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Isolation and hybrid genome assembly of Bacillus subtilis SNUY1

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Disclaimer

This protocol was developed and optimized specifically for the isolation and genome sequencing of Bacillus subtilis SNUY1 from a Doenjang sample. The described materials and methods are provided as a reference framework for similar studies but are not intended as universal procedures. Laboratories are encouraged to adjust parameters such as media composition, incubation temperature, and DNA quantification settings according to their strain characteristics and facility protocols.

Abstract

This protocol minimally documents the procedures used to isolate Bacillus subtilis SNUY1 from Doenjang, perform pre-sequencing taxonomic identification (near-full-length 16S rRNA; Sanger), and report DNA concentration and purity measured for whole-genome sequencing. Values are reported as dataset-specific, non-prescriptive information.

Guidelines

1. Quantify extracted DNA using the QuantiFluor dsDNA System (Promega) with a Victor Nivo reader (PerkinElmer).
2. Assess purity with a NanoPhotometer (IMPLEN) to verify the A260/280 ratio.
3. Representative values for the SNUY1 preparation (approximately 439 ng/µL DNA and an A260/280 ratio of ~1.8) are provided for reference only; actual concentrations may vary depending on strain, sample quality, and instrument calibration.

Materials

Agar/broth media: LA/LB, NA/NB, TSA/TSB, MRS
Sterile distilled water (DW)
Glycerol (molecular biology grade)
PrepMan Ultra (Thermo Fisher Scientific)
QuantiFluor dsDNA System (Promega) and Victor Nivo reader (PerkinElmer)
NanoPhotometer (IMPLEN)

Section 1. Sampling and strain isolation (used for SNUY1)

1 Homogenize 1 g of Doenjang in 9 mL sterile distilled water; perform serial 10-fold dilutions.

2 Spread on LA, NA, TSA, and MRS agar; incubate at 30°C and 37°C until well-isolated colonies appear.

3 Inoculate picked colonies into 5 mL LB, NB, TSB, or MRS; incubate 24 h at 30°C and/or 37°C (pre-cultures).

4 Inoculate the corresponding broth at 1% (v/v) and incubate 24 h (main cultures). Prepare glycerol stocks at 25% (v/v) and store at −80°C. For genome DNA preparation, subculture SNUY1 on TSA and grow in TSB at 30°C.

Section 2. Pre-sequencing taxonomic identification (near-full-length 16S rRNA; Sanger)

5 Extract DNA from a single colony (PrepMan Ultra; per manufacturer).

6 PCR primers: 27F (5′-AGAGTTTGATCMTGGCTCAG-3′), 1492R (5′-TACGGYTACCTTGTTACGACTT-3′). Cycling: 94°C 5 min; 30×(94°C 30 s, 55°C 30 s, 72°C 90 s); 72°C 10 min.

7 Sanger sequencing with internal primers 785F (5′-GGATTAGATACCCTGGTA-3′) and 907R (5′-CCGTCAATTCMTTTGAGTTT-3′).

8 Analyze the assembled sequence using BLASTN for taxonomic identification. Example result (for reference only): The best match for the tested isolate was Bacillus subtilis (post-assembly ANI using pyani v0.2.7: 99.87% ANI, 98.3% alignment coverage vs GCF_045037735.1).

Section 3. DNA concentration and purity (reporting only)

9 Quantify extracted DNA using the QuantiFluor dsDNA System (Promega) with a Victor Nivo reader (PerkinElmer).

10 Assess purity with a NanoPhotometer (IMPLEN) to verify the A260/280 ratio.

11 Representative values for the SNUY1 preparation (approximately 439 ng/µL DNA and an A260/280 ratio of ~1.8) are provided for reference only; actual concentrations may vary depending on strain, sample quality, and instrument calibration.

Protocol references

Genome assembly for B. subtilis SNUY1 available under GenBank accession CP199918.

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