# Product Application


## Highly purified DNA extraction from Tree Leaves for NGS Analysis

**Kit:** Maxwell® RSC PureFood GMO and Authentication Kit (Catalog# AS1600)
**Analyses :** NanoDrop、Quantus Fluorometer/QuantiFluor ONE dsDNA System
**Sample Type(s):** Leaves of Cedar, Japanese cypress, Black pine and Japanese larch
**Input:** 100mg
**Protocol:**

**Specially prepared buffer**
**Incubation Buffer :** 50 mM Tris, pH 8.0, 5 mM EDTA, 0.35M sorbitol, 10% (wt/vol) polyethylene glycol (Mr 6,000), 0.1% 2-mercaptoethanol

1. Crush 100mg of frozen leaves in tube with bead crusher
2. Add 1ml of Incubation Buffer into the tube and then suspend well by vortex. Centrifuge the tube at 14,000g x 2 minutes. Then remove the supernatant.
3. Add 1ml of CTAB Buffer, 20ul of RNase and 40ul of Proteinase K to the pellet, and then suspend by vortex. Incubate the tube at 65℃ x 30 minutes.
4. Centrifuge the tube at 14,000g x 10 minutes. Transfer 300ul of the supernatant into well#1 of Maxwell RSC cartridge.
5. Add 300ul of Lysis buffer into well#1.
6. Run Maxwell RSC with PureFood GMO and Authentication method.

**Results:**

DNA concentration was measured by NanoDrop and Quantus Fluorometer/QuantiFluor ONE dsDNA.

Elution volume: 50ul

| Sample | Sample amount(mg) | Protocols | NanoDrop | Quantus | A260/A280 | A260/A230 |
|---|---|---|---|---|---|---|
| **Cedar1** | 100 | Standard protocol of AS1600 | 368 | - | 1.87 | 1.57 |
| **Cedar2** | 100 | | 321 | - | 1.83 | 1.41 |
| **J. Cypress1** | 100 | | N.A. | - | | |
| **J. Cypress2** | 100 | | N.A. | - | | |
| **Black Pine1** | 100 | | 200 | - | 2.02 | 1.80 |
| **Black Pine2** | 100 | | 207 | - | 2.01 | 1.87 |
| **J. Larch1** | 100 | | 183 | - | 2.04 | 2.12 |
| **J. Larch2** | 100 | | 171 | - | 2.10 | 2.14 |
| **Cedar1** | 100 | Modified protocol with Incubation Buffer | 302 | 168 | 1.93 | 2.19 |
| **Cedar2** | 100 | | 299 | 176 | 1.94 | 2.20 |
| **J. Cypress1** | 100 | | 277 | 148 | 1.95 | 2.11 |
| **J. Cypress2** | 100 | | 245 | 146 | 1.94 | 2.15 |
| **Black Pine1** | 100 | | 204 | 98 | 1.95 | 2.04 |
| **Black Pine2** | 100 | | 179 | 82 | 1.95 | 1.96 |
| **J. Larch1** | 100 | | 250 | 154 | 1.93 | 2.13 |
| **J. Larch2** | 100 | | 207 | 116 | 1.94 | 2.11 |

**Conclusion :**

High purity and high yield of DNAs were extracted with the modified protocol with the Incubation Buffer. These DNAs are sufficient high quality for NGS analysis. On the other hand, there were issues with the standard protocol. The low value of A260/A230 in Cedar samples suggests that contaminations are included. As the eluent of Japanese Cypress was colored, it’s impossible to quantitate by NanoDrop.

The electrophoresis of extracted DNA showed a major band in the lower molecular weight region (the data is not shown here). This band is anticipated to be RNA. In order to avoid the RNA contamination, the protocol will be needed to improve further.