DNA EXTRACTION USING ZR FUNGAL/BACTERIAL DNA MINIPREP (Manufactured by Zymo Research)

1. Add 2mLs of bacterial cells broth to to a ZR BashingTM Lysis Tube. Add 750ul Lysis Solution to the tube.

2. Secure in a bead fitted with 2 ml tube holder assembly and process at maximum speed for > 5 minutes.

3. Centrifuge the ZR BashingBeadTM Lysis Tube in a microcentirifuge at > 10,000 x g for 1 minute.

4. Transfer up to 400 ul supernatant to a Zymo-SpinTM IV Spin Filter (orange top) in a Collection Tube and centrifuge at 7,000 x g for 1 minute.

5. Add 1,200 ul of Fungal/Bacterial DNA Binding Buffer to the filterate in the Collection Tube from Step 4.

6. Transfer 800 ul of the mixture from Step 5 to a Zymo-SpinTM IIC Column in a Collection Tube and centrifuge at 10,000 x g for 1 minute.

7. Discard the flow through from the Collection Tube and repeat Step 6.

8. Add 200 ul DNA Pre-Wash Buffer to the Zymo-Spin TM IIC Column in new Collection Tube and centrifuge at 10,000 x g for 1 minute

9. Add 500 ul Fungal/Bacterial DNA Wash Buffer to the Zymo-SpinTM IIC Column and centrifuge at 10,000 x g for 1 minute

10. Transfer the Zymo-SpinTM IIC Column to a clean 1.5 ml microcentrifuge tube and add 100ul (35 ul minimum) DNA Elution Buffer directly to the column matrix. Centrifuge at 10,000 x g for 30 seconds to elute the DNA.

Electrophoresis for DNA and PCR

1. Measure 1 g of agarose (for DNA) ; 1.5g of agarose for PCR
2. Mix agarose powder with 100 mL 1xTAE in a microwavable flask.
3. Microwave for 3-5 min until the agarose is completely dissolved (but do not over boil the solution, as some of the buffer will evaporate and thus alter the final percentage of agarose in the gel.
4. Let agarose solution cool down to about 60 °C (about when you can comfortably keep your hand on the flask), about 5 mins.
5. Add 10µL EZ vision DNA stain. EZ vision binds to the DNA and allows you to visualize the DNA under ultraviolet (UV) light.
6. Pour the agarose into a gel tray with the well comb in place

7. Place newly poured gel at 4 °C for 10-15 mins OR let sit at room temperature for 20-30 mins, until it has completely solidified.

Loading Samples and Running an Agarose Gel

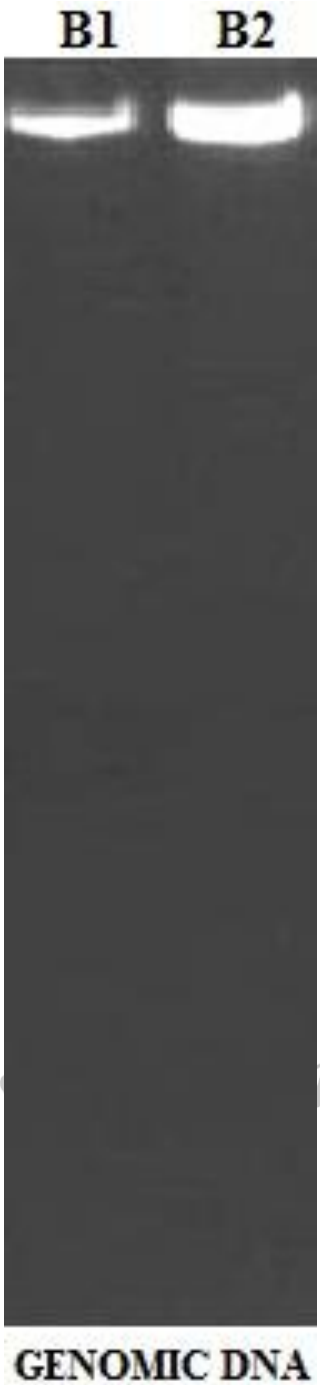
1. Add loading buffer to each of your DNA samples or PCR products
2. Once solidified, place the agarose gel into the gel box (electrophoresis unit).
3. Fill gel box with 1xTAE (or TBE) until the gel is covered.
4. Carefully load a molecular weight ladder into the first lane of the gel.
5. Carefully load your samples into the additional wells of the gel.
6. Run the gel at 80-150 V for about 1-1.5 hours
7. Turn OFF power, disconnect the electrodes from the power source, and then carefully remove the gel from the gel box.
8. Visualize DNA fragments or PCR product under UV transilluminator.

PCR Analysis

The sizes of the PCR products were estimated by comparison with the mobility of a HyperLadder 1kb (Molecular weight ladder) that was ran alongside experimental samples in the gel. The size of the amplicon is about 1500bp.

PCR sequencing preparation cocktail consisted of 10 µl of 5x GoTaq colourless reaction, 3 µl of 25mM MgCl2, 1 µl of 10 mM of dNTPs mix, 1 µl of 10 pmol each of the 16S ribosomal gene forward primer (27F: AGAGTTTGATCMTGGCTCAG) and reverse primer (1525R: AAGGAGGTGWTCCARCCGCA) and 0.3units of Taq DNA polymerase (Promega, USA) made up to 42 µl with sterile distilled water 8µl DNA template. PCR was carried out in a GeneAmp 9700 PCR System Thermal cycler (Applied Biosystem Inc., USA) with a PCR profile consisting of an initial denaturation at 94°C for 5 min; followed by a 30 cycles consisting of 94°C for 30 s, 30secs annealing of primer at 56^{o}C and 72°C for 1 minute 30 seconds; and a final termination at 72°C for 10 mins. And chill at 4^{o}C.

IMAGE SHOWING GENOMIC DNA



GENOMIC DNA

16S GENE PCR AMPLIFICATION

Amplification results of the Bacteria 16S region.

PURIFICATION OF AMPLIFIED PRODUCT

After gel integrity, the amplified fragments were ethanol purified in order to remove the PCR reagents. Briefly, 7.6 µl of Na acetate 3M and 240 µl of 95% ethanol were added to each about 40µl PCR amplified product in a new sterile 1.5 µl tube eppendorf, mix thoroughly by vortexing and keep at -20°C for at least 30 min. Centrifugation for 10 min at 13000 g and 4°C followed by removal of supernatant (invert tube on trash once) after which the pellet were washed by adding 150 µl of 70% ethanol and mix then centrifuge for 15 min at 7500 g and 4°C. Again remove all supernatant (invert tube on trash) and invert tube on paper tissue and let it dry in the fume hood at room temperature for 10-15 min. then resuspend with 20 µl of sterile distilled water and kept in -20°C prior to sequencing. The purified fragment was checked on a 1.5% Agarose gel ran on a voltage of 110V for about 1hr as previous, to confirm the presence of the purified product.

SEQUENCING

The amplified fragments were sequenced using a Genetic Analyzer 3130xl sequencer from Applied Biosystems using manufacturers' manual while the sequencing kit used was that of BigDye terminator v3.1 cycle sequencing kit. Bio- Edit software and MEGA 6 were used for all genetic analysis.

Please note that Sequencing was done at the International Institute of Tropical Agriculture, Bioscience Center.

Sequence Results

Isolate B1 has 93.91% Pairwise Identity to Bacillus subtilis strain YLB-P1 with NCBI accession number KF220577

Isolate sequence for sample B1

GGGTATCATATGTCGACATGGCTCAGGTGCGGCGGAACCACCCCCTTAAAGTTTGCCGC
GGACCGGTGAGTAACACGTGGCTAACCTGCCTGTATAACTGGGATAACTCCGGGAAACC
GGGGCTAATACCGGATGGTTGTTTGAACCGCATGGTTCAAACATAAAAGGTGGCTTCGG
CTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCA
AGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACG
GCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGAC
GGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTAAAGCTCTGTTGTTAGGGAA
GAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGC
TAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTG
GGCGTAAAGGGCTCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCG
GGGAGGGTCATTGGTAACTGGGGAACTTGAGTGCAGAAGAGGAGAGTGGAATTCCACG
TGTAGCGGTGAAATGCGTAAAGATGTGAGGAACACCAGTGGCGAAGGCGACTCTCTGGT
CTGTAACTGACGCTGAGAGCGAAGCGTGGGGAGCGAACAGATTAGATACCCTGATAGTC
CACGCCGTAACGATGAGTGCTAGTGTTAGGGGTTTCCGCCTCCTTAGTGCTGCAGCTAAC
GCATTAAGCACTCCGCCTGGGGAGTACCGCTCGCATTACTGAACTCCAACGAGTGACGC

GGCCCGTCTCCAGCGGTGAGATCATGTAGCTTTCATGCGGAGCATCGCGACGACTTACA
GTCTTGGACTGCCTCTGAACATCTAGAGATAGGACGTCATCGTGGCAAGGACAGTGGTG
CATAGCTGTCAGTCACCTCGTTCATGGAACTGTAGTTATACCGACCAGCCACCTTGATCT
AAAGTGTCC

Isolate B2 has 96.36% Pairwise Identity to Bacillus sp. strain SFBL 30with NCBI accession number MK490811

Isolate sequence for sample B2

TGGTGGGGGACCCCTTAAAATTGATATGGCTCGGGTGCGGTGGGATCCCTCCTTATAGTT
TGATCGGGCGACGGGGGGCCTGCACCCCCCCAAAAGACCCATAAGACTGGCGATAACTG
CCGGGGAAACCGGCGGTCTAATACCGGATAACATTTTGAACCGCATGGTTCTAAATTGA
AAGGCGGCTTCGGCTGTCACTTATGGATGGACCCGCGTCGCATTAGCTAGTTGGTGAGGT
AACGGCTCACCAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGG
GACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGA
CGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGCTTTCGGGTCGTAAAACTCT
GTTGTTAGGGAAGAACAAGTGCTAGTTGAATAAGCTGGCACCTTGACGGTACCTAACCA
GAAAGCCACGGCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTAT
CCTGAATTATTGGGCGTAAAGCGCGCGCAGGTGGTTTCTTTAGTCTGATGTGAAAGCCCA
CGGCTCACCCGTGGAGGGTCATTGGAAACTGGGAGACTTGAGTGCAGAAGAGGAAAGT
GGATTTCCATGTGTAGCGGTGAAATGCGTAGAGATATGGAGGACACCAGTGGCGAGGCT
ACTTTCTGGCTGTAACTGACACGAGGCCGAAAGCTGGGGAGCAACAGATAGATACCTGT
AGTCACCCGTAACGATATGCTAGTGTAGAGTT