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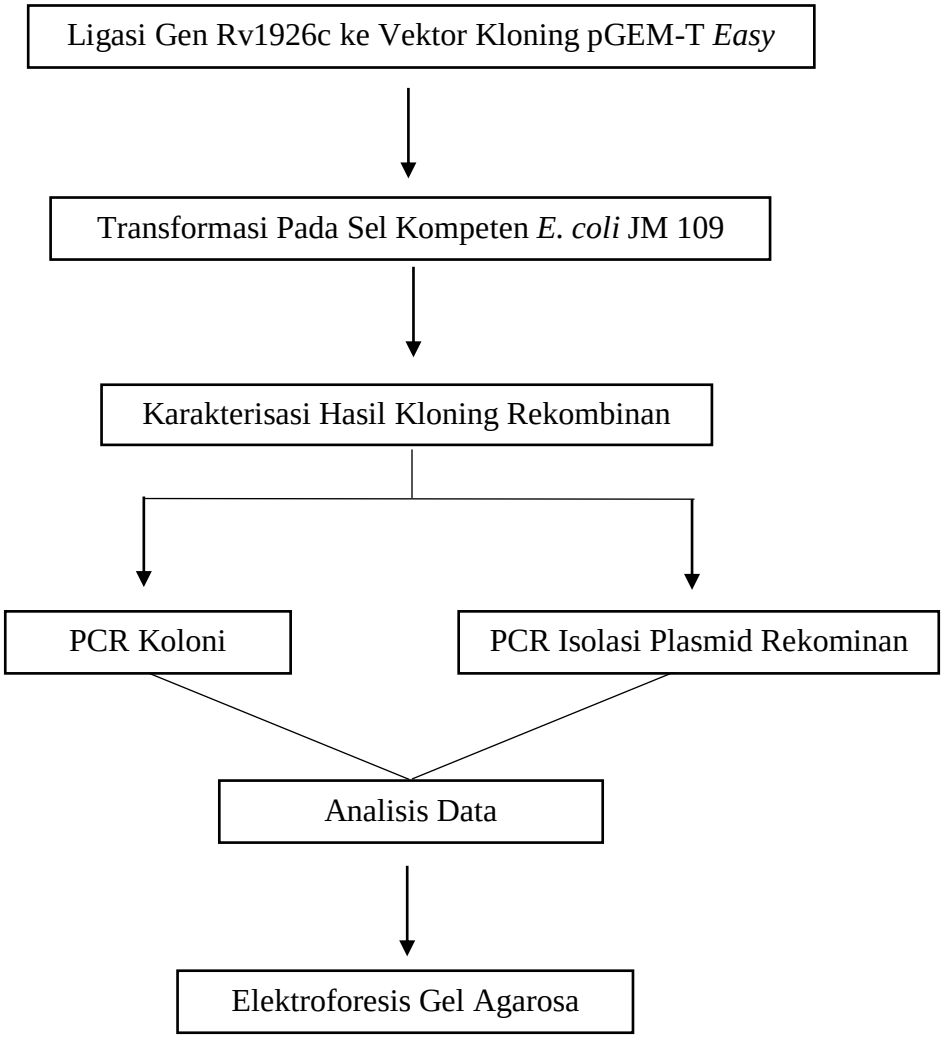
Lampiran 1. Skema Kerja Penelitian



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Lampiran 2. Kondisi PCR dan Primer untuk Amplifikasi

Amplifikasi Sebanyak 30 siklus

Pra Denaturasi	94ºC	10 Menit
Denaturasi	94°C	1 Menit
Annealing	56°C	1 Menit
Ekstensi	72°C	1 Menit
Ekstensi Akhir	72°C	10 Menit

Primer Forward

5'- CAGCAGGATCCCGCCTATCCCATCACCGGA - 3'

Primer Reverse

5'- GCCCAAGCTTCGGCTCCCAAATCAGCAG - 3'

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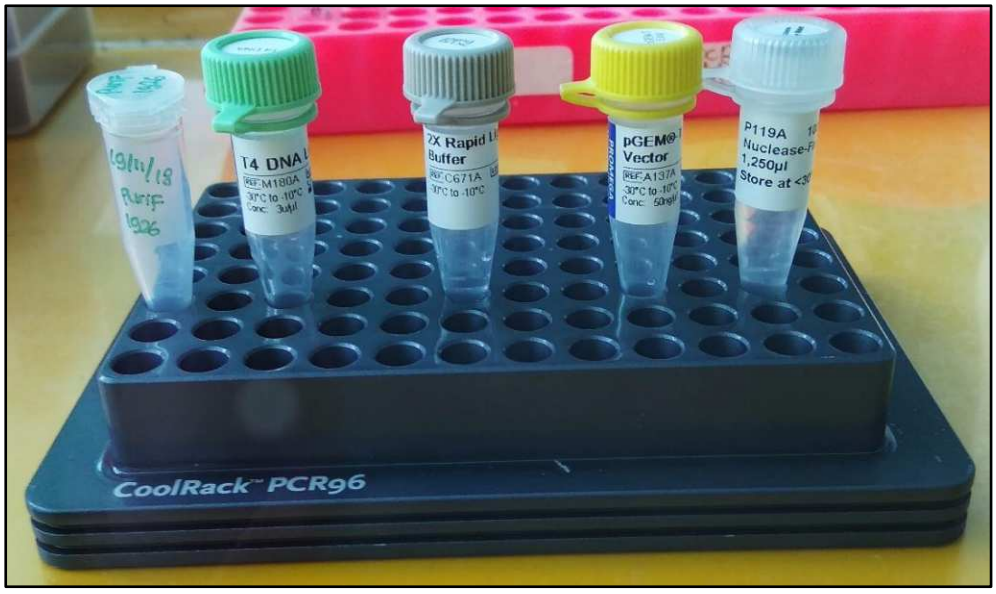
Lampiran 3. Komposisi Bahan

1. Reaksi Ligasi
   - 1 µl 2x *rapid ligation buffer*
   - 1 µl plasmid pGEM-T *Easy*
   - 5 µl produk PCR
   - 1 µl Enzim T4 DNA Ligase
   - 2 µl *nuclease free water*



2. Transformasi
   - 40 µl X- gal
   - 100 µl IPTG
   - 20 µl Ampisiln

3. Isolasi Plasmid Rekombinan

   uffer P1 250 µl

   uffer P2 250 µl

   uffer N3 350 µl

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- *Buffer* PE 750 µl
- *Buffer* EB 30 µl

4. Medium Luriah Bertani (LB)

a. LB Cair

- 1 g NaCl
- 0,5 g *Bacto tryton*
- 0,5 g *Bacto yeast*
- 100 mL Akuades

b. LB Padat

- 0,5 g NaCl
- 0,5 g *Bacto tryton*
- 1,75 g *Bacto agar*
- 0,25 g *Bacto yeast*
- 50 mL Akuades

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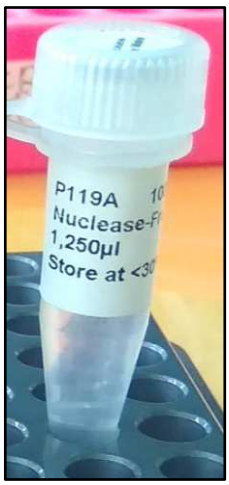
5. PCR Mix

- 12,5 µl enzimGo Taq Green Master Mix
- 0,5 µl Primer *forward*
- 0,5 µl Primer *reverse*
- 8,5 µl *Nuclease Free Water*
- 3 µl Sampel DNA



garosa 2 %

2 g Agarosa

0 mL *Buffer* TBE 1x

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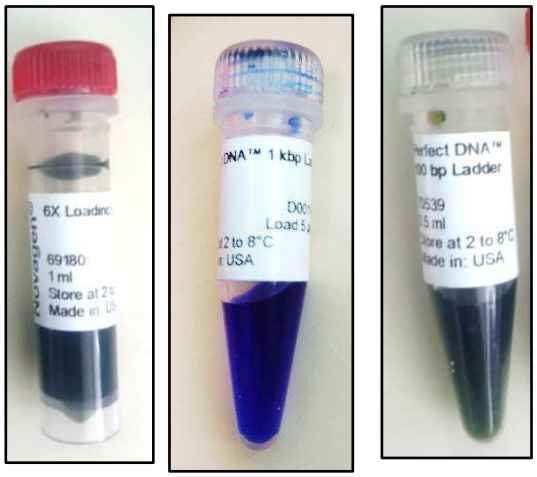
- 2 µl EtBr

7. Elektoforesis Gel

   - 2 µl *Loading Dye*

   - 10 µl Sampel DNA

   - 4 µl Marker 100 bp/ 1000 bp



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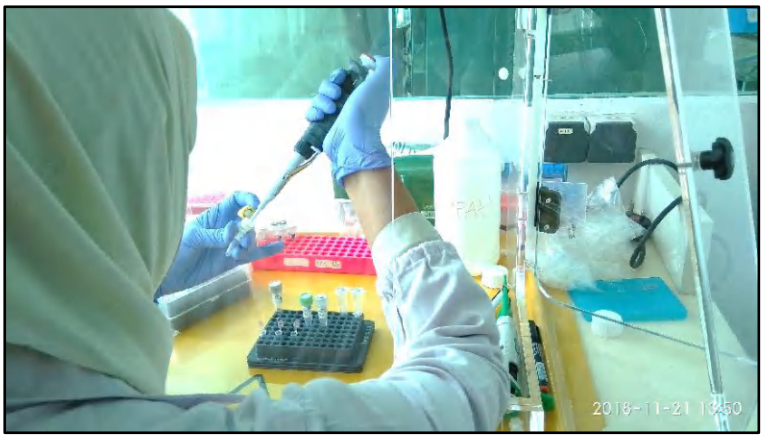
Lampiran 4. Prosedur Kerja

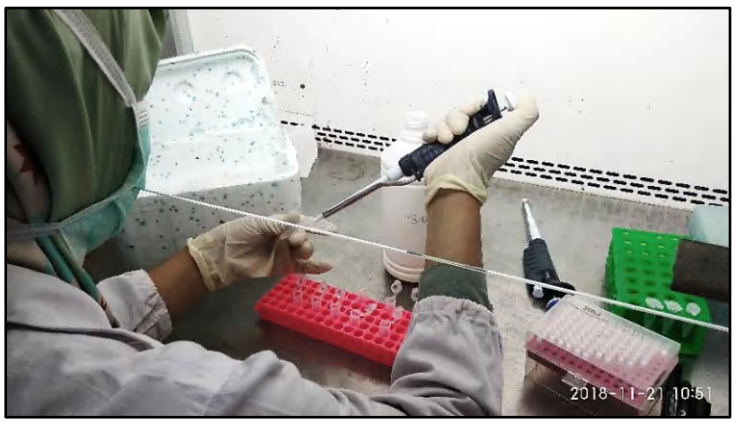
1. Ligasi Produk PCR yang Dimurnikan ke Vektor Kloning pGEM-T *Easy*



Ligasi DNA *orf* Rv1926c ke vektor kloning pGEM-T *Easy*

2. Persiapan Sel Kompeten

Kultur *Escherichia coli* JM 109 pada medium LB padat



coli JM 109 dipindahkan ke tabung eppendorf untuk siapa disentrifugasi

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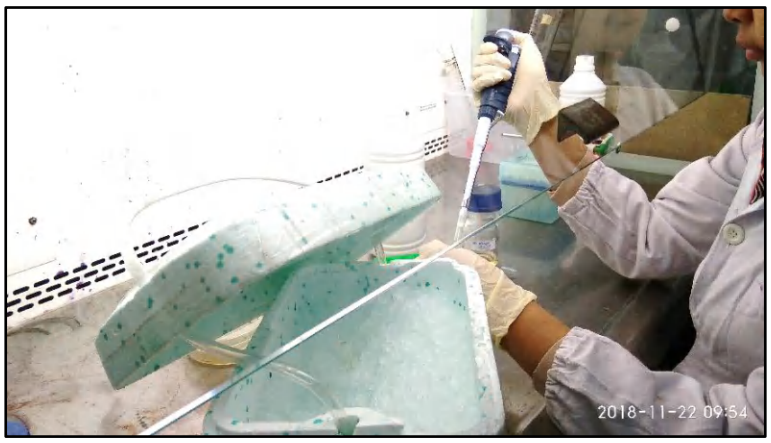
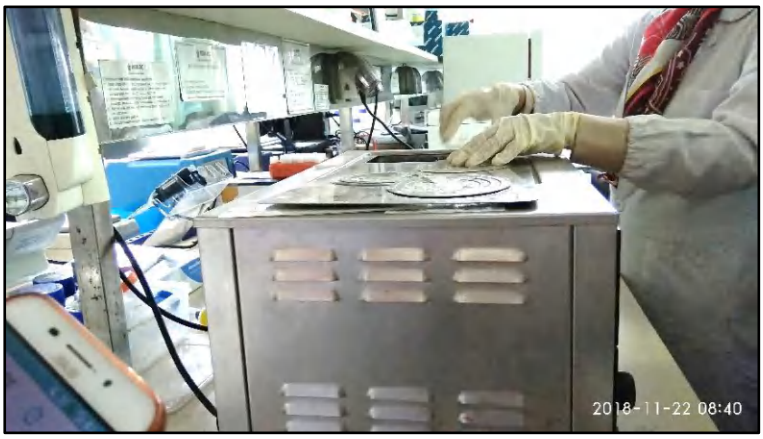
3. Transformasi Produk yang Diligasi ke *Escherichia coli* Strain Jm 109



Produk ligasi dimasukkan kedalam sel kompeten dan diinkubasi dalam es



Proses transformasi dengan menggunakan *Heat Shock*

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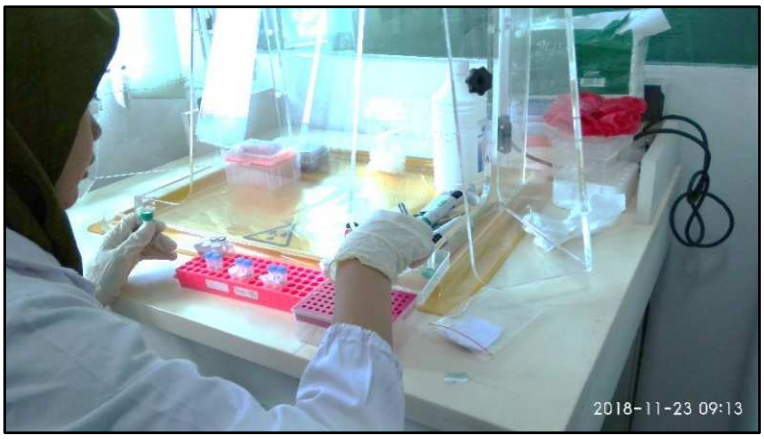
4. Karakterisasi

a. PCR Koloni



Persiapan reagen-reagen PCR Mix untuk PCR koloni

b. Isolasi Plasmid Rekombinan

Proses isolasi plasmid rekombinan dengan menggunakan kit miniprep (Qiagen)

Optimization Software:
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Lampiran 5. Genom Rv1926c

atgaagctcaccacaatgatcaagacggcagtagcggtcgtggccatggcggccatcgcgacctttgcggcaccg
gtcgcgttggctgcctatcccatcaccggaaaacttggcagtgagctaacgatgaccgacaccgttggccaagtcgt
gctcggctggaaggtcagtgatctcaaatccagcacggcagtcatccccggctatccggtggccggccaggtctgg
gaggccactgccacggtcaatgcgattcgcggcagcgtcacgcccgcggtctcgcagttcaatgcccgcaccgcc
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gaacaatcgaccggcaaaatctacttcgatgtcaccggcccatcgccaaccatcgtcgcgatgaacaacggcatgg
aggatctgctgatttgggagccgtag

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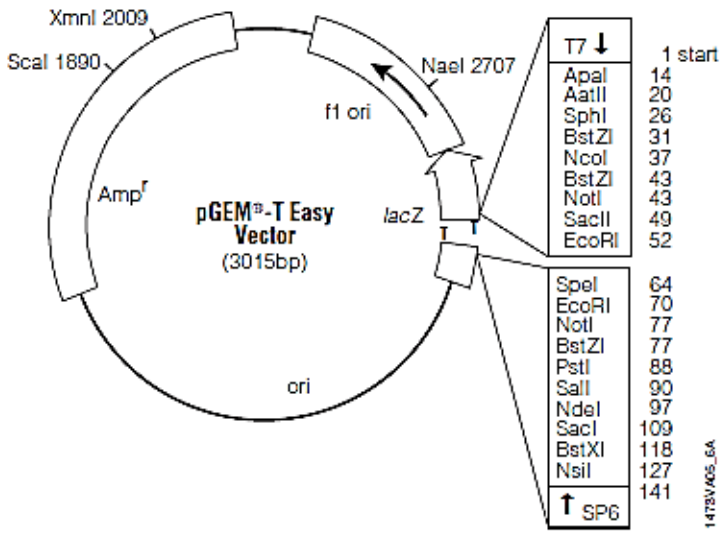
Lampiran 6. Peta Vektor pGEM-T *Easy*

Promega

5.D. pGEM®-T Easy Vector Map and Sequence Reference Points



pGEM®-T Easy Vector sequence reference points:

T7 RNA polymerase transcription initiation site	1
multiple cloning region	10–128
SP6 RNA polymerase promoter (–17 to +3)	139–158
SP6 RNA polymerase transcription initiation site	141
pUC/M13 Reverse Sequencing Primer binding site	176–197
*lac*Z start codon	180
lac operator	200–216
β-lactamase coding region	1337–2197
phage f1 region	2380–2835
lac operon sequences	2836–2996, 166–395
pUC/M13 Forward Sequencing Primer binding site	2949–2972
T7 RNA polymerase promoter (–17 to +3)	2999–3

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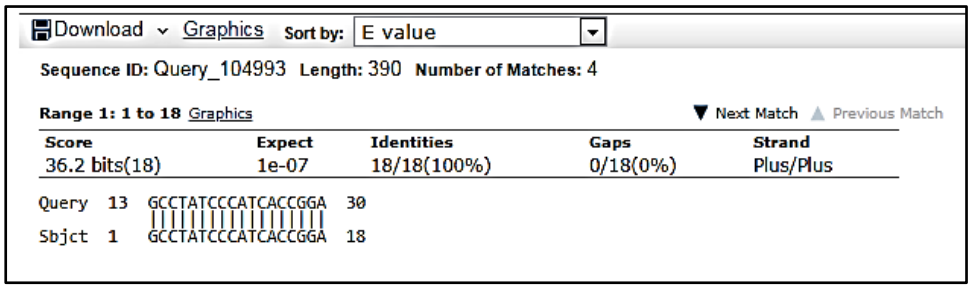
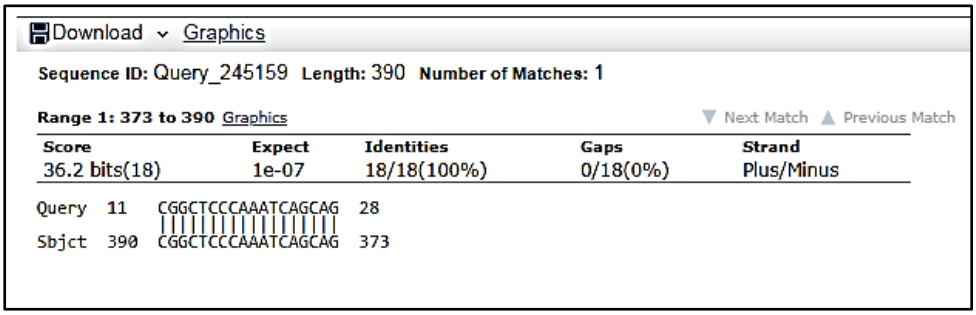
Lampiran 7. Hasil Blast dari Primer Forward dan Primer Reverse



Hasil BLAST *primer forward* Rv1926c



Hasil BLAST *primer reverse* Rv1926c

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