Comparison of prepIT®•C2D vs competitor “Q” in extraction of DNA from tissue

J. Niles
DNA Genotek, Ottawa, Ontario, Canada

prepIT®•C2D (PT-C2D) Genomic DNA MiniPrep Kit from DNA Genotek can be used for the purification of tissues. When compared to competitor “Q”, samples from different tissues had better DNA yields, higher molecular weight and equivalent ratios ($A_{260}/A_{280}$).

**Introduction**

The purpose of this technical bulletin is to compare the extraction performance of PT-C2D to a leading sample preparation kit and evaluate the DNA yield, purity, molecular weight and suitability for downstream applications. This comparison was performed using four different tissue types (liver, heart, brain and kidney) from mice.

**Materials and methods**

**Sample collection**

Four tissues (liver, heart, brain and kidney) were excised from six mice. The tissues were then kept frozen at -20°C prior to purification.

**Purification**

Each tissue was cut to a weight of approximately 20 mg for a total of 12 samples (6 samples per purification method). For extraction using PT-C2D, the tissue samples were purified according to the PT-C2D tissue purification protocol. In brief, 350 µL of phosphate buffered saline (PBS) was added to each tissue sample. The tissue was homogenized using a rotor-stator homogenizer before adding RNase A, Proteinase K and Lysis Buffer. After a 10 minute lysis and centrifugation step the supernatant was added to a MiniPrep Column and centrifuged. The filtrate was discarded and the column washed once with Wash Buffer 1 and twice with Wash Buffer 2. The DNA was eluted in 200 µL of Elution Buffer.

The same types of tissues were purified according to the competitor “Q” purification protocol. In brief, the tissues were homogenized in PBS using a rotor-stator homogenizer, followed by two lysis steps, a binding step and centrifugations combined with washing steps. The DNA was also eluted in 200 µL of elution buffer.

**DNA analysis**

The samples were quantified using fluorescence (PicoGreen) and the $A_{260}/A_{280}$ ratios were determined via UV absorbance. To evaluate the molecular weight of the DNA, a 0.8% agarose gel was run and the samples were compared to a Lambda-Hind III digest ladder. Finally real-time PCR was performed using primers for Dystrophin (DMD).

**Results**

The average yields and $A_{260}/A_{280}$ ratios are summarized in Table 1 for comparison of PT-C2D and competitor "Q". For liver, heart and kidney there was a significant increase (P values <0.005) in the average total yield, while the brain averages were not significantly different between the two purification kits.

The molecular weight of 4 samples from each tissue tested (liver, heart, brain and kidney) is shown in Figure 1. For each tissue type the 2 samples from PT-C2D and 2 samples from competitor "Q" purifications are shown. A distinct high molecular weight band is observed in all samples purified using PT-C2D when compared to competitor "Q".

Lastly the samples were tested for performance using real-time PCR. In Figure 2, the real-time PCR amplification profile of the DMD fragment using the brain DNA from both PT-C2D (A) and competitor "Q" (B) is presented.
### Purification protocol

<table>
<thead>
<tr>
<th>Purification protocol</th>
<th>Average DNA yield per 20 mg of tissue (ug)</th>
<th>Average ( A_{260}/A_{280} ) ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Heart</td>
</tr>
<tr>
<td>PT-C2D</td>
<td>6.60</td>
<td>5.41</td>
</tr>
<tr>
<td>Competitor “Q”</td>
<td>2.02</td>
<td>2.96</td>
</tr>
</tbody>
</table>

*Table 1: Average DNA yield and \( A_{260}/A_{280} \) ratio.*

---

### Discussion and conclusion

The average yields for liver, heart and kidney from PT-C2D purification were significantly higher than the average yields obtained from purification with competitor “Q.” The brain samples were not significantly different between the two purifications. Across all tissue types the high molecular weight band was better defined in samples purified with PT-C2D than with competitor “Q,” which showed varying amounts of smearing. The ratios for all samples were not significantly different between the two methods of purification. The DNA from both purifications also performed well in the real-time PCR reactions demonstrating that the DNA is of good quality and integrity. In summary the PT-C2D purification process performs equivalently to a leading column-based purification kit for the extraction of tissue samples.

---

**Reference**

1. prepIT•C2D Genomic DNA MiniPrep Kit Tissue sample protocol. DNA Genotek. PD-PR-00259.