Processing FFPE Punches On The Ionic® Purification System

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OVERVIEW

The Ionic® Purification System has demonstrated advantages over traditional solid-phase (column or bead) methods when it comes to extraction of RNA and DNA from Formalin-Fixed Paraffin Embedded (FFPE) tissue. When extracting nucleic acids from FFPE, the Ionic system routinely delivers 2-5 fold increases in yield as well as lower length and GC biases. Many users analyze whole sections of FFPE tissue in the form of scrolls while others analyze a sub-section of the tissue via various FFPE block sampling methods such as macrodissection, punching, or laser-capture microdissection.

Here we outline the technique for processing punches of FFPE tissue on the Ionic System. FFPE punches are prepared by using a large-gauge needle to remove a core from a desired portion of an FFPE block. This method is useful when a study calls for sampling a specific area of the FFPE tissue (ex. tumor, stroma, or normal regions) for downstream analysis. Since the surface area to mass ratio of punches is much lower than for FFPE scrolls or sections, punches require an augmented deparaffination and lysis process as part of the Ionic protocol. Outlined below is the protocol for processing FFPE punches on the Ionic system

Taking Punches from FFPE Tissue Blocks

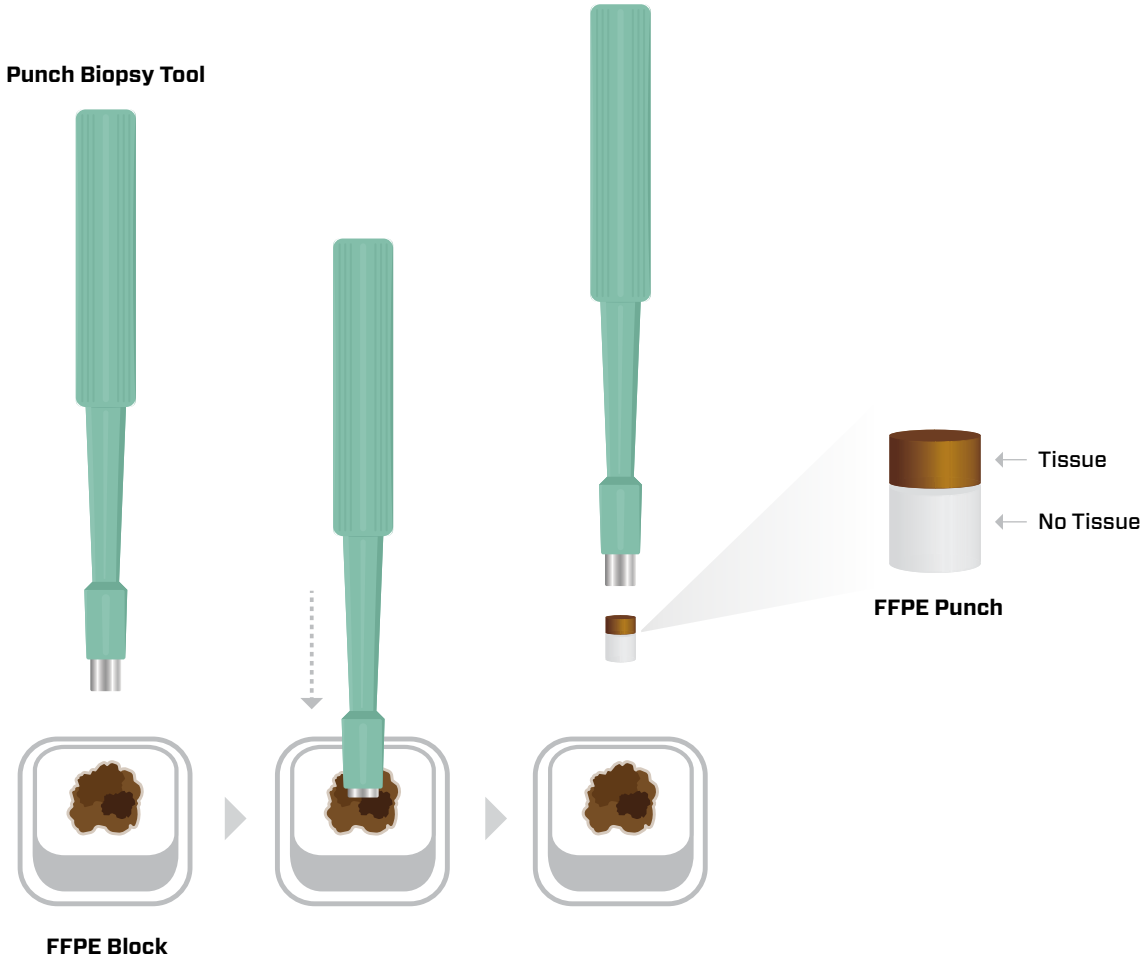

FIGURE 1: FFPE tissue punch steps

Ionic Kit Selection

This protocol is intended for use with the **Ionic® FFPE to Pure DNA Kit** from Purigen Biosystems.

Sample Requirements

1. This protocol is designed to process **up to four FFPE tissue punch samples per run** on the Ionic system.
2. Each punch sample is expected to come from a standard 2 mm biopsy punch that produces a cylindrical core of approximately 5 mm in depth. Cores with smaller diameters are also acceptable.

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Prepare ThermoMixer

IMPORTANT
Different incubation parameters are recommended depending on your downstream application. Purigen recommends programming a ThermoMixer with the selected parameters prior to starting the lysis reaction.

1. Place an appropriate heating block on the ThermoMixer to accommodate 1.5 mL microtubes. Use of the ThermoMixer with its lid is recommended.
2. Select an appropriate incubation program:

   Option 1: Recommended for hybrid capture-based next generation sequencing:

   1. Incubate at 65°C for 10 min at 1000 rpm.
   2. Incubate at 56°C for 60 min at 1000 rpm.
   3. Incubate at 70°C for 8–16 hours (overnight) at 1000 rpm.
   4. Hold at 8°C for up to 24 hours.

   Option 2: Recommended for **amplification-based analyses** (*e.g.*, amplicon-based next generation sequencing):

   1. Incubate at 65°C for 10 min at 1000 rpm.
   2. Incubate at 56°C for 60 min at 1000 rpm.
   3. Incubate at 90°C for 60 min at 1000 rpm.
   4. Hold at 8°C for up to 24 hours.

Prepare Samples for Lysis

1. For each sample, cut a single punch into multiple pieces with a sterile razor blade and transfer to a 1.5 mL LoBind Eppendrf tube.

Prepare Lysis Buffer and Master Mix

1. Allow the **Lysis Buffer** to reach room temperature. Vortex for 3 seconds and centrifuge briefly.

IMPORTANT
It is important that the Lysis Buffer is at **room temperature** before vortexing and using.

2. Place **Proteinase K** on ice.
3. Add 80 µL of **Proteinase K** directly to each of the 2 tubes of Lysis Buffer provided with the kit. Vortex for 3 seconds to mix and centrifuge briefly.

Lysis Procedure

IMPORTANT
All centrifuge steps should occur at **room temperature**.

1. Centrifuge microtubes containing tissue samples at >10,000 RPM for 5 min, to move the tissue to the bottom of the tube.
2. Add 400 µL of **Mineral Oil** to each tube. A pipette tip can be used to help to push down any portion of the tissue that is not submerged in solution.
3. Using a P1000 pipette, accurately dispense 440 µL of prepared **Lysis Master Mix** into each tube.
4. Place tubes into ThermoMixer and start the chosen Lysis program.
5. Once the ThermoMixer has reached 8°C, samples can be removed. The lysis mixture will remain stable at 8°C until sample tubes can be removed.
6. Allow samples to reach room temperature for 10 minutes.
7. Centrifuge the samples for 5 minutes at room temperature, at maximum speed (>10,000 x g). The lysis mixture will separate into two phases with the lysate containing nucleic acid contained in the lower phase.
8. For each sample, using a P1000 pipette, transfer 440 µL of lysate from the lower phase of each sample tube into a new 1.5 mL LoBind Eppendorf tube. A total of 440 µL should be transferred. A small amount of mineral oil can be transferred with the aqueous phase.

NOTE
Remove the lower phase by pipetting out in a slow manner to avoid excess transfer of mineral oil. Keep the end of the pipette tip in contact with the bottom of the tube. It is ok to include a small amount of the mineral oil layer (less than 20 µL) when removing the lysate (lower phase) from each sample tube. A small amount of mineral oil will not impact purification on the Ionic system.

9. Remove **RNAse A** from freezer (-20ºC).
10. Add 10 µL **RNAse A** (10 mg/mL) to each tube of transferred lysate.
11. Briefly vortex and spin each tube.
12. Incubate tubes for a minimum of 5 minutes at room temperature.
13. If you do not plan to purify the samples within 30 minutes, store at **4°C for up to 24 hrs**.
14. Ensure samples are at room temperature, vortexed and spun down 10 seconds before beginning the purification procedure.
15. Divide each lysate and run on two lanes of an Ionic chip (200 µL/lane). Follow guidance outlined in the Ionic® FFPE to Pure DNA Kit Protocol to set up the run on the Ionic Instrument. The protocol can be downloaded from the **Purigen Documentation Page** on our website.

IMPORTANT
Sample tubes can be stored at **4ºC for a maximum of 24 hours** prior to purification. Sample tubes should be stored at -20°C when stored by more than 24 hours.

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