**Extraction:**

Before starting the extraction:

- A pathologist must analyse each sample to verify and define the tumour area.
- The percentage of **tumour cells** should be at least 20% per sample in order to obtain significant results.

**Deparaffination:**

**Paraffin Slides**

1. Immerse the glass slides in *Xylene* for 5'.

2. Immerse the glass slides in *Ethanol 96%* for 5'.

3. Immerse the glass slides in **fresh Ethanol 96%** for 5'.
   - Withdraw the cover glass and scrape the slice with a scalpel.
   - Collect the sample on a 1.5ml tube.

4. **Centrifuge 5’ at max speed**.
   - Discard the supernatant.

5. **Centrifuge 2’ at max speed**.
   - Discard the supernatant.
   - Allow the ethanol to air-dry.

**Paraffin Rolls**

1. Discard as much paraffin as possible from the block before preparing the slices.

2. Take the paraffin rolls into a 1.5 ml tube.

3. Add 500 µl of mineral oil. Make sure the oil covers the sample completely.

4. Incubate 2’ at 95°C and 550 rpm.

5. Centrifuge 2’ at 8000 rpm.

6. Carefully aspirate the oil avoiding to touch the sample.

7. Repeat steps 3-6.

8. Add the **lysis buffer** making sure it completely covers the sample.

**Extraction:**

Continue with the extraction with the **QiAmp DNA FFPE Tissue Kit, Qiagen.**

- Follow the manufacturer instructions.
- **Elution volume:** 50 µl.
**Amplification:**

Before starting the amplification:

- The amount of genetic material added to each amplification tube should be **150 ng** in a max volume of 10 µl.
- It is recommended to dilute the extracted DNA to **30 ng/µl to add 5 µl to each amplification tube**.
- If DNA concentration is lower than 15 ng/µl the extraction should be repeated.
- It is recommended the use of conventional thermocycler or adjust the temperature ramp to the assays needs (see point 7.3 on the user’s manual).
- **Keep the amplification tubes at 4ºC at all times.**

1. Thaw just the number of amplification tubes according to the number of samples to analyze.

2. If possible, briefly centrifuge the amplification tubes.

3. Add **5-10 µl of extracted DNA** to each amplification tube.

4. Place the amplification tubes in the thermocycler only when the block has reached **95ºC**.

5. The amplification product must be kept at **4ºC and visualized within 5 days**.

### PCR program:

<table>
<thead>
<tr>
<th>Cycle Type</th>
<th>Temperature</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 cycle</td>
<td>95ºC</td>
<td>15'</td>
</tr>
<tr>
<td>40 cycles</td>
<td>94ºC</td>
<td>60''</td>
</tr>
<tr>
<td></td>
<td>66ºC</td>
<td>60''</td>
</tr>
<tr>
<td>1 cycle</td>
<td>72ºC</td>
<td>10'</td>
</tr>
<tr>
<td>1 cycle</td>
<td>4ºC</td>
<td>∞</td>
</tr>
</tbody>
</table>

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