TECHNICAL NOTE

Recommended workflow Devyser BRCA and FFPE samples

INTRODUCTION

The combination of formalin fixation and paraffin embedding (FFPE) is a commonly used method for archiving pathological tissue specimens. Archives of FFPE tissue sections can be many years old and are a valuable tool to study diseases such as cancer. The ability to extract nucleic acid from FFPE tissue sections enables researchers to correlate the disease state and tissue morphology of a patient with a particular genetic trait. However, DNA extraction from these tissues is associated with several challenges, mainly due to the formalin fixation, which results in cross-linking between proteins and DNA as well as between different DNA strands. The result is purified DNA that is often significantly degraded and fragmented. The degree of fragmentation depends on tissue type, age of the sample and specific conditions used during fixation. These factors can lead to issues with downstream applications, many of which incorporate PCR. Thus, the protocol for DNA purification from FFPE tissues must efficiently extract highly fragmented DNA and reverse cross-linking caused by formalin fixation.

The purpose of this technical note is to enable improved usability of Devyser BRCA in combination with severely compromised FFPE samples, highlighted by the easy to use and streamlined Devyser workflow for simultaneous library preparation and pooling for sequencing (**Figure 1**).

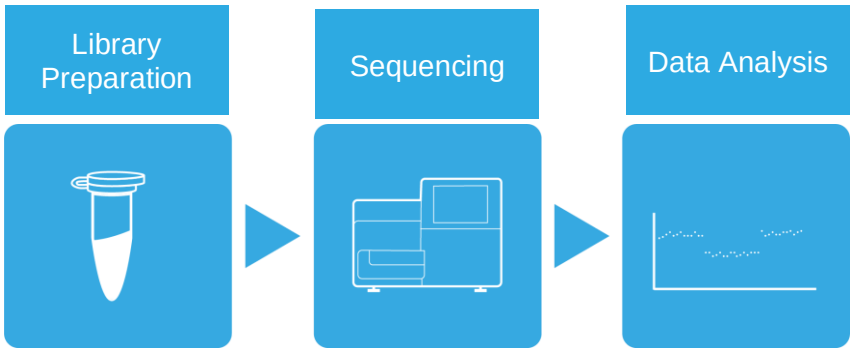

Figure 1: Devyser workflow – The streamlined NGS workflow for BRCA detection integrates library preparation, sequencing, and data analysis.

WORKFLOW

After completed PCR2, each FFPE sample should be purified separately before pooling and sequencing. For preparation of **Wash** solution, follow section 7.3.1 of Devyser BRCA IFU (7-A037).

Library Purification
Required kit: **Devyser Library Clean (8-A204)**
Required kit component: **Clean (4-A255), Wash (4-A254)**
Required (not provided): **DynaMag™-96 Side Magnet or equivalent**

A. For each FFPE sample, transfer 23 µL of the corresponding PCR2 library to a new PCR strip
B. Briefly centrifuge the **Clean** tube to collect the content
C. Firmly tap the **Clean** tube. Make sure that the bead pellet is re-suspended and that the content is homogenous. If necessary, briefly vortex the tube but avoid extensive vortexing
D. Add 23 µL re-suspended Clean to each of the libraries and mix by pipetting up and down at least 10 times
E. Incubate the mixtures for 3 minutes
F. Place the PCR strip onto the magnet until all beads are pelleted and the solutions are clear
G. While keeping the PCR strip on the magnetic rack, carefully remove and discard the solution by using a pipette (P20) that pipettes a maximum of 20 µL
Note! It is important to avoid touching the bead pellet during this step
H. Add 150 µL of prepared **Wash** solution to the PCR strip tubes without removing it from the magnet
I. Move the PCR strip from one position to another in the magnet. This results in the beads moving though the **Wash** solution. Perform the procedure twice
J. Carefully remove as much **Wash** solution as possible by pipetting from the bottom of the tube. **Note! It is important to avoid touching the bead pellet and the walls of the tube and that as much Wash as possible is removed**
K. Leave the PCR strip on the magnet until all **Wash** solution has evaporated and the bead pellet has changed from being luster to lusterless, approximately 2-3 minutes
L. Remove the PCR strip from the rack

Library elution
Required kit: **Devyser Library Clean (8-A204)**
Required kit component: **Dilution buffer (4-A245)**

A. Briefly centrifuge the **Dilution buffer** to collect the content
B. Add 12 µL Dilution buffer to the PCR strip tubes (from above) and re-suspend the pellet by pipetting up and down
C. Place the tubes onto the magnet until all beads are pelleted
D. While keeping the tubes on the magnet, transfer the cleared supernatant, containing the purified libraries, to new PCR strip tubes

Library quantification
Required kit: **Qubit 1x dsDNA Assay Kit**
Quantify the library as described in the valid user manual for Qubit 1X dsDNA HS Assay Kits for details

A. Ensure that all Qubit 1X dsDNA HS solutions are at room temperature
B. Mix 190 µL Qubit 1X dsDNA HS working solution with 10 µL of Qubit™ standard 1
C. Mix 190 µL Qubit 1X dsDNA HS working solution with 10 µL of Qubit™ standard 2
D. Mix **198 µL** Qubit 1X dsDNA HS working solution with **2 µL** of the purified libraries
E. Briefly vortex, centrifuge and incubate each tube for 2 minutes at room temperature
F. Measure the concentration (ng/µL) of the purified libraries on a Qubit Fluorometer

Library dilutions and pooling
Required kit: **Devyser Library Clean (8-A204)**
Required kit component: **Dilution buffer (4-A245)**

A. Dilute the purified libraries to a final concentration of **0.33 - 0.41 ng/µL** using the **Dilution buffer**
B. Mix the diluted libraries with a 1:1 ratio to have final volume of **at least 20 µL**
C. Measure the concentration of the diluted library pool to confirm the concentration
Note! 10µL library pool should be mixed with 190 µL Qubit 1X dsDNA HS working solution

Sequencing
According to section 8 of Devyser BRCA IFU (7-A037).