Nitrogen Agarose Plates

David Gresham 1/15/08

1. Make 100mM stock of each nitrogen source
2. Make 10x N-lim salts (1L)
3. Make 1000mL of 2x salts, 4% glucose and 2x vitamins and minerals:
   - 200mL of 10x salts
   - 100mL of 40% glucose
   - 2mL of 1000x vitamins
   - 2mL of 1000x metals
   - 696mL of ddH2O
   filter sterilize
4. To make 200mL of agarose media mix together (using sterile technique):
   - 100mL of 2x salts, 4% glucose and 2x vitamins and minerals solution
   - depending on nitrogen source in order to get 800uM nitrogen final conc.

<table>
<thead>
<tr>
<th>100mM stock</th>
<th>2x washed agarose</th>
</tr>
</thead>
<tbody>
<tr>
<td>200uM</td>
<td>400uL 100mL</td>
</tr>
<tr>
<td>400uM</td>
<td>800uL 100mL</td>
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<tr>
<td>800uM</td>
<td>1.6mL 100mL</td>
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</tbody>
</table>

5. Use a pipette man to dispense 35mL of media into a 96well format plate (should be enough media for 5 plates)