**BIOTECH Project Resource Center Loan Checklist**

Teacher: Date loaned:

School: # groups:

students/classes: Independent:

**Materials for DNA extraction and PCR of 16S bacteria (samples to be sequenced)**:

|  |  |  |
| --- | --- | --- |
| Number | Item | Returned? |
| 1 | hot water bath with lid |  |
| 2 one liter bottles  | plastic bottles of TAE (can be poured back into containers) |  |
| 1 bottle per 4 gels | glass bottles of agarose (125 ml 0.8% agarose in TAE) |  |
| 1 | thermometer in plastic holder |  |
| 8 | P20 adjustable microliter pipettes |  |
| 8 | P200 adjustable microliter pipettes |  |
| 1 pipet box per group,  | pipette tip boxes (each box holds 96 pipet tips) |  |
| 4 | Gel electrophoresis boxes, with 2 stoppers and 1 comb each |  |
| 4 | pairs of red and black wires |  |
| 2 dual power supplies  | power supplies for gel electrophoresis |  |
| 1 | edvotek insta stain (EtBr)  |  |
| 1 | plastic tray (to hold gels during staining) |  |
|  | Latex and nitrile gloves |  |
|  | EtBr waste bag |  |
| bacteria on plates | bacteria |  |
| 1/sample | 100l Chelex in 1.7 ml tube |  |
| 1/ sample | pestle |  |
| 1/sample | Lid locks |  |
| 1/ sample | 1μL inoculating loops |  |
| 1 | Heat Block |  |
| 1  | centrifuge |  |
| 1 tube /group | GoTaq (Taq polymerase, dNTPs, PCR buffer, MgCl2 and loading buffer) (20l in each PCR tube) |  |
| 1 tube/group | forward 2M 16S gene primers (60 l for 4 samples) |  |
| 1 tube/group | Reverse 2M 16S gene primers (60 l for 4 samples) |  |
| 1 tube | Molecular Weight Marker (1 kb plus) (10l per gel) |  |
| 8 | PCR tube rack |  |
|  | PCR machine |  |
| 1 | biohazard bag |  |

When you are reloading the bins, please check off each item in the 'Returned?' column as a double-check that all those little pieces of equipment get packed. Thanks!

If you have questions about experiments or materials, please feel free to contact the BIOTECH Project at:

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**• Primer dilution**

All primer stocks are 100 µM. Lab working stocks are 10 µM (1 in 10 dilution of the primer stock). Most classroom primers are sent out at 2 µM (1 in 5 dilution of the lab working stock).

To make a 1 in 10 dilution combine 50 µl of 100µM primer stock with 450 µl of nuclease free water.

To make a 1 in 5 dilution combine 100 µl of 10 µM primer stock with 400 µl of nuclease free water.

**Chelex 100 mix for DNA extraction**

10% Chelex in 50 mM Tris pH 11

1g of Chelex powder 100 (100-200 mesh, sodium form from BioRad)

Add 50 mM Tris pH 11 to make 10 ml of solution

Mix thoroughly before each use as the Chelex will settle.

**50 mM Tris**

Mix 0.605 grams of Tris with deionized sterile water to make 100 ml of solution, pH to 11 with NaOH.

**• Tris-acetate-EDTA buffer for dye electrophoresis**

10X stock (per liter)

48.4 g Tris base

11.4 ml glacial acetic acid

3.72 g EDTA

Add distilled water to make total volume 1 liter. Dilute to make 1X working solution (100ml stock, 900 ml distilled water).

**• 0.8% Agarose gel for dye electrophoresis**

In 250 ml Pyrex bottle, combine:

125 ml 1X TAE

1 g agarose

Microwave uncovered to melt agarose, be careful not to boil over on microwave or your hand. Cover loosely and store at room temperature.