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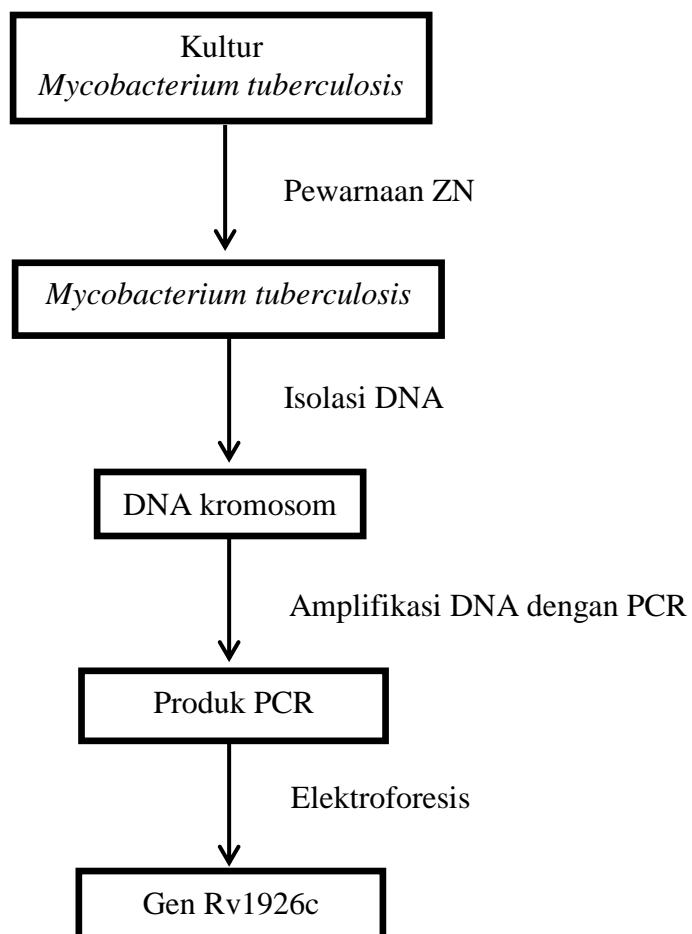


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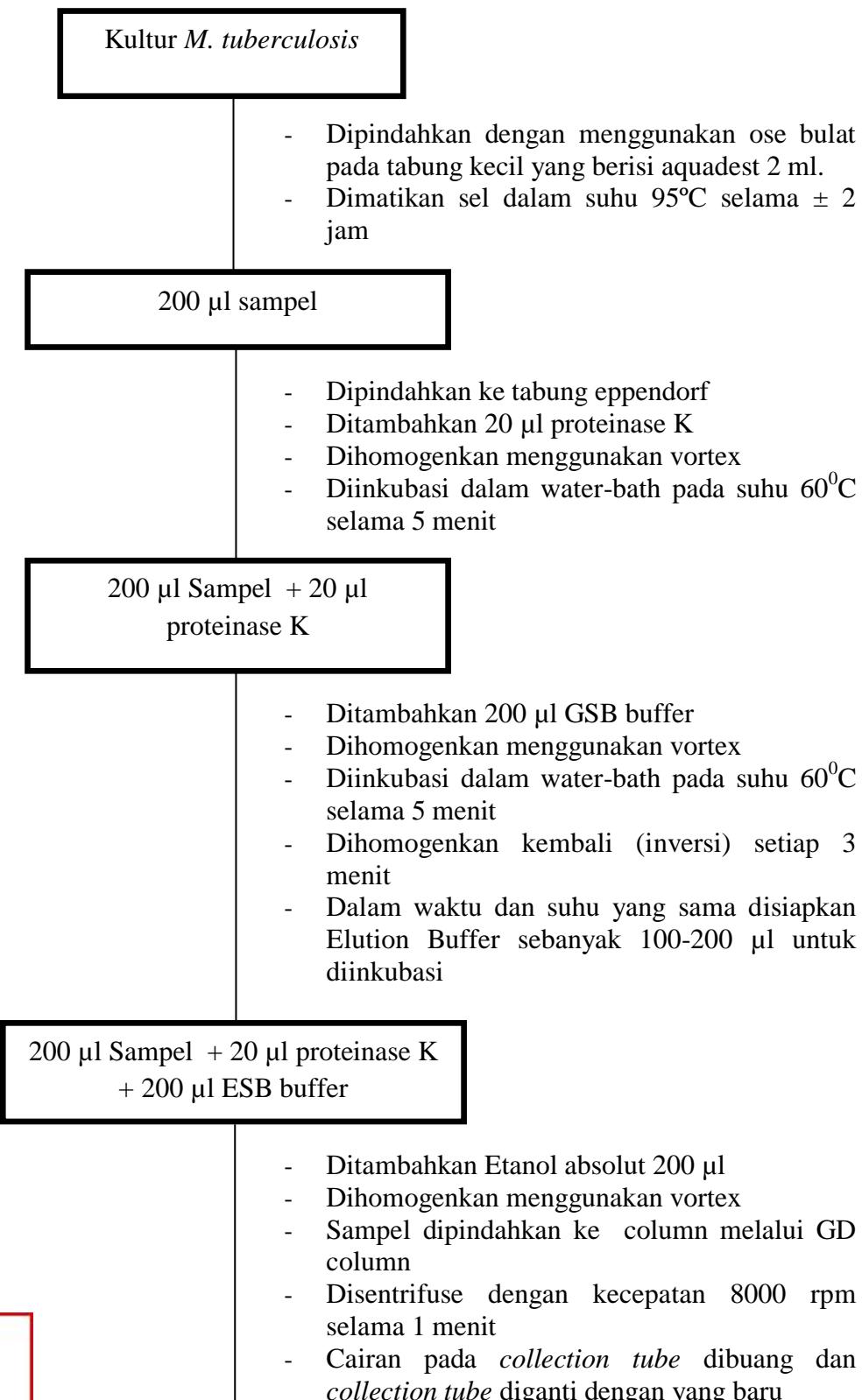


Lampiran

1. Bagan Kerja Penelitian



2. Skema Kerja Ekstraksi DNA dengan gSYNC DNA Extraction Kit (Geneaid)



200 µl Sampel + 20 µl proteinase K +
200 µl ESB buffer + 200 µl etanol
absolut

- Ditambahkan W₁ buffer 400 µl
- Disentrifuse dengan kecepatan 8000 rpm selama 1 menit
- Cairan pada *collection tube* dibuang dan *collection tube* diganti dengan yang baru

200 µl Sampel + 20 µl proteinase K +
200 µl ESB buffer + 200 µl etanol
absolut + 400 µl W₁ buffer

- Ditambahkan wash buffer 600 µl
- Disentrifuse dengan kecepatan 8000 rpm selama 1 menit
- Cairan pada *collection tube* dibuang
- Dilakukan kembali sentrifuse kering

Natan + 600 µl Wash
Buffer

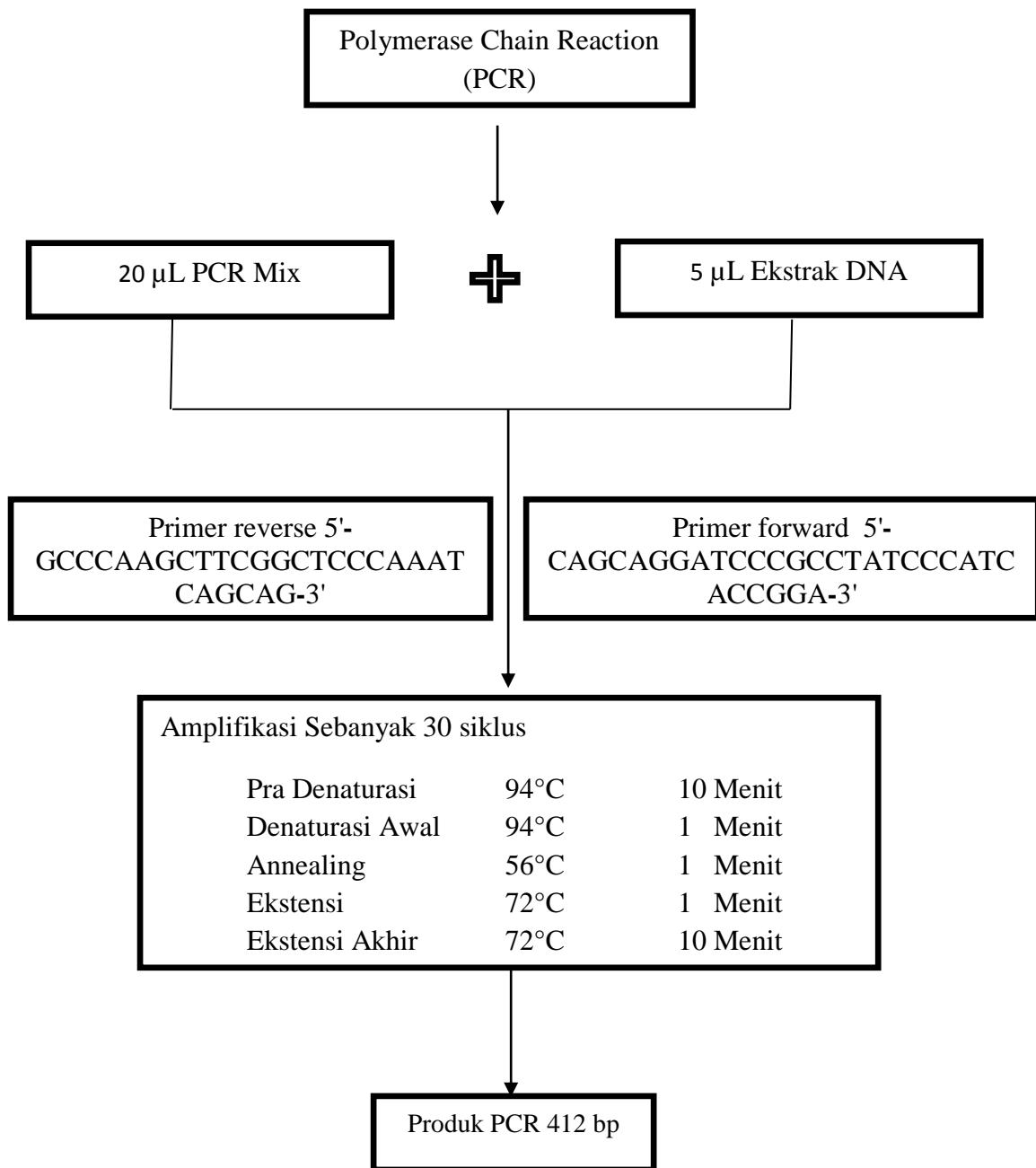
- Dipindahkan column ke ependorf 1,5 ml.
- Ditambahkan 100 µl pre-heated elution buffer
- Didiamkan selama 3 menit
- Disentrifugasi selama 1 menit dengan kecepatan 8000 rpm

DNA kromosom

- Disimpan pada suhu -20°C



3. Bagan Kerja PCR



Optimization Software:
www.balesio.com

4. Komposisi Bahan

a. Media Lowenstein Jensen

LJ	37.5 gr
Aquades	600 ml
Telur Bebek	250 ml
Gliserol	3 ml

b. Pewarnaan Ziehl-Neelsen (ZN)

Carbol Fuhsin	0.3 %
HCL Alkohol	3 %
Metylen Blue	0.3 %

c. Ekstraksi/ Isolasi DNA

Proteinase K	20 µl
ESB Buffer	200 µl
Elution Buffer	100 – 200 µl
Etanol Absolut	200 µl
W ₁ Buffer	400 µl
Wash Buffer	600 µl
Pre-heated elution buffer	100 µl

d. PCR Mix

Komposisi : Enzim GoTaq Green Master Mix	12.5 µl (1.5 U)
Primer (Forward)	1.0 µl (10 pmol)
Primer (Reverse)	1.0 µl (10 pmol)
Nuclease free water (DDH ₂ O)	5.5 µl
Sampel DNA	5 µl

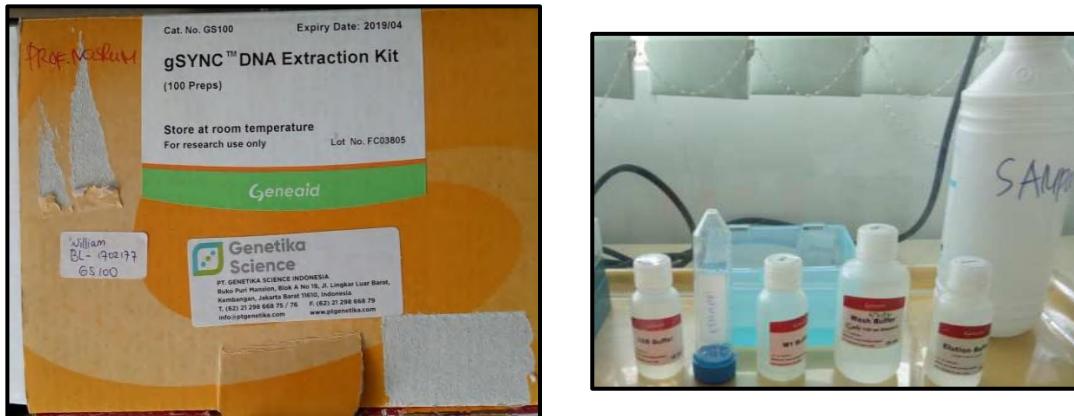
e. Gel Agarosa 2 %

Komposisi : Agarosa	1.2 gr
0.5x Tris-Buffer-EDTA	60 ml
Ethidium Bromide	2 µl



Optimization Software:
www.balesio.com

5. Foto bahan yang digunakan



(a) KIT yang digunakan dalam isolasi DNA



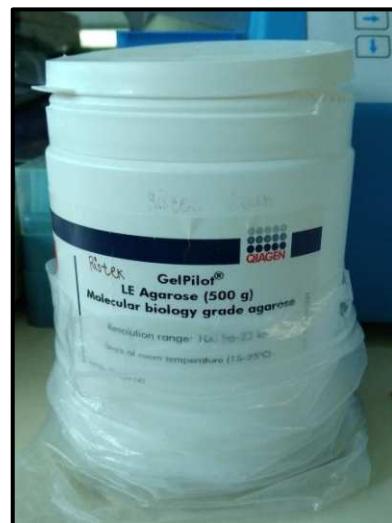
(b) KIT yang digunakan dalam purifikasi



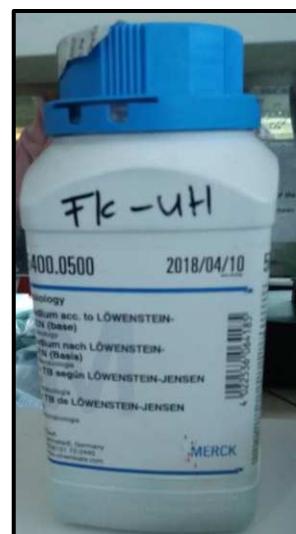
(c) Primer Forward dan primer Reverse



(d) Marker DNA dan Loading buffer



(e) Gel agarosa



(f) Bubuk LJ

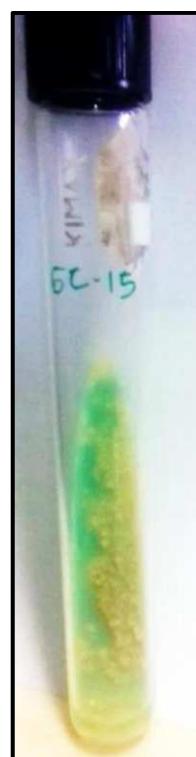


(g) Larutan TBE 0.5x

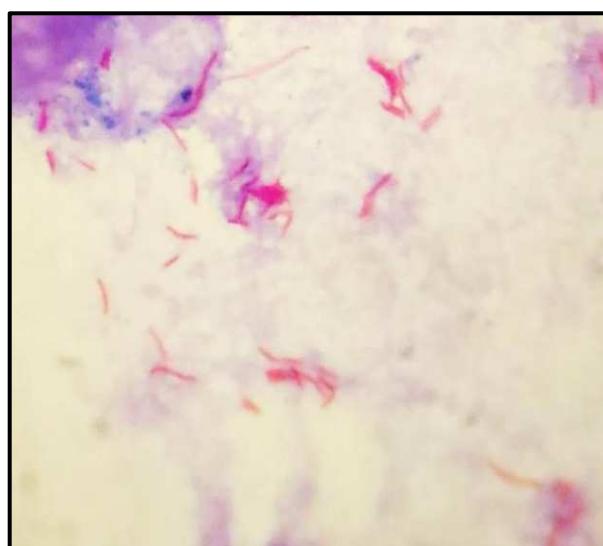


(h) Ethidium bromida (EtBr)

6. Prosedur kerja



(A) Koloni bakteri yang ditumbuhkan di media LJ



(A) Hasil pengamatan di bawah mikroskop



(A)



(B)



(C)



(D)



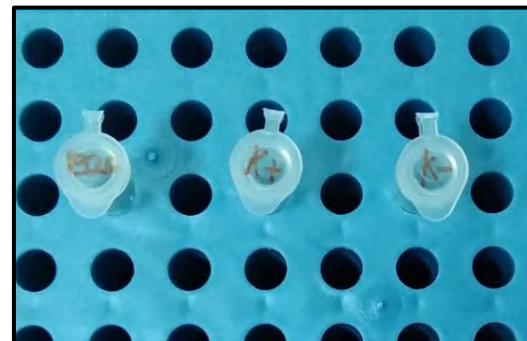
(E)

Keterangan Foto Isolasi DNA

- (A). Kit Isolasi yang digunakan yaitu gSYNC DNA Extraction Kit (Geneaid)
- (B). Proses penambahan buffer ke dalam tabung eppendorf.
- (C). Sampel dan campuran buffer kit isolasi DNA di dalam tabung eppendorf kemudian dimasukkan dalam water-bath.
- (D). Proses sentrifugasi.
- (E). Hasil Isolasi DNA.



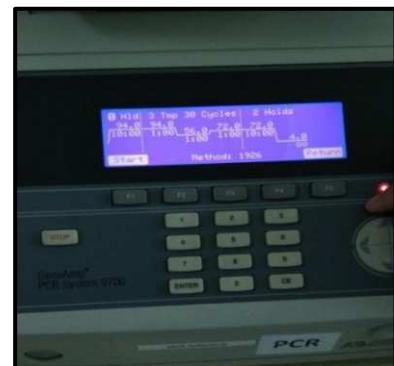
(A)



(B)



(C)



(D)



(E)

Keterangan Foto Amplifikasi Gen Rv1926c

(A).Proses pengerajan PCR Mix.

(B). Sampel yang akan diamplifikasi.

(C) Proses PCR.

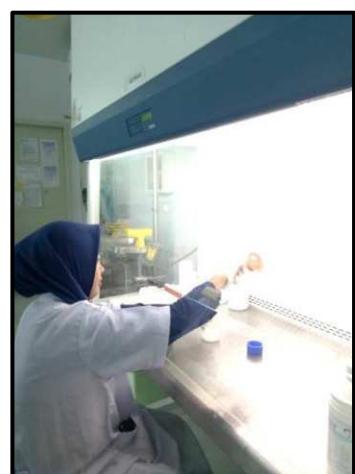


imasi PCR.

l Amplifikasi.



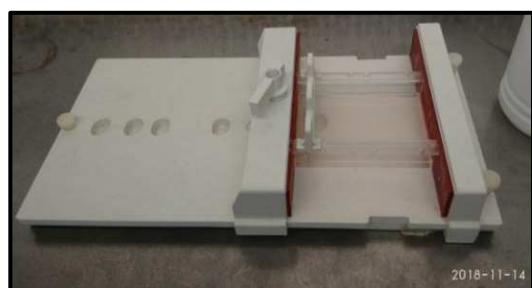
(A)



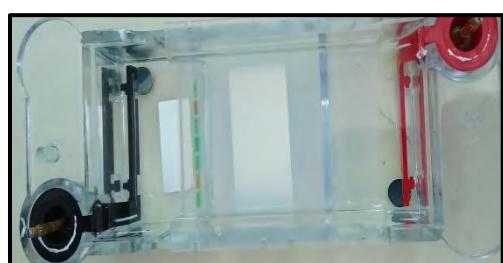
(B)



(C)



(D)



(E)



(F)

Keterangan Foto Elektroforesis

1. Pembangkitan gel agarosa 2%.



2. Campuran larutan gel agarosa dengan larutan TBE 0.5x dalam botolo reagen.

3. Pengentalan gel agarosa di dalam microwave.

- (D). Gel agarosa padat yang berada dalam cetakan gel dengan sisir gel yang telah diatur.
- (E). Gel agarosa yang terendam larutan TBE dan berisi sampel pada sumur gel yang akan dielektroforesis.
- (F). Mesin elektroforesis dijalankan dan ditunggu hingga \pm 2 jam.

