DNA Isolation Protocol

(with Thermo Scientific GeneJET Genomic DNA Purification Kit)

Materials and reagents:

- Thermo Scientific GeneJET Genomic DNA Purification Kit
- pipettes
- water bath
- vortex
- microcentrifuge
- microcentrifuge tube(s)
- 50% ethanol

Isolation:

1. Add 200 µl of Lysis Solution and 20 µl of Proteinase K Solution to the cell pellet. Mix thoroughly by vortexing or pipetting to obtain a uniform suspension.
2. Incubate the sample at 56 °C while vortexing occasionally until the cells are completely lysed (10 min).
3. Add 20 µl of RNase A Solution, mix by vortexing and incubate the mixture for 10 min at room temperature.
4. Add 400 µl of 50% ethanol and mix by pipetting or vortexing.
5. Transfer the prepared lysate to a GeneJET Genomic DNA Purification Column inserted in a collection tube. Centrifuge the column for 1 min at 6000 x g (8000 rpm). Discard the collection tube containing the flow-through solution. Place the GeneJET Genomic DNA Purification Column into a new 2 ml collection tube.
6. Add 500 µl of Wash Buffer I. Centifuge for 1 min at 8000 x g (9200 rpm). Discard the flow-through and place the purification column back into the collection tube.
7. Add 500 µl of Wash Buffer II to the GeneJET Genomic DNA Purification Column. Centrifuge for 3 min at maximum speed (≥ 12000 x g) (11000 rpm). If residual solution is seen in the purification column, empty the collection tube and re-spin the column for 1 min at maximum speed. Discard the collection tube containing the flow-through solution and transfer the GeneJET Genomic DNA Purification Column to a sterile 1.5 ml microcentrifuge tube.
8. Add 200 µ of Elution Buffer to the center of the GeneJET Genomic DNA Purification Column membrane to elute genomic DNA. Incubate for 2 min at room temperature and centrifuge for 1 min at 8000 x g (9200 rpm).
9. Discard the purification column. Store the DNA at -20 °C for further use.

Quantitation of DNA with a Multi-Mode Microplate Reader:

- Drop 2 x 2 µl Elution Buffer (as Blank) on the first row and 2 x 2 µl DNA solution on the second row of microplate insert (Take 3).

- Measure the sample on 260/280nm using the Gene5. program /Nucleic Acid Quantification./

- Measure A_{260} and A_{280} of the DNA solution. Calculate the DNA content assuming that one A_{260} unit equals 50 µg of double-stranded DNA per ml.

  The A_{260}/A_{280} ratio of the isolated DNA is within the 1.9-2.1 range.