1593899

Query  369      CGGCGGTCCGGATCCTGGGACCTGGGTGTCCACCCGCTGGTGCGACCCCAATGCACCCGA  428
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593900  CGGCGGTCCGGATCCTGGGACCTGGGTGTCCACCCGCTGGTGCGACCCCAATGCACCCGA  1593959

Query  429      CCAACAGTGGGATAGCGTGCCG  450
               |||||||||||||||||||||
Sbjct  1593960  CCAACAGTGGGATAGCGTGCCG  1593981
```

D. Hasil BLAST Sampel R4

Download GenBank Graphics

Mycobacterium tuberculosis H37Rv, complete genome

Sequence ID: CP003248.2 Length: 4411709 Number of Matches: 1

Range 1: 1593545 to 1593981 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
800 bits(433)	0.0	436/437(99%)	1/437(0%)	Plus/Plus

```
Query  14       GGGT-CTTGTCGTGGTCGGTGCGGTGTTCGATGTGGCGGTGCTAAACGCCGGTGCGGCTA  72
               |||| ||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593545  GGGTCCTTGTCGTGGTCGGTGCGGTGTTCGATGTGGCGGTGCTAAACGCCGGTGCGGCTA  1593604

Query  73       GTGCCGACGGCCCGGTCCAGCTGAAGAGCCGATTGGGCGATGTTTGCCTGGACGCCCCGA  132
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593605  GTGCCGACGGCCCGGTCCAGCTGAAGAGCCGATTGGGCGATGTTTGCCTGGACGCCCCGA  1593664

Query  133      GTGGGAGCTGGTTCAGCCCGCTGGTGATCAACCCCTGCAATGGGACCGACTTTCAGCGCT  192
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593665  GTGGGAGCTGGTTCAGCCCGCTGGTGATCAACCCCTGCAATGGGACCGACTTTCAGCGCT  1593724

Query  193      GGAATCTCACCGATGACCGGCAGGTCGAGAGCGTGGCCTTCCCCGGGGAATGCGTGAATA  252
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593725  GGAATCTCACCGATGACCGGCAGGTCGAGAGCGTGGCCTTCCCCGGGGAATGCGTGAATA  1593784

Query  253      TCGGAAATGCTTTGTGGGCGCGCCTGCAGCCCTGTGTGAACTGGATCAGCCAGCACTGGA  312
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593785  TCGGAAATGCTTTGTGGGCGCGCCTGCAGCCCTGTGTGAACTGGATCAGCCAGCACTGGA  1593844

Query  313      CTGTCCAGCCCGACGGCCTGGTCAAGAGTGATCTTGATGCCTGCCTCACGGTTCTCGGCG  372
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593845  CTGTCCAGCCCGACGGCCTGGTCAAGAGTGATCTTGATGCCTGCCTCACGGTTCTCGGCG  1593904

Query  373      GTCCGGATCCTGGGACCTGGGTGTCCACCCGCTGGTGCGACCCCAATGCACCCGACCAAC  432
               ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593905  GTCCGGATCCTGGGACCTGGGTGTCCACCCGCTGGTGCGACCCCAATGCACCCGACCAAC  1593964

Query  433      AGTGGGATAGCGTGCCG  449
               |||||||||||||||||
Sbjct  1593965  AGTGGGATAGCGTGCCG  1593981
```

E. Hasil BLAST Sampel R5

Download GenBank Graphics

Mycobacterium tuberculosis H37RvSiena, complete genome

Sequence ID: CP007027.1 Length: 4410911 Number of Matches: 1

Range 1: 1593548 to 1593980 GenBank Graphics ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
800 bits(433)	0.0	433/433(100%)	0/433(0%)	Plus/Plus

```
Query  17       CCTTGTCGTGGTCGGTGCGGTGTTCGATGTGGCGGTGCTAAACGCCGGTGCGGCTAGTGC  76
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593548  CCTTGTCGTGGTCGGTGCGGTGTTCGATGTGGCGGTGCTAAACGCCGGTGCGGCTAGTGC  1593607

Query  77       CGACGGCCCGGTCCAGCTGAAGAGCCGATTGGGCGATGTTTGCCTGGACGCCCCGAGTGG  136
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593608  CGACGGCCCGGTCCAGCTGAAGAGCCGATTGGGCGATGTTTGCCTGGACGCCCCGAGTGG  1593667

Query  137      GAGCTGGTTCAGCCCGCTGGTGATCAACCCCTGCAATGGGACCGACTTTCAGCGCTGGAA  196
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593668  GAGCTGGTTCAGCCCGCTGGTGATCAACCCCTGCAATGGGACCGACTTTCAGCGCTGGAA  1593727

Query  197      TCTCACCGATGACCGGCAGGTCGAGAGCGTGGCCTTCCCCGGGGAATGCGTGAATATCGG  256
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593728  TCTCACCGATGACCGGCAGGTCGAGAGCGTGGCCTTCCCCGGGGAATGCGTGAATATCGG  1593787

Query  257      AAATGCTTTGTGGGCGCGCCTGCAGCCCTGTGTGAACTGGATCAGCCAGCACTGGACTGT  316
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593788  AAATGCTTTGTGGGCGCGCCTGCAGCCCTGTGTGAACTGGATCAGCCAGCACTGGACTGT  1593847

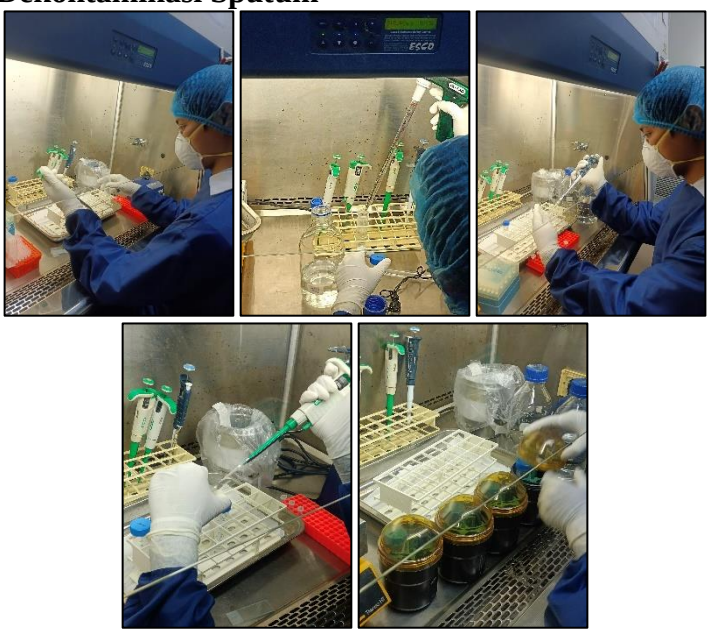
Query  317      CCAGCCCGACGGCCTGGTCAAGAGTGATCTTGATGCCTGCCTCACGGTTCTCGGCGGTCC  376
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593848  CCAGCCCGACGGCCTGGTCAAGAGTGATCTTGATGCCTGCCTCACGGTTCTCGGCGGTCC  1593907

Query  377      GGATCCTGGGACCTGGGTGTCCACCCGCTGGTGCGACCCCAATGCACCCGACCAACAGTG  436
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  1593908  GGATCCTGGGACCTGGGTGTCCACCCGCTGGTGCGACCCCAATGCACCCGACCAACAGTG  1593967

Query  437      GGATAGCGTGCCG  449
                |||||||||||||
Sbjct  1593968  GGATAGCGTGCCG  1593980
```

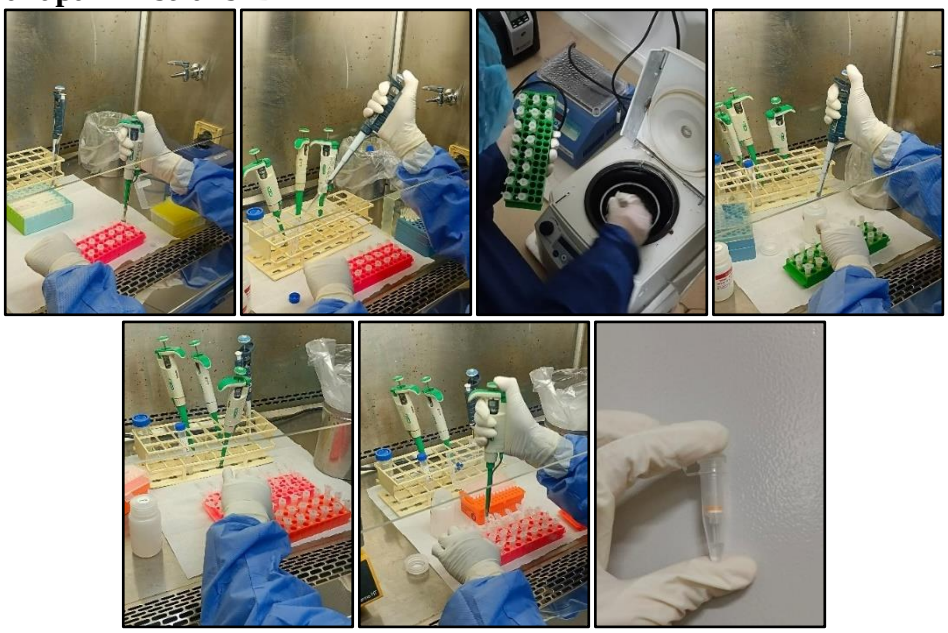
Lampiran 6. Dokumentasi Penelitian

A. Tahapan Dekontaminasi Sputum

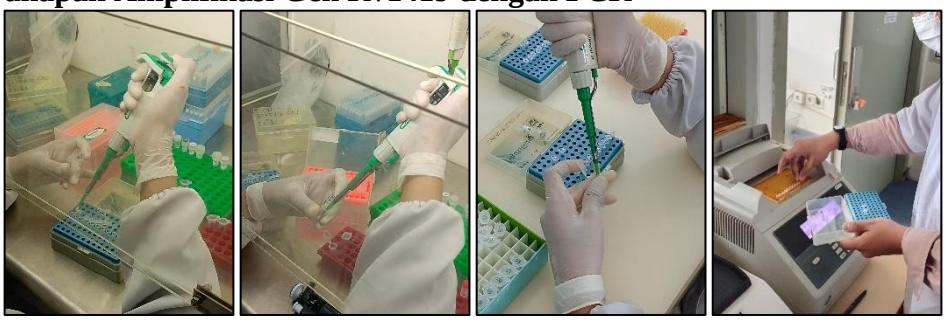


B. Tahapan Pewarnaan Ziehl-Neelsen

C. Tahapan Ekstraksi DNA



D. Tahapan Amplifikasi Gen Rv1419 dengan PCR



E. Tahapan Elektroforesis