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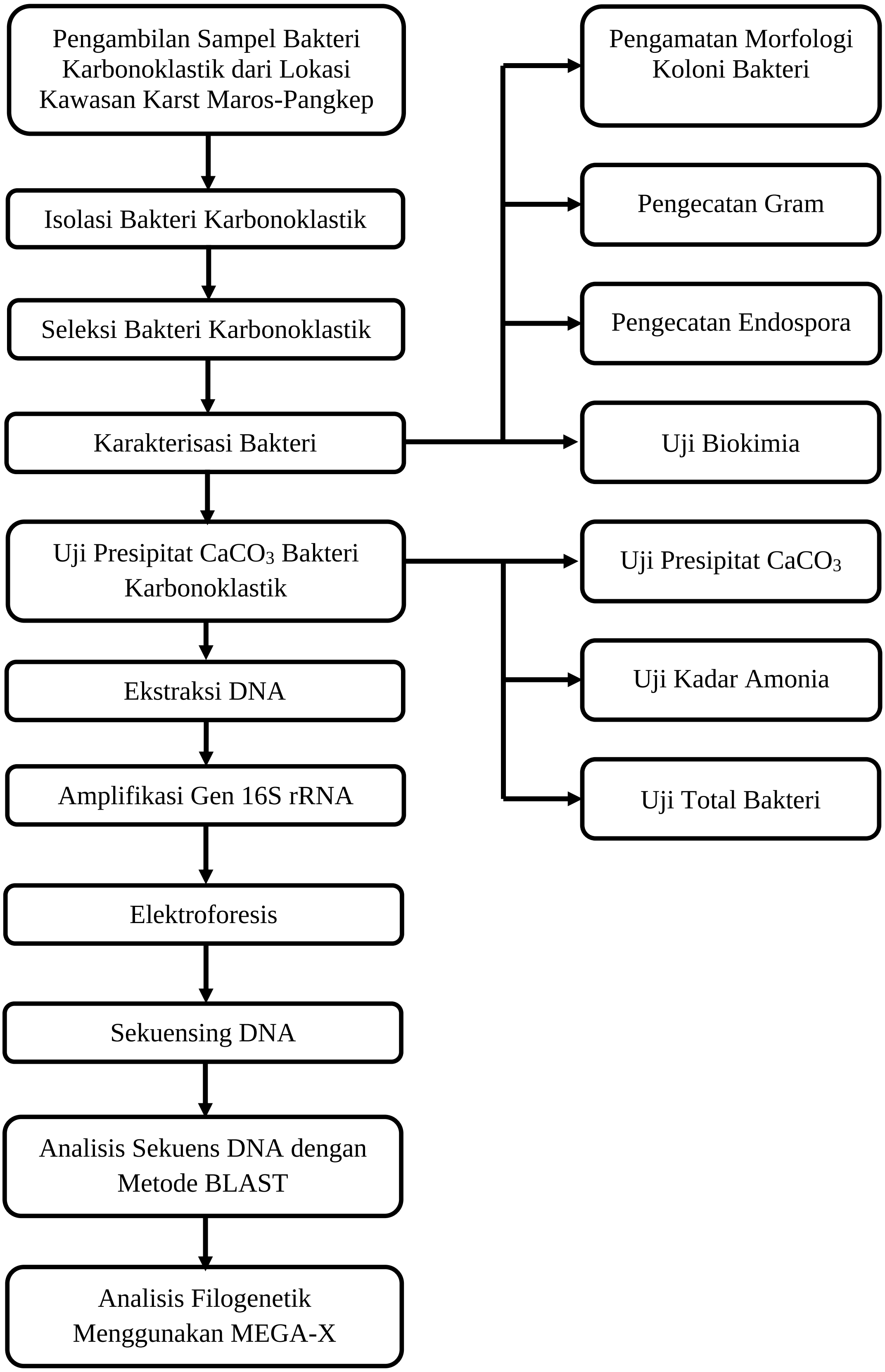
**Lampiran 1.** Skema Kerja Penelitian



**Lampiran 2.** Skema Kerja Pengambilan Sampel, Isolasi dan Seleksi Bakteri Karbonoklastik

**Pengambilan Sampel Bakteri**

- Sampel diambil menggunakan *cotton swab*
- Dimasukkan ke dalam larutan NaCl fisiologis
- Disimpan dalam *cool box* dan dibawa ke laboratorium untuk pengerjaan

↓

**Isolasi Bakteri**

- Sampel diambil sebanyak 1 ml
- Diencerkan dengan akuades steril hingga pengeceran $10^{-3}$
- Sampel pengenceran diambil 1 ml, kemudian diinokulasikan pada medium Nutrient Agar.
- Diinkubasi selama 1×24 jam

↓

**Hasil Isolasi**

- Memilih setiap koloni yang berbeda
- Menyentuhkan jarum ose yang steril pada koloni kemudian digores pada medium Nutrient Agar
- Diinkuubasi selama 1×24 jam

↓

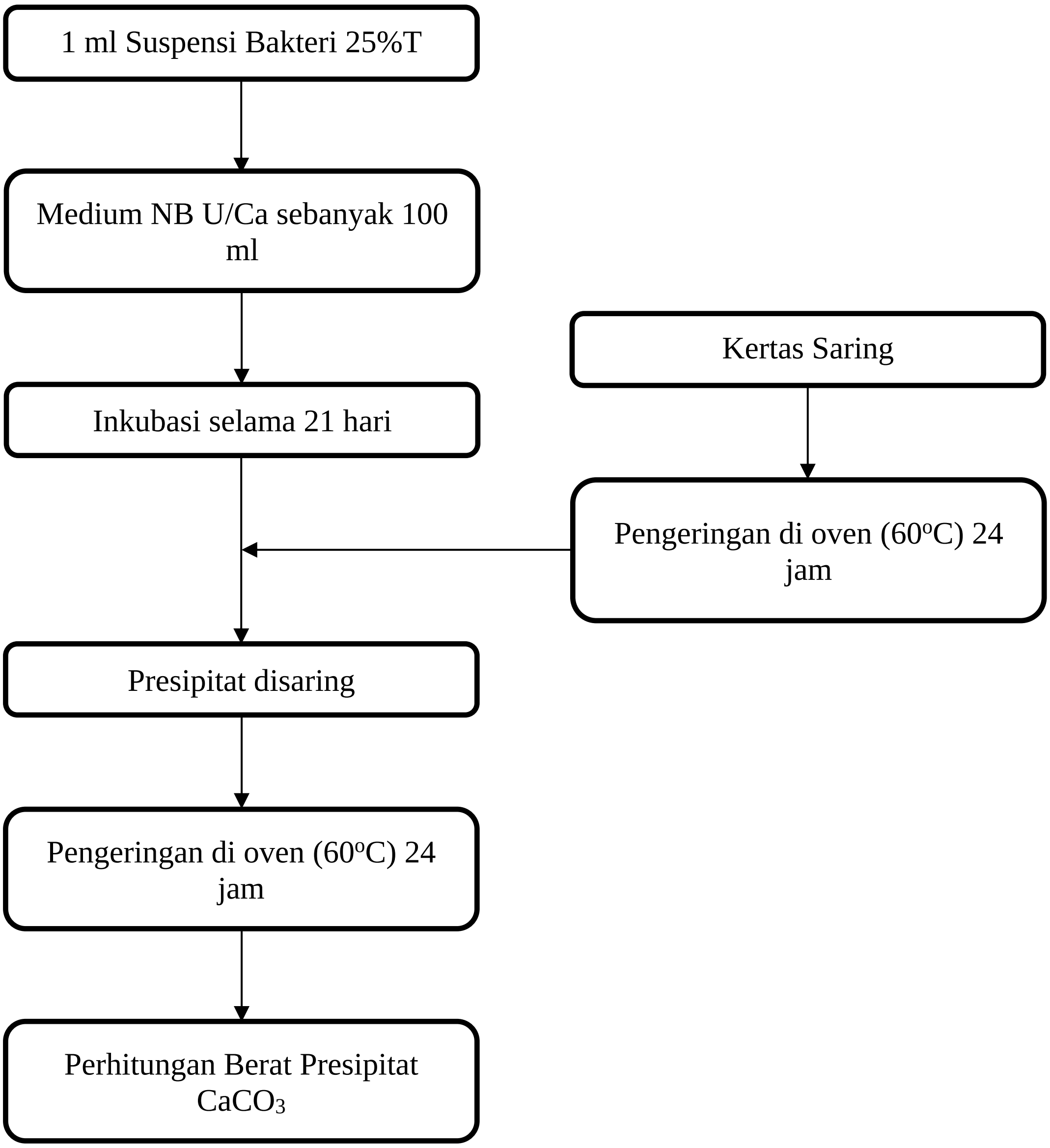
**Isolat Murni**

- Diambil sebanyak 1 isolat murni menggunakan ose
- Diinokulasikan ke medium *Calcium Carbonat Presipitation* Agar dengan Teknik *quadran streak*
- Diinkubasi selama 3×24 jam
- Dilakukan pengamatan menggunakan mikroskop stereo dengan ciri koloni membentuk kristal di sekitarnya

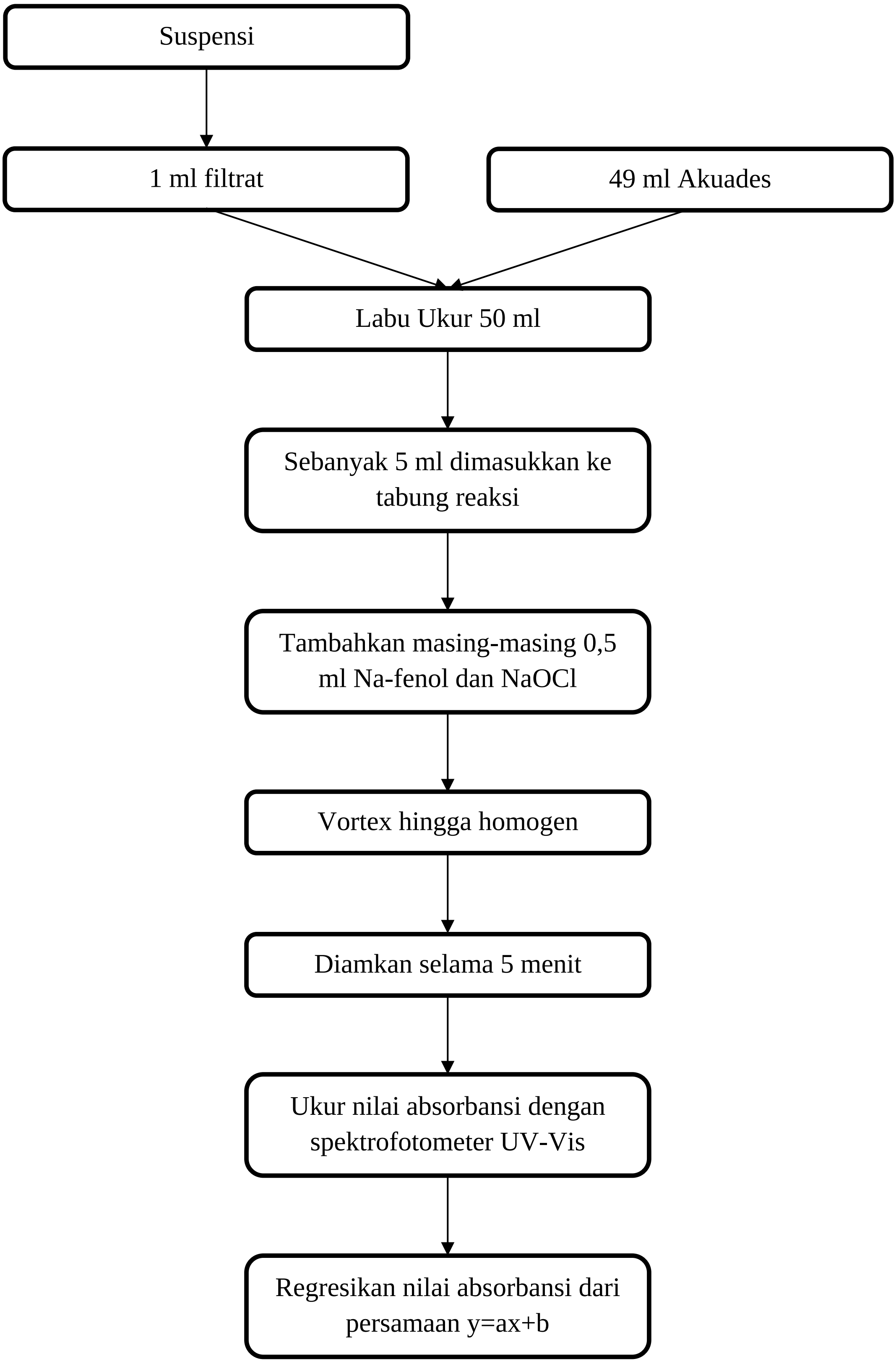
↓

**Seleksi Bakteri Karbonoklastik**

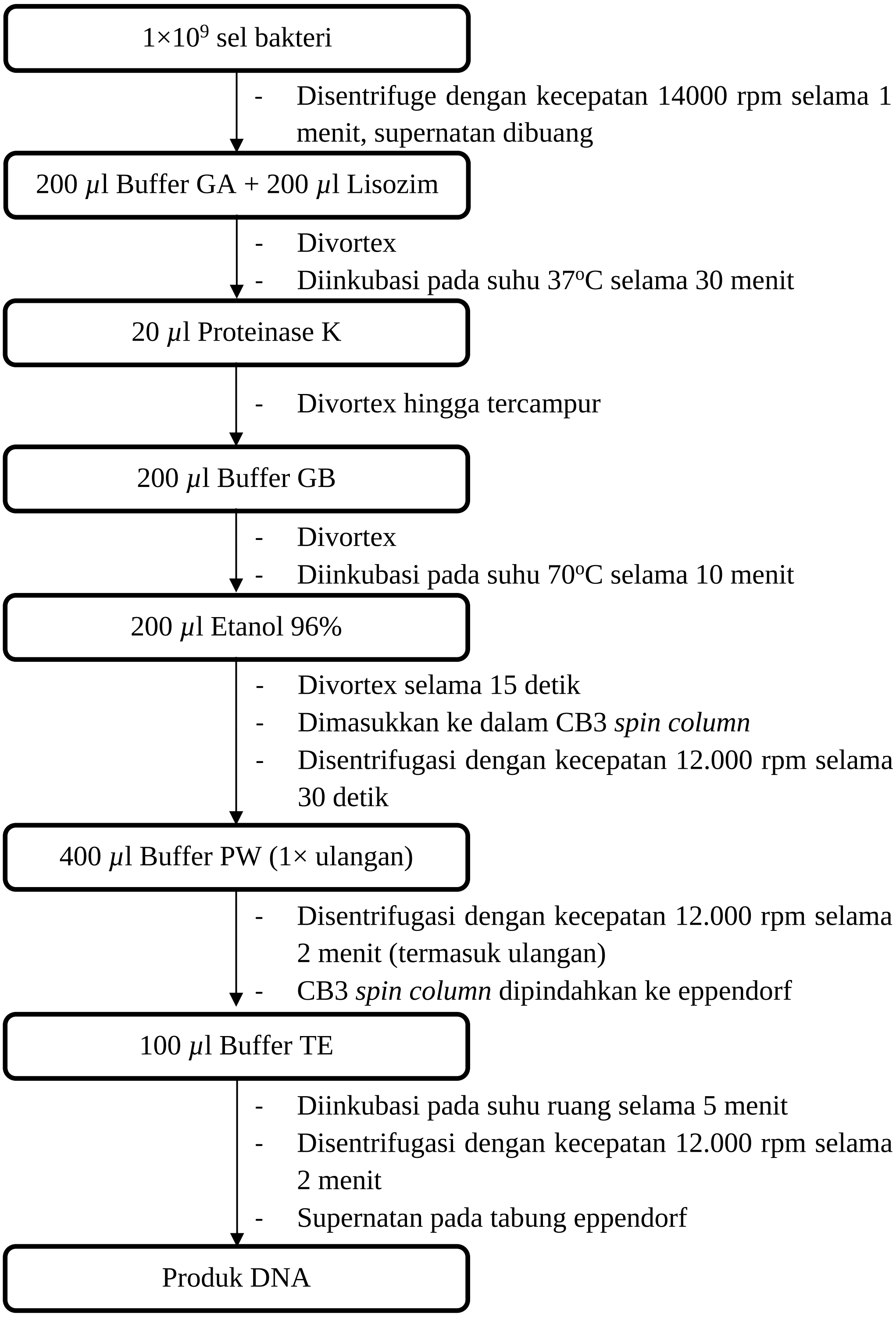
**Lampiran 3.** Skema Kerja Uji Presipitat $CaCO_3$ yang dihasilkan Bakteri Karbonoklastik



**Lampiran 4.** Skema Kerja Uji Kadar Amonia yang dihasilkan Bakteri Karbonoklastik



**Lampiran 5.** Skema Kerja Ekstraksi DNA Bakteri



**Lampiran 6.** Skema Kerja Amplifikasi DNA dengan PCR

**Sampel DNA**

- Dibuat campuran reaksi PCR yaitu 8 $\mu$l 1× Mytaq HS Red Mix, 2 $\mu$l ddH2O dan masing-masing 1 $\mu$l DNA gen 16S rRNA Primer 63F dan 1387R

↓

**12 $\mu$l campuran reaksi PCR**

- Dimasukkan ke dalam tabung eppendorf

↓

**5 $\mu$l ekstraksi DNA**

- Dimasukkan ke dalam tabung Eppendorf yang telah berisi campuran reaksi PCR

↓

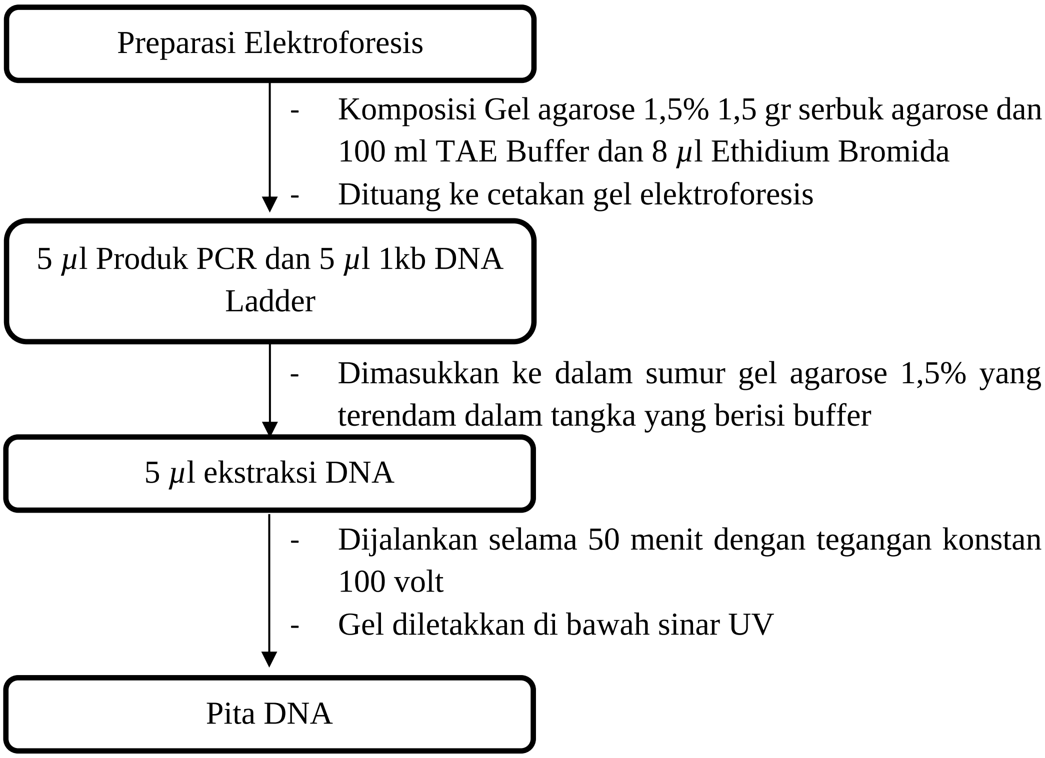
**Amplifikasi dengan mesin PCR**

- Diamplifikasi sebanyak 35 siklus
- Setiap siklus
  Predenaturasi pada suhu 95$^{o}$C selama 5 menit
  Denaturasi pada suhu 95$^{o}$C selama 1 menit
  *Anneling* pada suhu 55$^{o}$C selama 45 detik
  *Extending* pada suhu 72$^{o}$C selama 1 menit
  *Post Extending* pada suhu 72$^{o}$C selama 10 menit

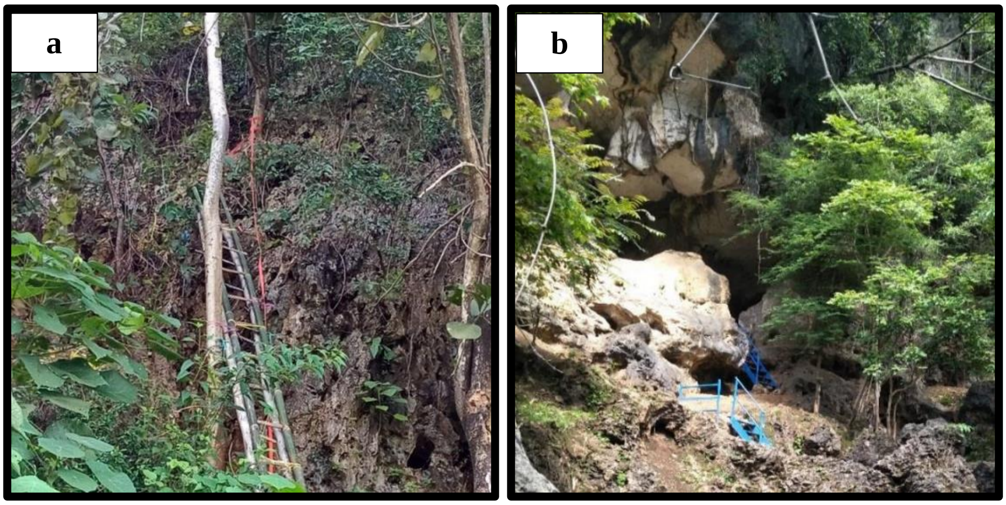
↓

**Produk PCR**

**Lampiran 7.** Skema Kerja Visualisasi Produk PCR dengan Elektroforesis

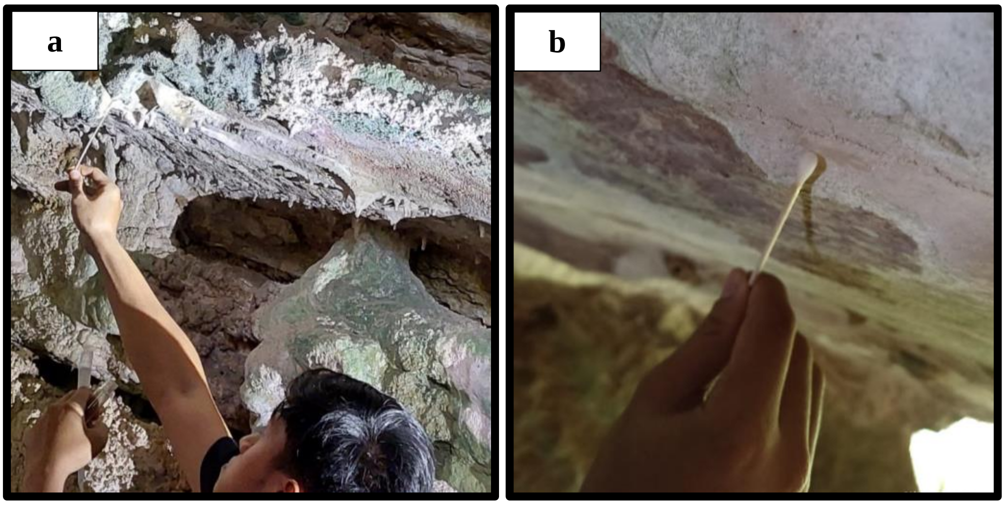


**Lampiran 8.** Tempat Pengambilan Sampel




Tempat Pengambilan Sampel (a) gua Parewe, (b) gua Bulu Sipong

**Lampiran 9.** Pengambilan Sampel




Titik Pengambilan Sampel (a) gua Parewe, (b) gua Bulu Sipong

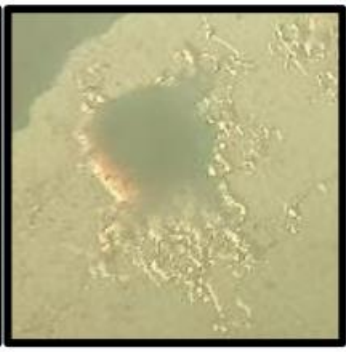
**Lampiran 10.** Hasil Seleksi Bakteri Karbonoklastik

Isolat Ps1-c

Isolat Ps1-d

Isolat Ps8-a

Isolat Ps8-b



Isolat Ps8-c

Isolat Ps8-d

Isolat BSs2-a

Isolat BSs2-c

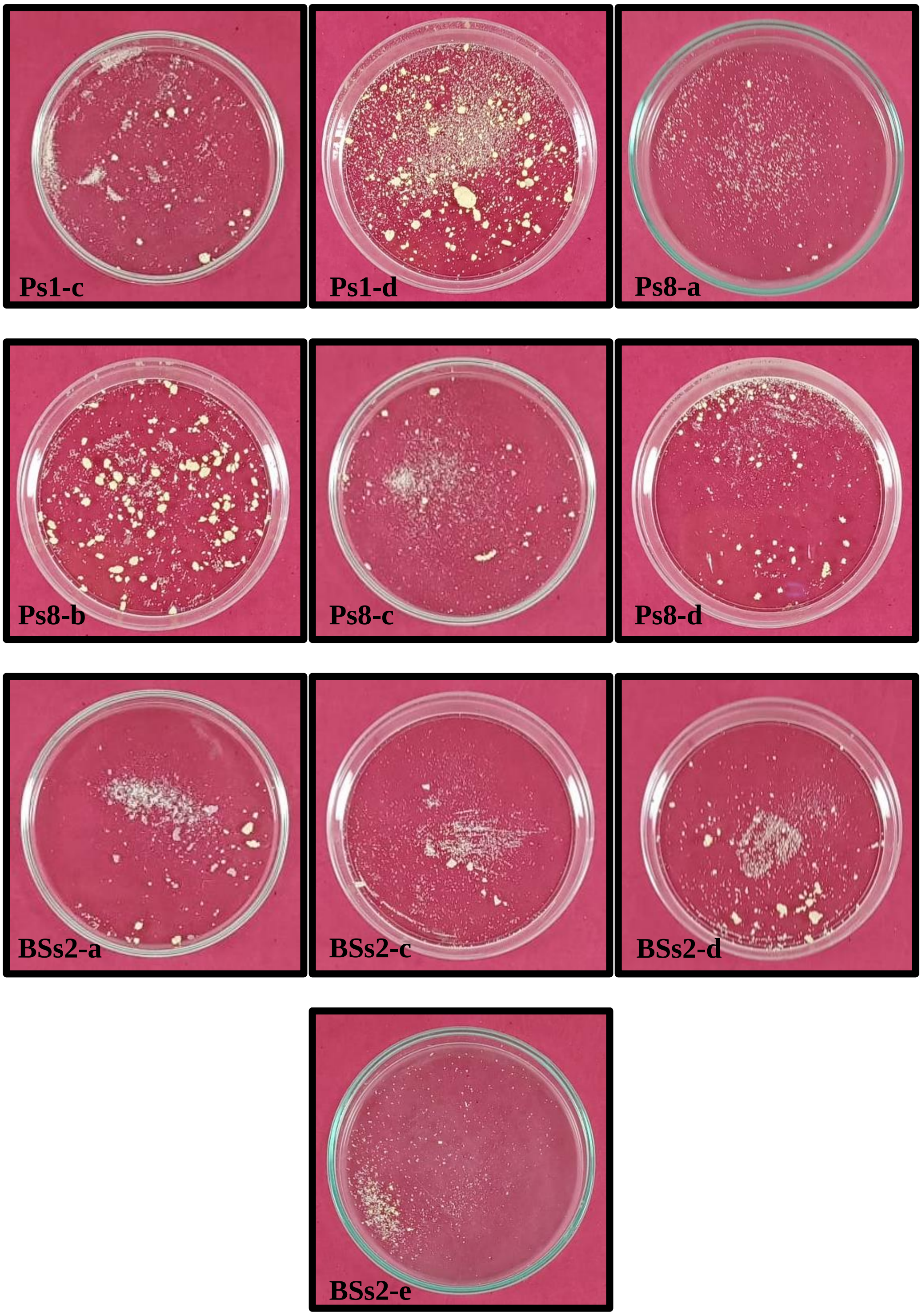
Isolat BSs2-d

Isolat BSs2-e

**Lampiran 11.** Uji Potensi Presipitat $CaCO_3$ oleh Bakteri Karbonoklastik


Kultur Isolat Bakteri Karbonoklastik pada Medium NB U/Ca

**Lampiran 12.** Presipitat yang Dihasilkan oleh Bakteri Karbonoklastik



**Lampiran 13.** Hasil Perhitungan Berat Presipitat $CaCO_3$

| Isolat | Berat Presipitasi (mg) | | |
|---|---|---|---|
| | **Berat presipitat dan Berat Kertas Saring (Wfc)** | **Berat Kertas Saring (Wf)** | **Berat Presipitat (Wc)** |
| Ps1-c | 1,388.70 | 1,326.70 | 62.00 |
| Ps1-d | 1,774.90 | 1,530.40 | 244.50 |
| Ps8-a | 1,341.70 | 1,288.00 | 53.70 |
| Ps8-b | 1,731.90 | 1,552.40 | 179.50 |
| Ps8-c | 1,411.50 | 1,355.70 | 55.80 |
| Ps8-d | 1,456.80 | 1,367.20 | 89.60 |
| BSs2-a | 1,442.10 | 1,383.40 | 58.70 |
| BSs2-c | 1,472.50 | 1,433.20 | 39.30 |
| BSs2-d | 1,463.10 | 1,401.00 | 62.10 |
| BSs2-e | 1,653.70 | 1,609.40 | 44.30 |

**Lampiran 14.** Hasil Perhitungan Kadar Analisa Amonia

| No | Isolat | Pengenceran | | | Absorbansi | Slope | Intercep | y | Kadar N-NH3 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Sampel | H2O | P | x | a | b | | ppm | mMol |
| 1 | Ps1-c | 1 | 49 | 50 | 0.176 | 41.106 | 0.1403 | 7.375 | 368.748 | 26.339 |
| 2 | Ps1-d | 1 | 49 | 50 | 0.368 | 41.106 | 0.1403 | 15.267 | 763.365 | 54.526 |
| 3 | Ps8-a | 1 | 49 | 50 | 0.457 | 41.106 | 0.1403 | 18.926 | 946.287 | 67.592 |
| 4 | Ps8-b | 1 | 49 | 50 | 0.129 | 41.106 | 0.1403 | 5.443 | 272.149 | 19.439 |
| 5 | Ps8-c | 1 | 49 | 50 | 0.174 | 41.106 | 0.1403 | 7.293 | 364.637 | 26.046 |
| 6 | Ps8-d | 1 | 49 | 50 | 0.125 | 41.106 | 0.1403 | 5.279 | 263.928 | 18.852 |
| 7 | BSs2-a | 1 | 49 | 50 | 0.142 | 41.106 | 0.1403 | 5.977 | 298.868 | 21.348 |
| 8 | BSs2-c | 1 | 49 | 50 | 0.116 | 41.106 | 0.1403 | 4.909 | 245.430 | 17.531 |
| 9 | BSs2-d | 1 | 49 | 50 | 0.120 | 41.106 | 0.1403 | 5.073 | 253.651 | 18.118 |
| 10 | BSs2-e | 1 | 49 | 50 | 0.154 | 41.106 | 0.1403 | 6.471 | 323.531 | 23.109 |

**Lampiran 15.** Hasil Karakterisasi Isolat Bakteri Karbonoklastik

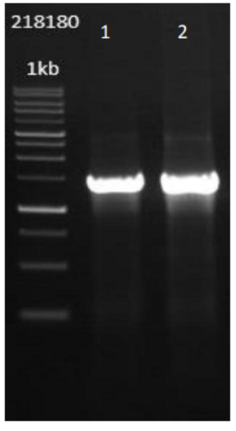
| Isolat | Morfologi Koloni | | | | Morfologi Sel | | | Uji Biokimia | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | SIM | | | | MR-VP | | |
| | Bentuk | Elevasi | Tepi | Warna | Bentuk | Sifat Gram | Endo spora | Motilitas | $H_2S$ | Indol | Sitrat | MR | VP | Katalase |
| Ps1-c | Irregular | Raised | Lobate | Putih gading | Basil | - | + | + | - | - | - | + | + | + |
| Ps1-d | Irregular | Raised | Lobate | Putih susu | Basil | - | + | + | - | - | - | + | + | + |
| Ps8-a | Irregular | Raised | Lobate | Putih gading | Basil | + | + | + | - | - | - | + | - | + |
| Ps8-b | Irregular | Raised | Lobate | Putih gading | Basil | + | + | + | - | - | - | + | - | + |
| Ps8-c | Irregular | Raised | Lobate | Putih gading | Basil | + | + | + | - | - | - | + | - | + |
| Ps8-d | Irregular | Raised | Lobate | Putih gading | Basil | + | + | + | - | - | - | + | - | + |
| BSs2-a | Irreguler | Raised | Lobate | Putih gading | Basil | - | + | + | - | - | - | + | - | + |
| BSs2-c | Irreguler | Raised | Lobate | Putih gading | Basil | - | + | + | - | - | - | + | - | + |
| BSs2-d | Irregular | Raised | Lobate | Putih gading | Basil | - | + | + | - | - | - | + | - | + |
| BSs2-e | Irregular | Raised | Lobate | Putih gading | Basil | - | + | + | - | - | - | + | - | + |

**Lampiran 16.** Hasil Elektroforesis Gen 16 rRNA dengan Primer 63F dan 1387R Isolat Bakteri Ps1-d dan Ps8-b pada 1300 bp

**Agarose Quantification Report**

**Order status:** PASS Orders On-Hold. Customer to give confirmation to process / reject FAIL sample(s).

| Rank | SampleID | OrderID | S.Name | S.Type | S.Size | CommentsAQ | AQ Status | SuggestionAQ |
|---|---|---|---|---|---|---|---|---|
| 1 | 2922349 | 218180 | 8B | Unpurified PCR Product | 1300 | Gel Extraction. | PASS | |
| 2 | 2922350 | 218180 | 1D | Unpurified PCR Product | 1300 | Gel Extraction. | PASS | |




| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Condition: | | | 0.8% | agarose gel | | | | | | | | | |
| Amount of DNA ladder loaded per lane: | | | 0.1ug | each | | | | | | | | | |
| Volume of sample loaded per lane: | | | 1uL | each | | | | | | | | | |
| **1kb DNA Ladder (bp):** | 250 | 500 | 750 | **1,000** | 1,500 | 2,000 | 2,500 | **3,000** | 4,000 | 5,000 | 6,000 | 8,000 | 10,000 |
| **1kb DNA Ladder (ng/0.1ug):** | 9 | 6 | 4.6 | 18.4 | 4 | 6.8 | 6.8 | **18.4** | 3.6 | 5.6 | 5.6 | 5.6 | 5.6 |

Note: The DNA ladder is not applicable for sizing comparison of non-linear DNA samples (e.g. plasmid DNA)

**Lampiran 17.** Identifikasi Jenis Bakteri Menggunakan Marka Molekuler

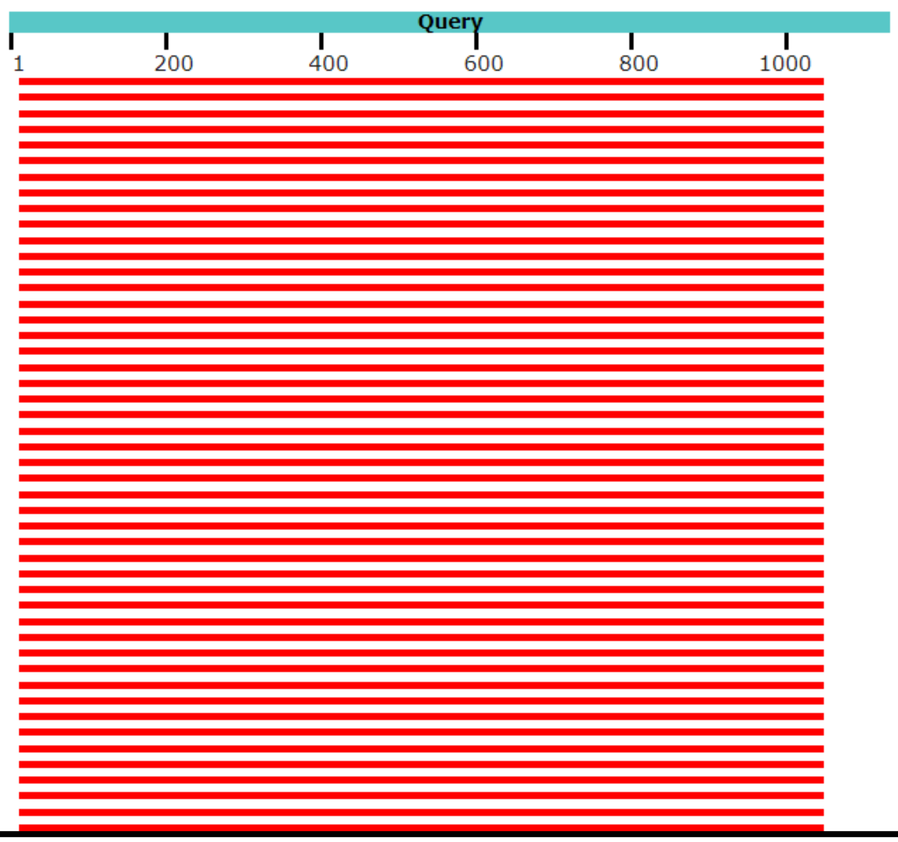
1. Hasil Sekuensing Isolat Bakteri Ps1-d

**Sequences producing significant alignments** — Download — Select columns — Show 100

select all — 100 sequences selected — GenBank — Graphics — Distance tree of results — MSA Viewer

| Description | Scientific Name | Max Score | Total Score | Query Cover | E value | Per. Ident | Acc. Len | Accession |
|---|---|---|---|---|---|---|---|---|
| Bacillus sp. (in: Bacteria) strain LMRE75 16S ribosomal RNA gene, partial sequence | Bacillus sp. (in: firmicutes) | 1784 | 1784 | 91% | 0.0 | 97.59% | 1375 | MK571702.1 |
| Bacillus cereus strain bk 16S ribosomal RNA gene, partial sequence | Bacillus cereus | 1784 | 1784 | 91% | 0.0 | 97.59% | 1462 | KX941838.1 |
| Bacillus thuringiensis strain R10-3 16S ribosomal RNA gene, partial sequence | Bacillus thuringiensis | 1779 | 1779 | 91% | 0.0 | 97.49% | 1268 | MN696520.1 |
| Bacillus cereus strain AHSM124 16S ribosomal RNA gene, partial sequence | Bacillus cereus | 1779 | 1779 | 91% | 0.0 | 97.49% | 1367 | MK074711.1 |
| Bacillus albus strain 214 16S ribosomal RNA gene, partial sequence | Bacillus albus | 1779 | 1779 | 91% | 0.0 | 97.49% | 1161 | MK389454.1 |
| Bacillus pumilus strain CGAPGPBS-051 16S ribosomal RNA gene, partial sequence | Bacillus pumilus | 1779 | 1779 | 91% | 0.0 | 97.49% | 1518 | KY495223.1 |
| Bacillus sp. (in: Bacteria) 16S ribosomal RNA gene, partial sequence | Bacillus sp. (in: firmicutes) | 1779 | 1779 | 91% | 0.0 | 97.49% | 1219 | KX570944.1 |
| Bacillus cereus strain TV5 16S ribosomal RNA gene, partial sequence | Bacillus cereus | 1779 | 1779 | 91% | 0.0 | 97.49% | 1357 | KT818808.1 |
| Bacillus sp. (in: Bacteria) strain NBAIR_BS10 16S ribosomal RNA gene, partial sequence | Bacillus sp. (in: firmicutes) | 1779 | 1779 | 91% | 0.0 | 97.49% | 1391 | OP800101.1 |
| Bacillus thuringiensis strain JK0716S 16S ribosomal RNA gene, partial sequence | Bacillus thuringiensis | 1777 | 1777 | 91% | 0.0 | 97.49% | 1398 | KF135459.1 |
| Bacillus sp. (in: Bacteria) strain MAIDO-R18b-8 16S ribosomal RNA gene, partial sequence | Bacillus sp. (in: firmicutes) | 1775 | 1775 | 91% | 0.0 | 97.40% | 1431 | MW632003.1 |
| Bacillus thuringiensis strain PWN2B 16S ribosomal RNA gene, partial sequence | Bacillus thuringiensis | 1773 | 1773 | 91% | 0.0 | 97.39% | 1160 | MK026873.1 |
| Bacillus cereus strain PL22-16A chromosome, complete genome | Bacillus cereus | 1773 | 24762 | 91% | 0.0 | 97.40% | 5282712 | CP115856.1 |
| Bacillus sp. (in: firmicutes) strain GH2 16S ribosomal RNA gene, partial sequence | Bacillus sp. (in: firmicutes) | 1773 | 1773 | 91% | 0.0 | 97.40% | 1454 | OQ223395.1 |
| Bacillus sp. (in: firmicutes) strain BR50 16S ribosomal RNA gene, partial sequence | Bacillus sp. (in: firmicutes) | 1773 | 1773 | 91% | 0.0 | 97.40% | 1141 | OQ221818.1 |
| Bacillus cereus strain DPP(A)_SGAM_SXC 16S ribosomal RNA gene, partial sequence | Bacillus cereus | 1773 | 1773 | 91% | 0.0 | 97.40% | 1488 | OQ216888.1 |
| Bacillus thuringiensis serovar tenebrionis strain NB125 chromosome, complete genome | Bacillus thuringiensis serovar tenebri... | 1773 | 24768 | 91% | 0.0 | 97.40% | 5605440 | CP114392.1 |
| Bacillus thuringiensis serovar tenebrionis strain NB176-1 chromosome, complete genome | Bacillus thuringiensis serovar tenebri... | 1773 | 24801 | 91% | 0.0 | 97.40% | 5606443 | CP114399.1 |
| Bacillus thuringiensis serovar tenebrionis strain NB-176 chromosome, complete genome | Bacillus thuringiensis serovar tenebri... | 1773 | 24834 | 91% | 0.0 | 97.40% | 5428075 | CP114406.1 |
| Bacillus cereus strain BC-01 chromosome BC01, complete sequence | Bacillus cereus | 1773 | 24790 | 91% | 0.0 | 97.40% | 5317157 | CP115307.1 |

**Distribution of the top 257 Blast Hits on 100 subject sequences**

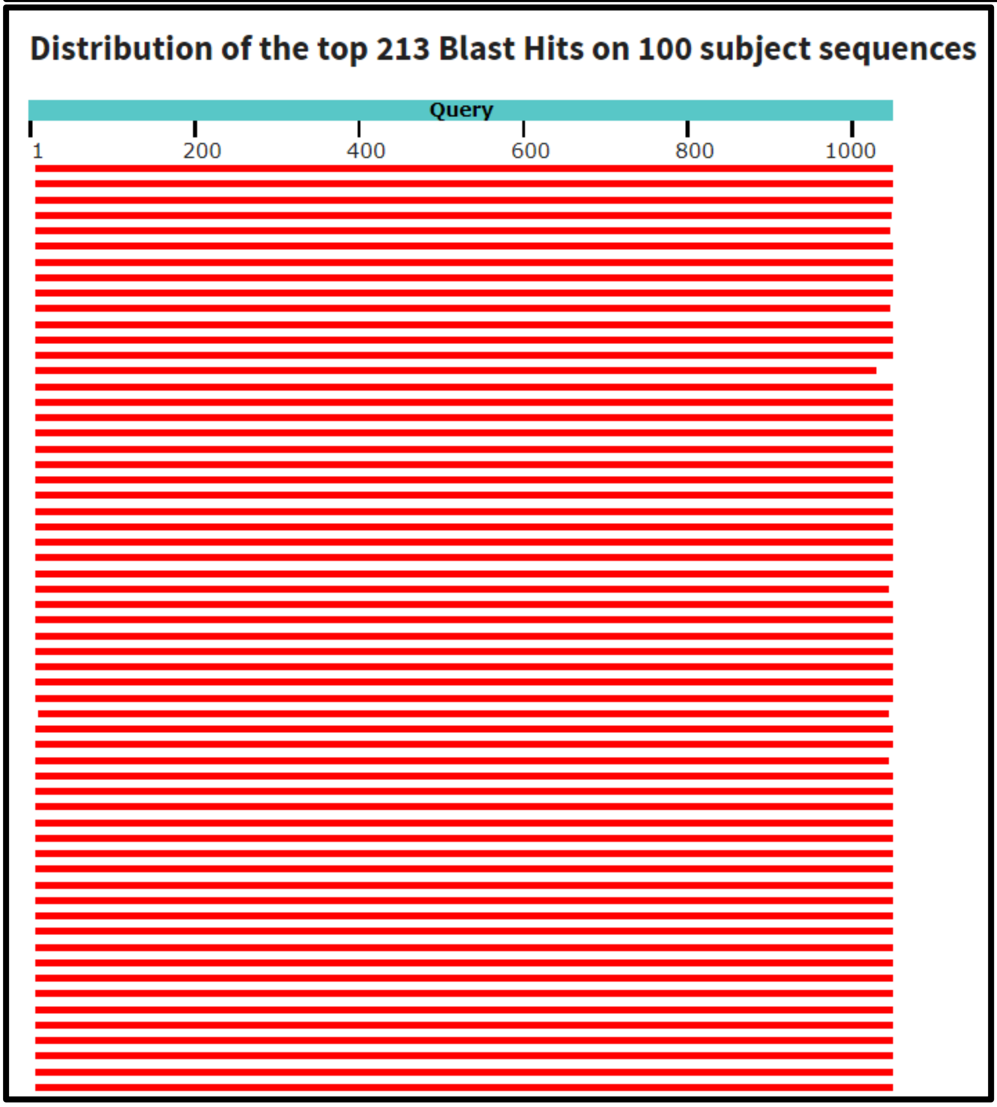


2. Hasil Sekuensing Isolat Bakteri Ps8-b

**Sequences producing significant alignments** — Download — Select columns — Show 100

select all — *100 sequences selected* — GenBank — Graphics — Distance tree of results — MSA Viewer

| Description | Scientific Name | Max Score | Total Score | Query Cover | E value | Per. Ident | Acc. Len | Accession |
|---|---|---|---|---|---|---|---|---|
| Bacillus sp. (in: Bacteria) NCCP-428 gene for 16S rRNA, partial sequence | Bacillus sp. (in: firmicutes) | 1829 | 1829 | 99% | 0.0 | 98.37% | 1134 | LC488913.1 |
| Bacillus cereus strain 151007-R3_A11_43_27F 16S ribosomal RNA gene, partial seque... | Bacillus cereus | 1823 | 1823 | 99% | 0.0 | 98.27% | 1138 | KY820937.1 |
| Bacillus cereus strain 151007-R3_M03_15_27F 16S ribosomal RNA gene, partial sequ... | Bacillus cereus | 1823 | 1823 | 99% | 0.0 | 98.27% | 1147 | KY820902.1 |
| Bacillus cereus strain 151007-R3_O03_16_27F 16S ribosomal RNA gene, partial sequ... | Bacillus cereus | 1820 | 1820 | 99% | 0.0 | 98.27% | 1141 | KY820924.1 |
| Bacillus tropicus strain B11 16S ribosomal RNA gene, partial sequence | Bacillus tropicus | 1820 | 1820 | 98% | 0.0 | 98.36% | 1188 | OL445008.1 |
| Bacillus thuringiensis strain BTH1 16S ribosomal RNA gene, partial sequence | Bacillus thuringiensis | 1818 | 1818 | 99% | 0.0 | 98.18% | 1273 | MK561610.1 |
| Bacillus albus strain 214 16S ribosomal RNA gene, partial sequence | Bacillus albus | 1818 | 1818 | 99% | 0.0 | 98.18% | 1161 | MK389454.1 |
| Bacillus cereus strain GOM11 16S ribosomal RNA gene, partial sequence | Bacillus cereus | 1818 | 1818 | 99% | 0.0 | 98.18% | 1496 | MG753797.1 |
| Bacillus thuringiensis strain ILBB297 16S ribosomal RNA gene, partial sequence | Bacillus thuringiensis | 1818 | 1818 | 99% | 0.0 | 98.18% | 1118 | KT340482.1 |
| Uncultured Bacillus sp. clone RZA-04 16S ribosomal RNA gene, partial sequence | uncultured Bacillus sp. | 1816 | 1816 | 98% | 0.0 | 98.27% | 1226 | OP364863.1 |
| Bacillus cereus strain SSPR6 16S ribosomal RNA gene, partial sequence | Bacillus cereus | 1814 | 1814 | 99% | 0.0 | 98.08% | 1452 | MF521558.1 |
| Bacillus cereus gene for 16S ribosomal RNA, partial sequence, strain: C4 | Bacillus cereus | 1814 | 1814 | 99% | 0.0 | 98.08% | 1449 | LC146716.1 |
| Bacillus cereus strain AS_RJ(w) 16S ribosomal RNA gene, partial sequence | Bacillus cereus | 1814 | 1814 | 99% | 0.0 | 98.08% | 1112 | OQ195697.1 |
| Bacillus sp. (in: Bacteria) strain VK33 16S ribosomal RNA gene, partial sequence | Bacillus sp. (in: firmicutes) | 1814 | 1814 | 97% | 0.0 | 98.73% | 1432 | MW436403.1 |
| Uncultured Bacillus sp. clone SRM02 16S ribosomal RNA gene, partial sequence | uncultured Bacillus sp. | 1812 | 1812 | 99% | 0.0 | 98.08% | 1474 | MT573330.1 |
| Bacillus thuringiensis strain KF935650.1 16S ribosomal RNA gene, partial sequence | Bacillus thuringiensis | 1812 | 1812 | 99% | 0.0 | 98.08% | 1096 | MT427644.1 |
| Bacillus sp. (in: Bacteria) strain N2-3-1 16S ribosomal RNA gene, partial sequence | Bacillus sp. (in: firmicutes) | 1812 | 1812 | 99% | 0.0 | 98.08% | 1200 | MN696501.1 |



**Lampiran 18.** Foto Prosedur Penelitian

Tahapan Isolasi Bakteri Karbonoklastik

Tahapan Seleksi Bakteri Karbonoklastik

Pengukuran Nilai Absorban Larutan Kurva Standar dan Kadar Amonia Kultur Bakteri Menggunakan Spektrofotometer UV-Vis


Tahapan Identifikasi Bakteri Karbonoklastik dengan Menggunakan Marka Molekuler