Maize miniprep DNA extraction

Day 1

- Turn on the lyophilizer chamber set up to -40 °C (stabilizes in 1-1.5 h);
- Place the leaves on the top chamber (young leaves yield more/better DNA);
- Turn the vacuum on;
- Hold the temperature as follows (for whole leaves) - 4 days;
  - -33 C for 10h
  - -23 for 8h
  - -3 for 36h
  - 22 for 48h
- Put the lyophilized leaf piece (~0.6 x 3.0 cm) in a tube along with ~5 glass beads;
  (if not extracting in the same day, store @ -20 C);
- Place the tubes in liquid nitrogen;
- Shake the tube for 30'' using the flour shaker;
- Take tube out, quick freeze again and put it back in the shaker;
- Shake until the tissue becomes a fine powder;
- Keep tubes tightly closed @ -20 C until extraction begins.

Day 2

- Add 300μl of CTAB extraction buffer (see recipe);
- Vortex tube to mix the buffer thoroughly;
- Move the tubes to the water bath (65°C) for 1h, invert the tube every 5';
- Cool down the tubes in ice for 10';
- Add 400ul of chloroform:octanol (24:1), invert the tube for 2';
- Keep in ice for 15';
- Spin for 20', 14000 rpm (1900 x g);
- Add 300 ul of isopropanol in a new tube;
- Transfer the aqueous supernatant to a new tube (with isopropanol);
- Invert for 50 times (precipitated DNA can be seen). Spin for 20';
- Pour off the alcohol (be careful: don’t loose your DNA pellet);
- Add 300μl of ethanol 70%. Invert the tube 50 times;
- Spin for 10’.
- Wash with 70% ethanol once again;
- Put the tube in a concentrator for 20’ with the heater on;
- Add 100μl of TE+RNAse buffer;
- Dissolve the DNA pellet overnight @ 4C;
- Measure the concentration with spectrophotometer or in an agarose gel.

- **CTAB Extraction Buffer**

<table>
<thead>
<tr>
<th>Stock</th>
<th>To 100ml</th>
<th>To 200ml</th>
<th>To 300ml</th>
<th>To 400ml</th>
<th>To 500ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O</td>
<td>73</td>
<td>146</td>
<td>219</td>
<td>292</td>
<td>365</td>
</tr>
<tr>
<td>1M TRIS-HCl pH 7.5</td>
<td>10</td>
<td>20</td>
<td>30</td>
<td>40</td>
<td>50</td>
</tr>
<tr>
<td>0.5M EDTA pH 8.0</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>5M NaCl</td>
<td>14</td>
<td>28</td>
<td>42</td>
<td>56</td>
<td>70</td>
</tr>
<tr>
<td>CTAB (1%) add just before use</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>BME (1%) add just before use</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>