## 3. Methods

### 3.1. Genomic DNA Extraction

One of the major limiting factors in the isolation of plant DNA is the efficient disruption of the cell wall. There is often a compromise between complete wall breakdown and the length and yield of DNA. Another problem often encountered with plant DNA isolation is the presence of contaminating carbohydrates and phenolics. These compounds are difficult to separate from the DNA and can interfere with the quantification of nucleic acids by spectrophotometry. More importantly, these contaminants also inhibit the activity of DNA modifying enzymes. To try to reduce this contamination problem, plant tissue that is low in such compounds should be used as source material. Young, fully expanded leaves are often suitable for this purpose.

In the method described below, which is based on that of Murray and Thompson *(2)*, the plant cell wall is disrupted by grinding the frozen plant tissue with a mortar and pestle. The ground tissue is then incubated in an aqueous solution containing chelating agents, to inhibit nuclease action, and a detergent, to solubulize membranous material. The success of the method is because of the fact that in the presence of high salt concentrations, the CTAB detergent binds nucleic acid to form soluble complexes. If the salt concentration is subsequently decreased, nucleic acid–CTAB complexes precipitate, but contaminating carbohydrates are left behind in solution. The resulting nucleic acids are therefore relatively free from compounds that can contaminate DNA preparations.

This protocol has routinely been used to extract DNA from tobacco leaves and whole mature *Arabidopsis* plants. A yield of up to 500 μg of genomic DNA can be expected from 5 g tobacco leaf material. A review of nucleic acid extraction protocols for different species of plants can be found in **ref. *3***.

1. Grind 5 g tobacco leaf material (*see* **Note 1**) in liquid nitrogen, in a precooled pestle and mortar.
2. Transfer the frozen powder to a 50-mL tube.
3. Add 1–2 mL per gram of 2X CTAB buffer preheated to 95°C. Mix thoroughly by gentle repeated inversion of the tube and transfer to a 56°C water bath (*see* **Note 2**).
4. Incubate for 20–60 min, mixing occasionally.
5. Allow the tubes to cool, and add an equal volume of chloroform:octanol.
6. Emulsify by gentle shaking and centrifuge for 10 min at 1000*g* (*see* **Note 3**).
7. Transfer the upper aqueous phase, which contains the DNA, to a fresh tube and add 0.1 vol 10% CTAB solution preheated in a 56°C water bath.
8. Mix thoroughly, until the solution clears, and repeat chloroform:octanol extraction and centrifugation.
9. To the new aqueous phase, add an equal volume of CTAB precipitation buffer, mix thoroughly, and allow to stand at room temperature for at least 20 min (*see* **Note 4**).