1. **Hazard Assessment**

To protect yourself from any possible hazards associated with this task wear eye protection. You should also wear latex, nitrile, or vinyl gloves and a lab coat with long sleeves. To protect your legs and feet wear closed shoes and long trowsers. Do not wear sandles, shorts or a short skirt. Wash your hands before eating and when leaving the laboratory. You should review the MSDS for any chemical used in this procedure. In case of a spill with a toxic chemical remove all contaminated clothing and wash affected areas with copious quantities of water. Check location of the nearest safety shower. Eyes should be washed copiously for 15 minutes.

This protocol is based on the Waller et al. 2006 [PCR protocol for SeM](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC1392674/?tool=pmcentrez)

1. **Reagent and Supplies**

**Reagent Amount SOP? Ready**

Lysozyme solution (pH 6.0) 8.2mL Yes \_\_\_\_\_\_\_\_\_

Proteinase K (20mg/mL) .5 mL Yes \_\_\_\_\_\_\_\_\_

Wash Solution Concentrate 80mL Yes \_\_\_\_\_\_\_\_\_

Buffer PB 20mL Yes \_\_\_\_\_\_\_\_\_

Buffer PE 30mL Yes \_\_\_\_\_\_\_\_

Buffer EB (10 mM Tris·Cl, pH 8.5) 2.0mL No \_\_\_\_\_\_\_\_\_

Lysis Solution C for cell lysis 20mL No \_\_\_\_\_\_\_\_\_

Column Preparation solution 60mL No \_\_\_\_\_\_\_\_\_

Ethanol (95%) for dissolution of 8.0mL No \_\_\_\_\_\_\_\_\_

all elements except for DNA

Wash Solution 1 50mL No \_\_\_\_\_\_\_\_

Elution Solution (10 mM Tris-HCl, 8.0mL No \_\_\_\_\_\_\_\_

0.5 mM EDTA, pH 9.0)

Forward Primer 40.0 µL No \_\_\_\_\_\_\_\_

Reverse Primer 40.0 µL No \_\_\_\_\_\_\_\_\_

Vent DNA Polymerase 8.0 µL No \_\_\_\_\_\_\_

dNTP 40.0 µL No \_\_\_\_\_\_\_

Distilled Water .6mL No \_\_\_\_\_\_\_

Sodium Acetate (3.0M) .4mL No \_\_\_\_\_\_\_

**Material**

GenElute Nucleic Acid Binding Columns in tube

Collection Tubes, 2.0mL capacity

55 C water bath

37 C water bath

Pipette tips

1.5mL microcentrifuge tubes

Microcentrifuge

QIAquick spin columns

1. **Bacterial Preparation and Suspension**

Prepare a solution of Mutanolysin and Lysozyme. Label

Suspend a single *S. equi* colony in 200µL of Mutolysin/Lysozyme solution

Incubate the solution for 1 hour and 37 C

1. **Cell Lysis**

Add 20 µL of Proteinase K to solution

Add 200 µL of Lysis solution C to solution

Vortex solution for 15 seconds

Incubate at 55 C for 10 minutes

1. **DNA Isolation**

Add 500 µL of column preparation solution to each GenElute Miniprep Binding Column seated in 2mL collection tube

Centrifuge at 12,000 x g for 1 minute

Discard Elute

1. **Binding Preparation**

Add 200 µL of ethanol to the lysate

Vortex solution for 10 seconds

1. **Lysate**

Add solution to the prepared binding column \*

Centrifuge at 6500 x g for 1 minute

Discard collection tube with eluate and place column in a new 2mL collection tube

\**Use wide tip pipet in order to not splice DNA*

1. **Wash**

Add 500 µL of wash solution 1c

Centrifuge at 6500 x g for 1 minute

Discard collection tube with eluate and place in a new 2mL collection tube

Add 500 µL of wash solution to column\*

Centrifuge at maximum speed for 3 minutes to dry column\*\*

Discard collection tube with eluate and place in a new 2mL collection tube

*\*Verify addition of ethanol to concentrate*

*\*\* If additional drying time is required, centrifuge for 1 additional minute*

1. **DNA Elution**

Pipet 200 µL of elution solution into the center of the column

Incubate column for 5 minutes at room temperature

Centrifuge for 1 minute at 6500 x g\*

*\*Template DNA remains in the eluate*

1. **Prepare PCR Ingredients**

Add 15 µL of distilled water to a .2ml PCR tube

Add 2 µL of 10x buffer solution to the tube

Add 1 µL of dNTP

Add 0.5 µL of forward primer 5′-CAGAAAACTAAGTGCCGGTG-3’

Add 0.5 µL of reverse primer 5′-ATTCGGTAAGAGCTTGACGC-3’

Add 0.2 µL of vent polymerase

Add 0.5 µL of template DNA

1. **Run PCR**

Place the PCR tube in the machine and set the cycle as follows (repeat x30)

|  |  |  |
| --- | --- | --- |
|  | Temperature | Time |
| STEP 1 | 94 C | 30 seconds |
| STEP 2 | 55 C | 30 seconds |
| STEP 3 | 72 C | 1 minute |
|  |  |  |

1. **PCR Product Purification**

Add 25 µL of Buffer PB to 5 µL of PCR reaction and mix\*

Put QIAquick column in 2mL collection tube

Apply Sample DNA to the column

Centrifuge Column in tube for 60 seconds at 17,900 x g

Discard the flow-through and place the column back in the 2mL tube

\*If orange or violet add 10 µL 3M sodium acetate

1. **PCR Product Wash**

Wash Column with .75mL of Buffer PE

Centrifuge for 60 seconds at 17,900 x g

Discard the flow-through and place the column back in the 2mL tube

Centrifuge for 60 seconds at 17,900 x g

Place column in a clean 1.5mL microcentrifuge tube

XIIII. **PCR Product Elution**

Add 50 µL of Buffer EB to the center of the QIAquick membrane

Centrifuge column for 60 seconds at 17,900 x g