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# Fast-COLD PCR Protocol for Enrichment of TP53 R181H Variant Before Sanger Sequencing

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**Protocol status:** Working

**We use this protocol and it's working**

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## Abstract

This protocol describes the wet-lab steps for enrichment of mosaic TP53 R181H variant using Fast-COLD PCR program before Sanger sequencing.

## Materials

Reagents:

- AmpliTaq Gold™ 360 Master Mix 1 mL, Applied Biosystems™, Catalog #4398876
- DNase/RNase-Free Water
- HPLC-purified oligos (Fwd and Rev primers)

Equipments:

- GeneAmp™ PCR System 9700 Fast Thermal Cycler

Primer sequences:

- Fwd: 5'-ACCAGCCCTGTCGTCTCT-3'
- Rev: 5'-GTGCAGCTGTGGGTTGATTC-3'



## Fast-COLD PCR Protocol

### 1 *Reagents/reaction tube:*

- 1.1 12.5  $\mu$ L AmpliTaq Gold™ 360 Master Mix
- 1.2 8.5  $\mu$ L DNase/RNase-Free Water
- 1.3 2  $\mu$ L 360 GC Enhancer
- 1.4 1  $\mu$ L DNA template (10 – 50 ng/ $\mu$ L)
- 1.5 0.5  $\mu$ L Fwd oligos (10  $\mu$ M)
- 1.6 0.5  $\mu$ L Rev oligos (10  $\mu$ M)

### 2 *Thermocycler program:*

- 2.1 Initial denaturation: 95°C, 10 min
- 2.2 First 15 cycles: Denaturation: 95°C, 30 s; Annealing: 59°C, 30 s; Extension: 72°C, 30 s
- 2.3 Last 20 cycles: Denaturation: 87°C, 30 s; Annealing: 59°C, 30 s; Extension: 72°C, 30 s
- 2.4 Final extension: 72°C, 7 min