Onion DNA Micro-Extraction For PCR

1. Crush approximately 2 cm of fresh young green leaf tissue in a ziploc plastic bag by rolling with a wallpaper roller or lead pipe.
2. Add 2.0 ml of 2× CTAB (hexadecyltrimethylammonium bromide) extraction buffer to bag. Thoroughly mix buffer with leaf sap and pour about 750 ml into a 1.5 ml centrifuge tube. Avoid the cell wall material.

To make:	1 liter
1 M Tris (pH 8.0)	100 ml
NaCl	81.8 g
0.5 M EDTA	40 ml
CTAB	20 g
β-mercaptoethanol	2 ml

Add β-mercaptoethanol after autoclaving.

3. Incubate at 50°C for at least 45 minutes. Mix by gentle inversion at least twice.
4. Add 750 ml of chloroform:isoamyl alcohol (24:1), and incubate at 50°C for an additional 45 minutes. Mix gently about every 10 minutes.
5. Centrifuge in 5 minutes in a microcentrifuge.
6. Harvest the supernant. If it is cloudy, extract again with chloroform:isoamyl alcohol.
7. Add an equal volume or more of isopropanol. Invert gently until two phases are no longer evident.
8. Store at –20°C for at least for 30 minutes, or usually overnight. This is a safe stopping point.
9. Centrifuge for 5 min. in microcentrifuge. Carefully pour off supernatant.
10. Wash pellet once with 1 ml of 76% ethanol, 10 mM ammonium acetate for at least 20 minutes.

76% Ethanol, 10 mM Ammonium acetate:
To make 475 ml, use 380 ml of 95% ethanol, 630 ml of 7.5 M NH_4OAc, and bring to 475 ml with H_2O.

11. Pour off ethanol and let pellet air dry long enough for ethanol to evaporate, but not long enough to completely dry pellet (smell of ethanol is no longer evident).
12. Add 100 µl of TE (10 mM Tris, pH 8.0, 1 mM EDTA) with Rnase (1 µl to 10 ml TE), and place at 50 C for hour to dissolve.
13. Centrifuge for 2 minutes to remove any remaining insoluble material.
14. Store DNA in freezer.