***Delftia* sp. 16S PCR Amplification Protocol**

Reagents and Materials:

* Individual PCR tubes (not strip capped PCR tubes)
* Microfuge Tubes
* p1000 pipette tips
* p200 pipette tips
* p20 pipette tips
* p10 pipette tips
* Ultra-clean (Read: DNA-free, RNA-free, Protein-free, DNAse-free, RNAse-free) deionized water

*The following should be kept on ice:*

* Genomic DNA (gDNA) of each *Delftia* sp. in question
* MyFiTM Mix, 25µL per reaction
* 1492R Primer, 20µM per reaction
  + 5’- AAG GAG GTG ATC CAG CCG CA -3’
* 27F Primer, 20µM per reaction
  + 5’- AGA GTT TGA TCC TGG CTC AG -3’

Steps prior to experimentation:

* Make sure both primers have been resuspended to a 100µM stock concentration from the original dry product
  + If either primer has not been resuspended to a 100µM concentration from the original dry product, use [www.idtdna.com](http://www.idtdna.com)‘s resuspension calculator
  + On the primer specification sheet, look for the number of nMoles of primer, just below “Amount of Oligo.” Enter this number into the top space next to nMoles
  + Enter “100” into the lower space next to µM
  + Click calculate
  + Resuspend as instructed with ultrapure (DNA and RNA free) diH2O in the biological safety cabinet
* Make sure a secondary dilution of 10µM has been created for each primer. This is the working stock (10 µM).
  + If such a dilution has not been created for each primer, create one using tenfold dilution procedures within a biological safety cabinet
    - Do not dilute the 100µM resuspension within the original container, but instead in a separate microfuge tube
* Make sure that there is a 16S program on the Thermal Cycler with the following specifications:
  + An initial denaturation step of 95ºC for one minute
  + A cyclical denaturation step of 95ºC for fifteen seconds
  + A cyclical annealing step 55ºC for fifteen seconds
  + A cyclical extension step of 72ºC for ninety seconds
  + A final extension step of 72ºC for five minutes
  + An indefinite incubation step at 4ºC
* Make sure that the concentration of each gDNA used for each isolate is at least 10ng/µL
  + If you are uncertain what concentration a solution of gDNA is, you may nanodrop it according to step seven of the “Genomic DNA Extraction using MoBio PowerBiofilm® Isolation Kit” protocols
* Calculate what volume of each gDNA solution is necessary to add 200ng of gDNA
* Calculate what volume of ultra-clean deionized water should be added to each reaction to result in a final volume of 50µL per reaction

Protocol:

1. Turn on the Thermal Cycler and navigate to the 16S program. Do not start the program.

*Complete the following within a Biological Safety Cabinet, utilizing proper protocols for BSC use. The phrase “cleanly” is used in the following protocols to emphasize when to use proper BSC protocols.*

1. Dampen a Kim-wipe with DNAse. Wipe down your workspace inside the hood with the Kim-wipe.
2. Remove the residue of the DNAse by spraying the workspace inside the hood with ethanol and collecting the fluid with a Kim-wipe.
3. **Cleanly** move a bag of PCR tubes into the hood. Aseptically remove enough PCR Tubes from the bag and reseal the bag. Close each PCR tube and place them in the PCR tube rack.
4. Appropriately label one PCR tube for each reaction. Label each PCR tube on the side of the tube. You may label the lid but do not label each tube by the lid alone. Ink on the lid may be smeared during thermal cycling.
5. **Cleanly** move your ultra-clean deionized water into the hood.
6. **Cleanly** move your bag of ultra-clean microfuge tubes into the hood. Aseptically remove enough microfuge tubes from the bag and reseal the bag. Close each microfuge tube and place them in the microfuge tube rack.
7. Label a microfuge tube “Ultra-clean water” with the date and your initials. Carefully aliquot 1mL of ultra-clean water into the microfuge tube.
8. Add the calculated volumes of water to each reaction.
9. **Cleanly** move your 27F and 1492R primer aliquots into the hood.
10. Add 2 µL (20µM) of 27F primer to each PCR tube.
11. Add 2 µL (20µM) of 1492R primer to each PCR tube.
12. Remove the 27F and 1492R primer aliquots from the hood and place them back on ice. **Cleanly** re-enter the hood and remove other unnecessary items in your work area (Read: PCR tube bag, Microfuge tube bag, et cetera).
13. **Cleanly** move your gDNA aliquots into the hood.
14. Add the calculated volume of each gDNA aliquot to each corresponding PCR tube.
15. Remove the gDNA aliquots and place them back on ice.
16. **Cleanly** move your 2X MyFiTM Mix into the hood.
17. Quickly add 25µL of 2X MyFiTM Mix to each PCR tube.
18. Quickly and **cleanly** remove the MyFiTM Mix and each PCR tube from the hood and place them on ice.
19. Keeping the PCR tubes on ice, transport them to the prepared Thermal Cycler.
20. Place the PCR tubes in the Thermal Cycler. Close the lid and slowly twist the circular cap clockwise until there is mild resistance. Excessive twisting will crush the PCR tubes.
21. Start the 16S program on the Thermal Cycler. Allow it to run. Set a timer to notify you once the program is finished.
22. **Cleanly** clean your workspace in the hood. Leave the hood organized, clean, and ready to be used. Remember:
    * 1. Avoid turning on the UV light while others are still working.
      2. Do not leave the UV light on for more than 30 minutes.
23. Remove the PCR tubes from the Thermal Cycler once the 16S program is finished. Place them in a PCR tube rack and store them at -20ºC.
24. Turn off the Thermal Cycler.

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