# N. Benthamiana DNA Extraction in Microcentrifuge Tubes

RS18-0239A.NIC

## Materials in PrecisionPak™

- Navy or PC2 Bead Lysis Kit
- FoamBlocker
- Plant DNA Extraction Kit

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### Supplied in the Kit:

| Plant DNA Extraction Kit Contents | |
|---|---|
| Solution LA (Lysing) | 60 mL |
| Solution PA (Precipitation) | 15 mL |
| Solution CA (Clean Up) | 30 mL |

### To Be Supplied by User:

- Molecular Grade Water

## Methods

### Homogenization

1. Add 500 µL of Solution LA into each lysis kit tube.
2. Add 1-2 $cm^2$ or 50 mg of plant material to the buffer-filled tubes. ***Note:*** *Cut sample into small pieces – larger sample size may affect complete homogenization with the recommended homogenization setting.*
3. Set the Bullet Blender at speed 12, time 3 minutes and homogenize the samples. If using other homogenizer models, refer to the manufacturer's instructions for settings.
4. Remove the tubes and visually inspect the samples to confirm complete homogenization (Figure 1). ***Note:*** *If unhomogenized tissue is seen, homogenize for additional 30 seconds.*

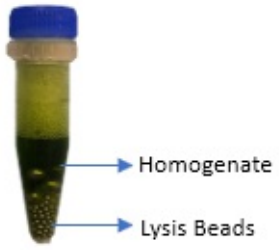


**Figure 1:** A sample after complete homogenization without any plant matter.

### Precipitation

1. Remove approximately 500 µL of the homogenate from the lysis tubes and transfer to a new tube containing an equal amount of Solution LA.
2. Add 100 µL of Solution PA to each tube and mix thoroughly by inversion.
3. Centrifuge the samples at 10,000 RPM for 5 minutes to pellet the contaminants.

### Cleaning

1. Transfer 750 µL of the supernatant that contains the DNA into new tubes with 750 µL of Solution CA. Mix thoroughly by inversion and incubate at room temperature for 5 minutes.
2. Centrifuge the solutions at 13,000 RMP for 7 minutes to pellet the DNA.
3. Remove the supernatant with a 1 mL pipette.



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4. Re-spin the tubes briefly and remove any remaining supernatant with a 200 µL pipette.
5. Add 30 µL of molecular grade water to the pellet.
6. Allow the DNA to rehydrate for 10 minutes (about 20% rehydration) or overnight (100% rehydration).
7. Analyze quality ($OD_{260}/OD_{280}$) and yield using a NanoDrop™ or other spectrophotometer and agarose gel (Data of gDNA is shown in Figure 2 and Table 1).
8. Isolated DNA can be stored at 4°C for up to a week or at -20°C for long term storage.



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