**DNA extraction (BOMB) V.5**

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**ABSTRACT**

DNA extraction (BOMB)

**MATERIALS**

1. Lysis master mix (870 µL/sample)

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>TE buffer</td>
<td>225 µL</td>
</tr>
<tr>
<td>Lysis buffer</td>
<td>375 µL</td>
</tr>
<tr>
<td>Ammonium acetate</td>
<td>270 µL</td>
</tr>
</tbody>
</table>

2. TE buffer

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris HCl pH8.0</td>
<td>10mM</td>
</tr>
<tr>
<td>EDTA</td>
<td>1mM</td>
</tr>
</tbody>
</table>

3. Lysis buffer

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
</tr>
</thead>
<tbody>
<tr>
<td>GITC</td>
<td>4M</td>
</tr>
<tr>
<td>Tris HCl pH8.0</td>
<td>50mM</td>
</tr>
<tr>
<td>SDS</td>
<td>0.5g</td>
</tr>
<tr>
<td>EDTA</td>
<td>20mM</td>
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</tbody>
</table>

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protocols.io https://dx.doi.org/10.17504/protocols.io.n2bvj6mdnlk5/v5

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**Protocol status:** Working
We use this protocol and it's working
Sample Collection

1. Add 200 µL of 0.5 mm beads to 2mL screw tube

2. Add 200 µL of 1 mm beads to 2mL screw tube
3. Add 870 µL Lysis master mix to 2mL screw tube. The final look:

Note

In 11F, 4°C fridge
Lysis master mix: 225 µL of TE buffer + 375 µL of lysis buffer + 270 µL of 10M ammonium acetate

4. Collect 20-50 mg of sample to 2mL screw tube
Note
You can collect up to 100 mg of sample if you can until you bump into the low DNA quality or PCR success rate; by then it means too many inhibitors in the sample and you have to lower the input.

Sample crush
5. Put the 2mL screw tube in mixmill for sample crush, at 3200 rpm

Note
Remember to balance if you have odd number of samples

Centrifugation
6. Put 2mL screw tube in centrifuge for centrifugation, at this condition:

DNA purification
7. Add 350 µL of isopropanol to the 1st well of 96 well plate
8  Add 100 µL of **magnetic beads (10mg/ml)** to the 1st well of 96 deep well plate.
9. Add 400 µL of isopropanol to the 2nd well of the 96 deep well plate.

10. Add 300 µL of 80% ethanol to the 3rd well of the 96 deep well plate.
Add 300 µL of 80% ethanol to the 4th well of 96 deep well plate.
12. Add 300 µL of DDW to the 5th well of 96 deep well plate.

13. Add 100 µL of DEPC-treated water to the 6th well of 96 deep well plate.
Add 300-500 µL of the sample (lysate) from the 1.5mL centrifuged tube to the 1st well of 96 deep well plate.
Note

Pipetting as many lysate as you can, as long as it's free of any cell debris (no solids in your tip)

15  Put the prepared 96 deep well plate in the automated DNA extraction machine and select the BOMB protocol

16  After the extraction is done, put on the 96 magnetic plate to pellet the magnetic bead residues.
17 Collect $100 \mu$L of the eluted sample (avoid getting magnetic bead) as the DNA template for downstream experiments