Mutations Investigation Transformation Materials

**Equipment**
Ice buckets  
Hot water bath  
Incubating shaker  
Incubator (for 37 °C)  
Incubator (or fridge, for 5 °C)  
Pipettes (from 10 to 900 µL)

**Consumables**
Sterile swab spreaders  
14 mL round-bottom tubes  
Petri dishes for agar plates  
Pipette tips  
1.5 mL microcentrifuge tubes

**Reagents**
Mutated pUC19 plasmid library  
XL-1 Blue competent *E. coli* cells (Agilent, product #: 200130)  
SOC medium (VWR, product #: 100219-988)  
LB agar containing:  
  - 100 µg/mL Ampicillin (Sigma-Aldrich, product #: A5354)  
  - 40 µM IPTG (VWR, product #: 100218-036)  
  - 80 µg/mL X-gal (VWR, product #: 100217-096)

**Safety Considerations**
- **XL-1 Blue E. coli Cells**: A non-pathogenic strain of *E. coli*, but should still be treated as a biohazard. Use appropriate safety gear and bleach or autoclave excess before disposal.  
- Protective gloves and eye protection should be used. In case of exposure, rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.

*BioSeq can provide materials for this procedure. Contact bioseq@tufts.edu to discuss options.*
Mutations Investigation Monoculture Materials

Equipment
Incubating shaker
Biohazard waste collection

Consumables
Sterile inoculating loops
14 mL round-bottom tubes

Reagents
LB medium containing:
  • 100 µg/mL Ampicillin (Sigma-Aldrich, product #: A5354)

Safety Considerations
  • Cultures contain XL-1 Blue E. coli Cells. Follow the same guidelines as in the transformation when performing this procedure.
  • Protective gloves and eye protection should be used. In case of exposure, rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.

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Mutations Investigation Plasmid Isolation Materials

**Equipment**

Vortex mixer
Centrifuge (for 1.5 or 2 mL tubes; for 16,000 x g)
Pipettes (for 2-900 µL)
Biohazard waste collection
Qubit Fluorometer

**Consumables**

MoBio Plasmid Isolation Kit (MoBio, product #: 12300-100):
- Spin Columns
- 2 mL Collection Tubes (can use 1.5 mL instead)
0.5 mL thin-walled tubes for Qubit (Thermofisher, product #: Q32856)

**Reagents**

MoBio Plasmid Isolation Kit (MoBio, product #: 12300-100):
- Solution 1
- Solution 2
- Solution 3
- Solution 4
- Solution 5

Qubit Broad Range (BR) kit (Thermofisher, product #: Q32850)
- BR Reagent
- BR Buffer
- BR Standard #1
- BR Standard #2

**Safety Considerations**

- **Plasmid Isolation** kit: Solutions 2 and 3 contain skin and eye irritants. Solution 4 is an ethanol solution and flammable. See kit’s safety data sheets (SDSs) for details.
- **Qubit BR** kit: the qubit reagent contains concentrated DMSO which is flammable and can cause skin/eye irritation. See kit’s safety data sheets (SDSs) for details.
- Protective gloves and eye protection should be used. In case of exposure, rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do.

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