***Arthrobacter globiformis* NRRL 2979 vs 2880 PCR Protocol**

NRRL and Pitt have at least 2 NRRL strains of *A. globiformis.* At least once, the strain *A. globiformis* NRRL 2880 SEA may have been sent (likely July 2019) in place of *A. globiformis* NRRL 2979 SEA. The phage plating efficiencies differ, so determining the host that you have is needed. Please verify which strain of *A. globiformis* you have in your freezers. Then make the appropriate bacterial host changes at phagesDB.

\*\**Disclaimer: This protocol is a diagnostic tool for SEA-PHAGES courses to use to help differentiate between two strains of bacteria. Since no controls are provided, results may differ from school to school. If confusing results are obtained, please reach out for assistance. \*\**

1. Order the following primers sequences to amplify and differentiate between the 2979 strain and the 2880 strain. This protocol is written with NEB Quick-Load® *Taq* 2X MM, but you can use any PCR mix and adjust accordingly.

|  |  |
| --- | --- |
| **Primer Name** | **Primer Sequence** |
| Arthro2880.1 | acttccggagataaaaccata |
| Arthro2880.2 | gcgttgtccattctgttaat |
| Arthro2880.3 | gccatcagtcagatttagga |
| Arthro2880.4 | acgatatacacatgtttaaaggaat |
| Arthro2979.5 | tggtctttatcgaaatcttgatac |
| Arthro2979.6 | ggctgcgtaagtatgtagtca |
| Arthro2979.7 | cgatggggtttataagcgt |
| Arthro2979.8 | gttctcctaagagctatttgatattg |

* Arthro2880.1, Arthro2880.2, Arthro2880.3, and Arthro2880.4 will have a PCR product with 2880 and no PCR product with 2979.
* Arthro2979.5, Arthro2979.6, Arthro2979.7, and Arthro2979.8 will have a PCR product with 2979 and no PCR product with 2880.

1. Once the primers have been ordered and reconstituted, obtain a PCR tube for heat killing of your bacteria.
2. Aliquot 20 mLs of diH2O to the PCR tube.
3. Using a sterile pipette tip, stab the edge of an isolated colony to pick up some bacteria. Submerge this into the PCR tube containing the water, pipette up and down to mix. The water should become slightly turbid.
4. Heat kill the PCR tube containing bacteria at 98oC for 15 minutes in the thermocycle to lyse the bacteria to release its DNA.
5. Obtain 4 more PCR tubes and label tubes 1-4 (see table below for what primers and what bacterial DNA will be in each numbered tube).
6. Obtain a tube of PCR Master Mix (NEB Quick-Load® *Taq* 2X MM used here) and the associated 2880 and 2979 specific primers to use for this PCR reaction.
7. To each of the PCR tubes, add the following quantities of reagents according to your master mix’s specifications (NEB Quick-Load® *Taq* 2X MM used here).

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **PCR Tube Number** | **diH2O (mLs)** | **Forward Primer (mLs)** | **Reverse Primer**  **(mLs)** | **PCR**  **MM**  **(mLs)** | **Template (Heat killed bacteria) (mLs)** |
| 1 | 10.5 | 0.5  (2880.1) | 0.5  (2880.2) | 12.5 | 1 |
| 2 | 10.5 | 0.5  (2880.3) | 0.5  (2880.4) | 12.5 | 1 |
| 3 | 10.5 | 0.5  (2979.5) | 0.5  (2979.6) | 12.5 | 1 |
| 4 | 10.5 | 0.5  (2979.7) | 0.5  (2979.8) | 12.5 | 1 |

1. Run PCR in thermocycle based on Master Mix’s specifications (We use an annealing temperature of 55oC).
2. Prepare a 5-lane agarose gel for electrophoresis.
3. Load 20 mLs of a DNA ladder into the first lane of the gel, followed by 25 mLs from each PCR tube.
4. Let the gel run for ~ 1 hour at 100 Vs.
5. Remove gel and image to see results.
6. If you see a 1233 bp product with the 2880.1 + 2880.2 primers and a 922 bp product with 2880.3 + 2880.4 primers, you have *A. globiformis* 2880 (Tubes 1 and 2).
7. If you see a 1130 bp product with the 2979.5 + 2979.6 primers and a 790 bp product with 2979.7 + 2979.8, you have *A. globiformis* 2979 (Tubes 3 and 4).

\**See next page for an annotated gel of each strain with PCR results ran with all 4 primers. Results obtained by individual schools should either resemble the top or bottom half of the gel, not both. \**

Graphical user interface

Description automatically generated with medium confidence