SNP Analysis Directly from Formalin-Fixed Paraffin-Embedded Human Ovarian Tissue Samples Using Thermo Scientific Phusion Human Specimen Direct PCR Kit

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Tech Note

Thermo Scientific™ Phusion™ Human Specimen Direct PCR Kit enables DNA amplification directly from various human tissues. Here we demonstrate a fast and easy way to perform SNP analysis from formalin-fixed paraffinembedded (FFPE) human tissues without the need for time consuming DNA purification.

Introduction

FFPE samples are complicated starting materials for both DNA purification and the subsequent PCR amplification due to significant nucleic acid degradation and crosslinking[1,2]. However, with Phusion Human Specimen Direct PCR Kit DNA fragments are readily amplified from FFPE samples without any prior DNA purification steps.

In this Technical Note a fragment of *FOXL2* gene[3,4] was amplified directly from human FFPE tissues using Phusion Human Specimen Direct PCR Kit. The SNP genotypes of each human individual were determined by sequencing the amplified DNA fragments.

Materials and Methods

- Thermo Scientific Phusion Human Specimen Direct PCR Kit (#F-150)
- SequiTherm™ EXCELL II DNA Sequencing Kit-LC (Epicentre #SE9101LC)
- Thermo Scientific™ PikoThermal™ Cycler 24-well (Cat #TCP0024)
- LI-COR 4200 IR2 Sequencer
- Thermo Scientific™ Piko™ PCR Plate, 24-well white (Cat #SPL0241)
- Thermo Scientific 8-cap strips, flat, optically clear (Cat #TCS1080)
- Human ovarian and breast FFPE slides
- Purified FFPE genomic DNA (C/G)

Human ovarian FFPE slides

- **Dilution protocol:** 100 µL of solution mixture prepared from Dilution Buffer and TE, pH 8.0 (1:1) was pipetted onto the FFPE tissue slides. The tissues were scraped off with a pipette tip and the mixtures were collected into a tube. Thermo Scientific™ DNARelease™ Additive (1.5 µL) was added to each sample. The samples were incubated for 1 hour at 60° C followed by 10 minutes at 98° C. The samples were cooled on ice and centrifuged for 2 minutes at 16,000 × g. Two µL of supernatants were used as a template in a 20 µL PCR reaction.

- **Control reactions:** 50 ng of purified FFPE genomic DNA (C/G) in a 20 µL PCR reaction (C+). The genomic DNA was purified using a traditional method (Proteinase K digestion, organic extraction and ethanol precipitation). C-:0.5 µL of water in a 20 µL PCR reaction (no template control).

Sequencing

- After PCR, the reactions were diluted 1:3 into water and 1 µL of the dilutions were used as templates in DNA sequencing reactions. The reactions were prepared according to the manufacturer's instructions.

- Note that the PCR products include both sample and PCR reagent-derived factors that may interfere with the Taq-based sequencing enzymes. The effect can be decreased by diluting the PCR product; however, sometimes it may be necessary to purify the PCR product prior to sequencing.

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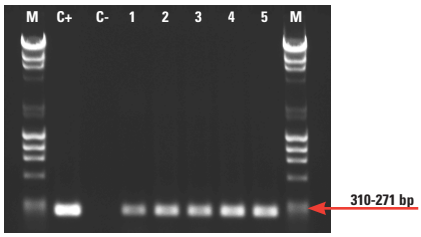

Figure 1. PCR from FFPE samples using the universal control primer mix to confirm gDNA integrity. Universal control primers amplify a 237 bp fragment from the highly conserved non-coding region upstream of the human *SOX21* gene. C+ is purified ovarian FFPE tumor DNA with genotype C/G and C- is the negative control (no template). Lane 1 is FFPE breast conduct and lanes 2-5 are FFPE ovarian tumor samples. M is DNA marker.

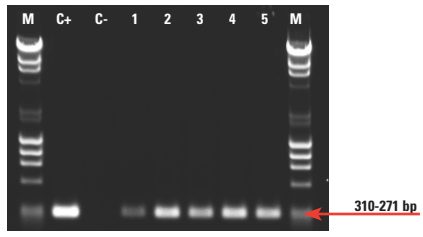

Figure 2. Amplification of the 269 bp fragment of *FOXL2* gene covering the SNP site of interest using Phusion Human Specimen Direct PCR kit. C+ is the purified ovarian FFPE tumor DNA with genotype C/G and C- is the negative control (no template). Lane 1 is FFPE breast conduct and lanes 2-5 are FFPE ovarian tumor samples. M is DNA marker.

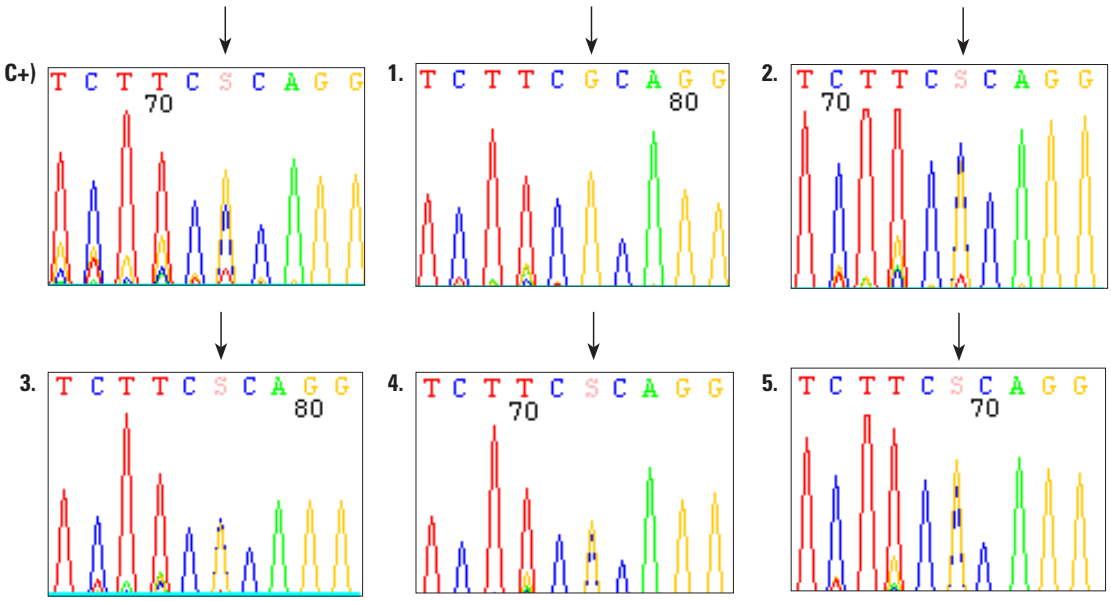

Figure 3. Sequencing of PCR products to identify sample genotype. C+) Purified human FFPE genomic DNA (C/G), **1.** FFPE breast conduct, **2.-5.** FFPE ovarian tumor samples. The results indicate that the FFPE breast conduct slide was G/G and all the FFPE ovarian tumor samples were heterozygous (C/G) to the corresponding SNP site. The C/G genotype was previously found in the same granulosa cell tumors, utilizing frozen tissue and conventional DNA analysis and sequencing[3].

Results and Discussion

In this technical note we show that human ovarian granulosa cell tumor FFPE samples can be used in SNP genotyping without the need for time consuming DNA purification when using the Phusion Human Specimen Direct PCR Kit.

First, we confirmed that the sample material was in an appropriate condition by amplifying the FFPE DNA with universal control primers supplied with the kit (Figure 1). This is extremely important since the quality of the FFPE samples varies from block to block. The results showed that the FFPE DNA allowed amplification of the 237 bp fragment indicating good DNA integrity and suitability for the Direct PCR approach.

For the SNP genotyping assay four ovarian tumor FFPE tissue slides and one breast FFPE tissue slide were tested using the kit's dilution protocol. A 269 bp fragment of *FOXL2* gene covering a SNP of interest was amplified with Thermo Scientific™ Phusion™ Hot-Start II High-Fidelity DNA Polymerase included in the kit (Figure 2). The unpurified PCR products were sequenced from 1:3 dilutions to resolve the SNP genotype of each human individual. The results indicate that the genotypes were as predicted (Figure 3). The SNP genotype of breast FFPE sample was G/G which is predictable since this particular SNP mutation has been consistently identified in granulosa cell tumors of ovary, but not in other tissue or tumor types (personal comm. of Dr. Mikko Anttonen, Dept. of Obstetrics and Gynecology and Children's Hospital, Helsinki University Central Hospital). The granulosa cell tumor samples appeared to be heterozygous to the SNP (C/G). The C/G genotype was previously found in the same granulosa cell tumors, utilizing frozen tissue and conventional DNA analysis and sequencing[3].

In this experiment the obtained PCR yields were sufficient without purification of the amplicon for sequencing. It may be necessary to purify the PCR products prior to sequencing if the FFPE DNA is highly fragmented and the PCR yields are low.

In conclusion, Phusion Human Specimen Direct PCR Kit enables amplification of DNA fragments directly from unpurified FFPE samples. This allows significant savings both in time and cost by avoiding DNA purification steps with hazardous solvents. However, since DNA is always fragmented in FFPE samples, it is essential to determine the quality of DNA prior to PCR by using the universal control primers provided with the kit.

Acknowledgements

The FFPE samples and the FOXL2 primers were provided by Drs. Mikko Anttonen and Noora Andersson, Department of Obstetrics and Gynecology and Children's Hospital, Helsinki University Central Hospital.

References

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