Please refer to the QIAamp DNA FFPE Tissue Kit protocol for more information on the QIAGEN web page given below.
http://www.qiagen.com/Products/Catalog/Sample-Technologies/DNA-Sample-Technologies/Genomic-DNA/QIAamp-DNA-FFPE-Tissue-Kit#resources

**Equipment and Reagents Required, but not Provided with OncoScan™ FFPE Assay Kit**

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, consult the appropriate material safety data sheets (MSDSs), available from the product supplier.

**Equipment Required**

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Vendor</th>
<th>P/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microtome (HM340E Electronic Rotary Microtome Package, with Universal Cassette Clamp and Disposable Blade Carrier E (ThermoFisher Cat#: 905190A))</td>
<td>VWR International</td>
<td>89219-568</td>
</tr>
<tr>
<td>Vortex Genie 2 (GS60)</td>
<td>VWR International</td>
<td>58815-234</td>
</tr>
<tr>
<td>Microcentrifuge, 5424 (EPPEO22620401)</td>
<td>VWR International</td>
<td>80094-126</td>
</tr>
<tr>
<td>Thermomixer R (22670107) (Qty Required: 2)</td>
<td>VWR International</td>
<td>21516-166</td>
</tr>
<tr>
<td>Block for 24 x 1.5 mL Tubes for Thermomixer R (22670522) (Qty Required: 2)</td>
<td>VWR International</td>
<td>21516-176</td>
</tr>
<tr>
<td>Pipet-Lite LTS Pipette, Single Channel, 0.5-10 μL</td>
<td>Rainin</td>
<td>L-10</td>
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<tr>
<td>Pipet-Lite LTS Pipette, Single Channel, 20-200 μL</td>
<td>Rainin</td>
<td>L-200</td>
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<tr>
<td>Pipet-Lite LTS Pipette, Single Channel, 100-1000 μL</td>
<td>Rainin</td>
<td>L-1000</td>
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<tr>
<td>Pipet-Lite LTS Pipette, 12-Channel, 2-20 μL</td>
<td>Rainin</td>
<td>L12-20</td>
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</table>
Consumables Required

Table E.2 Consumables Required

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Vendor</th>
<th>P/N</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP-35 High-Profile blades with PTFE coating</td>
<td>Fischer Scientific</td>
<td>3153735</td>
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<tr>
<td>Eppendorf tubes, 1.5 mL microcentrifuge tubes</td>
<td>VWR International</td>
<td>022363204</td>
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<tr>
<td>Non-Stick RNase-free Microfuge Tubes (1.5 mL)</td>
<td>Life Technologies</td>
<td>AM12450</td>
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<td>Greenpak LTS 20 μL Filter Tip, 960 Tips</td>
<td>Rainin</td>
<td>GP-L10F</td>
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<tr>
<td>Greenpak LTS 200 μL Filter Tip, 960 Tips</td>
<td>Rainin</td>
<td>GP-L200F</td>
</tr>
<tr>
<td>Greenpak LTS 1000 μL Filter Tip, 768 Tips</td>
<td>Rainin</td>
<td>GP-L200F</td>
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<tr>
<td>VWR PCR Plate, 96-Well, Flat Plate</td>
<td>VWR</td>
<td>82006-636</td>
</tr>
</tbody>
</table>

Reagents Required

Table E.3 Reagents Required

<table>
<thead>
<tr>
<th>Equipment</th>
<th>Vendor</th>
<th>P/N</th>
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<tbody>
<tr>
<td>QIAamp DNA FFPE Tissue Kit</td>
<td>QIAGEN</td>
<td>56404</td>
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<tr>
<td>QIAamp MinElute Columns</td>
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<td>1020901</td>
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<tr>
<td>Collection Tubes - 2 mL</td>
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<td>1016810</td>
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<tr>
<td>Buffer ATL</td>
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<td>1014758</td>
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<tr>
<td>Proteinase K</td>
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<td>19133</td>
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<tr>
<td>Buffer AL</td>
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<td>1014604</td>
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<tr>
<td>Buffer AW1</td>
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<td>1014790</td>
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<tr>
<td>Buffer AW2</td>
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<td>1014592</td>
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<tr>
<td>Buffer ATE</td>
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<td>1049476</td>
</tr>
<tr>
<td>Xylene</td>
<td>Sigma Aldrich</td>
<td>534056-500ML</td>
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<tr>
<td>Ethanol (96-100%)</td>
<td>Sigma Aldrich</td>
<td>459836</td>
</tr>
<tr>
<td>RNase A</td>
<td>QIAGEN</td>
<td>1007885</td>
</tr>
</tbody>
</table>

Optional Prerequisite

The yield of tissue from FFPE is highly dependent on the type of tissue and method used for the initial fixation. For optimal DNA yield, samples submitted for extraction should have a tissue size of 400-700 square mm.

Preparation of Buffers

Preparing Buffer ATL
- Check whether precipitate has formed in Buffer ATL. If necessary, dissolve by heating to 70 °C with gentle agitation.

Preparing Buffer AL
- Check whether precipitate has formed in Buffer AL. If necessary, dissolve by heating to 70 °C with gentle agitation.
Preparing Buffer AW1

Add 25 mL ethanol (96-100%) to the bottle containing 19 mL Buffer AW1 concentrate. Can be stored in room temperature up to 1 year.

Preparing Buffer AW2

Add 30 mL ethanol (96-100%) to the bottle containing 13 mL Buffer AW2 concentrate. Can be stored in room temperature up to 1 year.

Deparaffinization

1. From an FFPE block, prepare 10 micron slices. Place 5 slices in a 1.5 mL Eppendorf Safe-Lock Tube.

2. Turn on two thermal mixers. Set one to 56 °C and the other to 98 °C.
3. Add 1 mL of Xylene to the tube.
4. Vortex the tube at max speed for 10 seconds.
5. Spin down the tube at full speed (~14000 rpm) for 5 minutes.
6. Without disturbing the pellet remove the Xylene.
7. Add 1 mL of Ethanol to the tube.
8. Vortex the tube at max speed for 10 seconds.
9. Spin down the tube at full speed (~14000 rpm) for 5 minutes.
10. Without disturbing the pellet remove the Ethanol by using a P1000 pipette.
11. Repeat Step 7 through Step 10 once more.
12. Spin down the tube at full speed (~14000 rpm) for 3 minutes.
13. Use a P20 or P200 pipette to completely remove any residual Ethanol without disturbing the pellet.
14. Allow any remaining Ethanol to evaporate by letting the tube air dry for 10 minutes at room temperature.

Tissue Lysis

1. Add 180 µL of ATL Buffer to the tube after ensuring the residual ethanol has completely evaporated.
2. Vortex at full speed for 10 seconds.
3. Spin down the tube briefly and then place it onto the thermal mixer that is set at 98 °C.
4. Incubate the tube for 15 minutes with a 15 second mix at 1400 rpm every 1 minute.

5. After 15 minutes, stop the thermomixer program and turn off the thermomixer. Let the tube cool down for 5 minutes in the thermomixer before removing them.
6. Remove the tube carefully and slowly (as they may still pop open) from the thermal mixer and allow it cool at room temperature for 10 minutes.
7. Spin down the tube to remove any solution from the top of the tube.
8. Add 20 µL of Proteinase K to the tube.
9. Vortex the tube at max speed for 10 seconds, then spin down briefly.
10. Place tube on the thermal mixer that is set at 56 °C.
11. Incubate the tube for at least 3.5 hours with a 15 second mix at 1400 rpm every 1 minute.
12. After 3.5 hours, verify that all tissue has lysed.
   A. If tissue remains, incubate the samples overnight. If tissue still remains, add an additional 20 µL of Proteinase K and continue incubation for a minimum of 1 hour.
13. Spin down the tube and place them onto the thermal mixer that is set at 90 °C.
14. Incubate the tube for 1 hour with a 15 second mix at 1400 rpm every 1 minute.

**NOTE:** Thermomixer settings: Ensure the thermomixer is programmed to shake the samples for 15 sec after each minute at 1400 RPM.

15. After 1 hour, remove the tube from the thermal mixer and allow to cool at room temperature for 10 minutes.
16. Spin down the tube to remove any solution from the top of the tube.
17. Add 2 µL of RNase A to each tube.
18. Vortex the tube at max speed for 10 seconds, then spin down briefly. Allow to incubate for 2 minutes.

### DNA Purification

1. Remove a QIAamp MinElute column from the refrigerator and allow to warm to room temperature for 15 minutes.
2. Remove the ATL tube and equilibrate at room temperature.
3. Add 200 µL of Buffer AL to the sample tube, vortex at max speed for 10 seconds, then spin down briefly.
   A. If processing multiple samples, ensure that after adding Buffer AL, Ethanol is added as quickly as possible.
   B. Precipitate may form at this step, which does not affect the DNA yield.
4. Add 200 µL of Ethanol to each tube, vortex at max speed for 10 seconds, then spin down briefly.
5. Label the QIAamp MinElute column (in a 2 mL collection tube) appropriately.
6. Carefully transfer the entire lysate to the QIAamp MinElute column (in a 2 mL collection tube) without wetting the rim, close the lid, and centrifuge at 6000 x g (8000 rpm) for 1 min.
   A. Check to see that all the lysate has moved through the column. If lysate is still in the column centrifuge again at a higher speed for 1 minute.
7. Place the column into new collection tube and discard eluate.
8. Open the column and add 500 µL of Buffer AW1.
9. Load the column onto the centrifuge and spin at 8000 rpm (6000g) for 1 minute.
   A. Check to see that the entire Buffer AW1 has moved through the column. If Buffer AW1 is still in the column centrifuge again at a higher speed for 1 minute.
10. Place the column into new collection tube and discard eluate.
11. Open the column, and add 500 µL of Buffer AW2.
12. Load the column onto the centrifuge and spin at 8000 rpm (6000g) for 1 minute.
   A. Check to see that the entire Buffer AW2 has moved through the column. If Buffer AW2 is still in the column centrifuge again at a higher speed for 1 minute.
13. Place the column into new collection tube and discard eluate.
14. Load the column onto the centrifuge and spin at 14000 rpm (20000g) for 3 minutes to dry the membrane completely.
DNA Elution

1. Label a clean Nuclease-free 1.5 mL tube for DNA elution.
2. Place the column into the labeled 1.5 mL tube prepared for elution.
3. Add 50 µL of Buffer ATE to the center of the column membrane.
4. Close the lid and incubate the column at room temperature for 5 minutes.
5. Load columns onto the centrifuge and spin at 14000 rpm (20000g) for 1 minute to elute the DNA.
   A. Note: Caps for 1.5 mL tube will not be able to close at this step due to the columns. When loading onto the centrifuge rotate caps to the right to keep them from breaking during the spin.
   B. When eluting multiple samples, alternate positions in centrifuge to make room for caps.

Quantitation of Eluted FFPE DNA

1. Perform either of the two Affymetrix recommended and tested dsDNA quantitation protocols that are included in the user manual, to measure the concentration of the eluted FFPE DNA. Affymetrix strongly recommends using these protocols that have been tested at Affymetrix using the following kits for DNA quantitation.
   - Quant-iT™ PicoGreen® dsDNA Assay Kit (Catalog Number: P7589, LifeTechnologies™)
   - Qubit® dsDNA HS Assay Kit (Catalog Number: Q32851, LifeTechnologies™)
2. Record the results and the volume information in a spreadsheet.