**IMPALA CRE PCR Protocol**

**Purpose**

Method for conducting real time PCR on IMPALA isolates to detect CRE resistance genes.

**Materials**

* Lo bind Eppendorf tubes
* 1X TE Buffer
* Sterile loops
* P2 pipette & tips
* P10 pipette & tips
* P200 pipette & tips
* P1000 pipette & tips
* Molecular grade water
* Applied Biosystems 96 well plate & film
* Primer/probe mix for each gene of interest
  1. NDM (assay ID Ba4931076\_s1)
  2. OXA (assay ID Ba04930816\_s1)
  3. IMP (assay ID Ba04646131\_s1)
  4. KPC (assay ID Ba04646152\_s1)
  5. VIM (assay ID Ba04646155\_s1)
* TaqMan Fast Advanced Master Mix (Cat# 4444556)
* Positive controls for each gene of interest
* Negative control (CRE-EC(-))

**Procedure**

* Lysis Buffer Recipe

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **1X TE Buffer** |  |  |  |  |  |  |
|  |  |  | Final Volume: | | 50 | mL |
| Chemical | [Stock] | | [Final] | | Volume to Add | |
| Tris-HCl, pH 8.0 | 1000 | mM | 10 | mM | 0.500 | mL |
| EDTA | 500 | mM | 1 | mM | 0.100 | mL |
|  |  |  |  | Total: | 0.600 | mL |
|  |  |  |  | + ddH2O | 49.400 | mL |
|  |  |  | Final Volume: | | 50.000 | mL |

* Bacterial Cell Lysis
  1. If plated isolates are older than 2 weeks, subculture on blood agar and incubate for 24 hours at 37C.
  2. Add 5-10 colonies to 100uL lysis buffer.
  3. Briefly vortex samples for homogeneity.
  4. Incubate samples at 95C for 10 min.
  5. Freeze samples at -20C until ready for PCR.
* Real Time PCR
  1. 56 samples per plate (not completed in triplicate), 7 negative controls, 5 positive controls
     + NDM control = CRE505 (NDM-1)
     + OXA control = TBD
     + VIM control = CRE501 (VIM-1)
     + KPC control = CRE517 (KPC-3)
     + IMP control = TBD
  2. While samples thaw, prepare mastermix solution using the following calculation:
     + *Note: primers are premixed, so the forward primer and reverse primer for each target are in their own solution, along with the appropriate probe*

**Master Mix Recipe:**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **1 Rxn** | **68 Rxn** | **+10%** |
| Master Mix (2X) | 10 | 680 | 748 |
| Primer/Probe mix (20X) | 1 | 68 | 74.8 |
| Water | 7 | 476 | 523.6 |
| Total Volume= | 18 | 1224 | 1346.4 |
| Template | 2 | 2 | 2 |

**Thermocycling Settings:**

|  |  |  |  |
| --- | --- | --- | --- |
| **FAST Thermal Cycling** |  |  |  |
| Activation | 1X | 95C | 20 sec |
| Denaturation | 30X | 95C | 3 sec |
| Annealing/Extension | 30X | 60C | 20 min |