**Bacterial Colony PCR Protocol**

**Purpose**

Method for preparing bacterial isolates for varying types of colony PCR.

**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
* Master Mix
* Primer mixes
* Probe mixes

**Procedure**

* 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. Incubate samples at 95C for 10 min.
  4. Freeze samples at -20C until ready for PCR.
* PCR
  1. Procedure will vary depending on type of PCR, whether it is singleplex or multiplexed, etc.
  2. Please refer to guidelines for PCR prep given by the master mix supplier.
  3. 2.5uL of template DNA is typically used for colony PCR reactions.

**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 |