multiplexed
ICE COLD-PCR™

Overview of Multiplexed ICE COLD-PCR (MX-ICP)

Introduction

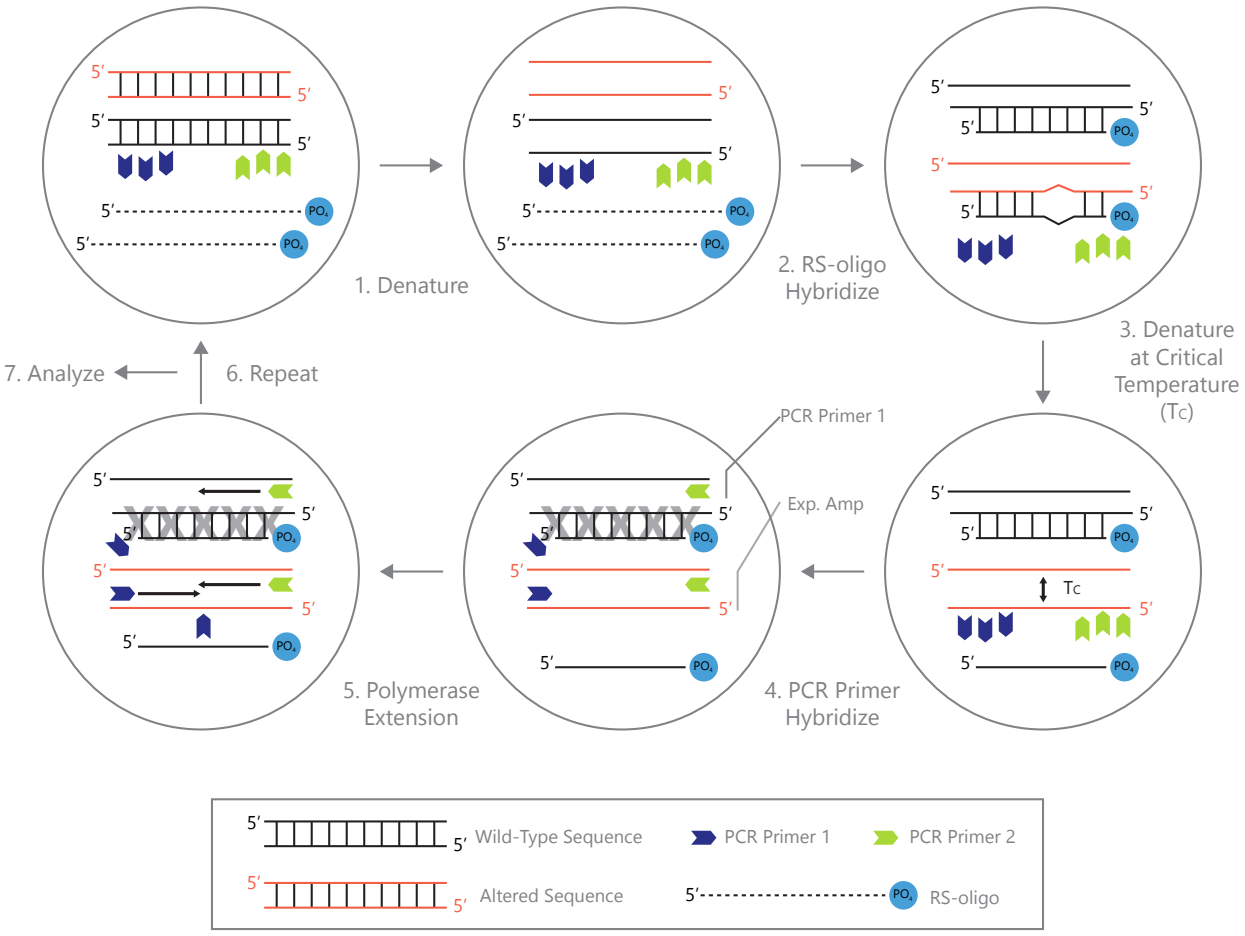
KRAS Exons 2, 3 and 4; NRAS Exons 2, 3 and 4; BRAF Exons 11 and 15; PIK3CA Exons 9 and 20; and EGFR Exons 12, 18, 19, 20 and 21 are regions of oncogenes that have been determined to be predictive and/or prognostic for many types of cancer including colorectal cancer (CRC), melanoma, breast cancer and Non-small Cell Lung Cancer (NSCLC). The ICEme Kit provides an assay for detecting all sequence and small insertion/deletion alterations in these exons.

This kit uses Transgenomic's proprietary primer sets for MX PCR amplification and MX-ICP mutation enrichment.

MX PCR as a pre-amplification step is used to amplify sufficient starting material for the analysis of multiple mutations in multiple genetic regions. The product from this pre-amplification is then used in the MX-ICP assay for enrichment of any alteration present at low levels in the starting material.

General MX-ICP Assay Process

Figure 1. MX-ICP Process Outline



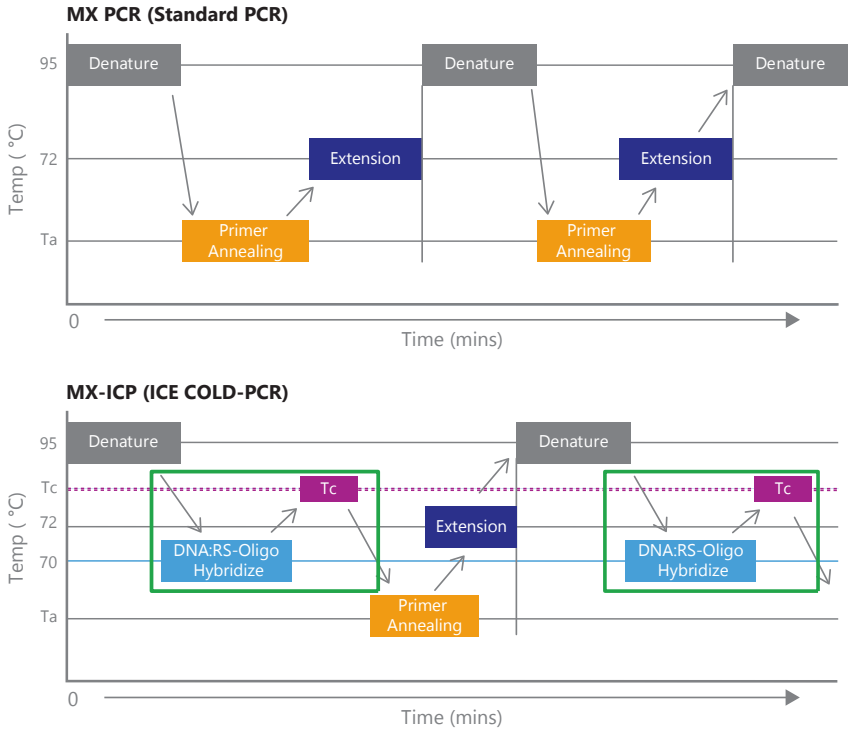
Steps in the ICE COLD-PCR Process

1. Denaturation. The MX-ICP reaction mix contains a Reference Strand oligonucleotide (RS-oligo) that is complementary to one of the Wild-Type amplicon strands. This RS-oligo spans the majority of the amplicon such that all mutations in this region will be enriched. At this step all DNA is denatured to single strands.
2. Hybridize. At the optimum hybridization temperature the RS-oligo, in molar excess of the template, hybridizes to both its complementary Wild-Type and mutant strands. The RS-oligo forms a heteroduplex with the mutant strand.
3. Differentially Denature at Tc. The MX-ICP reaction temperature is raised to a reaction-specific critical temperature (Tc). This Tc value is the temperature at which the majority of the mutant:RS-oligo heteroduplexes will denature and the majority of the Wild-Type:RS-oligo homoduplexes remain bound.
4. Anneal Amplification Primers. The reaction is cooled to allow the MX-ICP primers to anneal. The primers are in excess of the RS-oligo so they will preferentially hybridize to the mutant templates. As the Wild-Type:RS-oligo complexes were not denatured in Step 3 and one of PCR primers overlaps the region covered by the RS-oligo, PCR primers can only anneal to (1) the Wild-Type mutant strand that is not complementary to the RS-oligo and (2) both mutant strands.
5. Extension. DNA Polymerase extends all primer:template complexes. Both mutant strands will be extended resulting in exponential amplification of the mutant sequences. The Wild-Type strand complementary to the RS-oligo will not be extended although the non-complementary Wild-Type strand will be a template for DNA Polymerase; however this results in only linear amplification of this strand.
6. Repeat Steps 1-5. With each cycle mutant strands exponentially amplify while one Wild-Type strand linearly amplifies. The overall percentage of mutant strand increases with each cycle such that the mutant will eventually have a relative concentration high enough to be analyzed by standard sequencing methods.
7. Purify and Analyze. On completion of the MX-ICP thermal cycler program, the products are purified and can be analyzed by any downstream DNA sequencing platform.

Comparison

Two extra steps are added to a 3-step standard PCR thermal cycling program when performing MX-ICP for mutation enrichment.

Figure 2. General comparison of the thermal cycling profiles for MX PCR (standard PCR) and MX-ICP (ICE COLD-PCR)



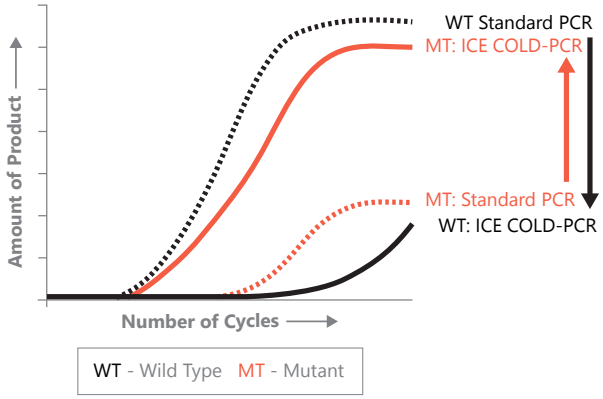


Figure 3. Outline of the enrichment process used in the ICEme Kit

In MX PCR Pre-Amplification the mutant (MT) to Wild-Type (WT) ratio stays the same.

In MX-ICP Enrichment the mutant (MT) to Wild-Type (WT) ratio increases resulting in enrichment of genetic alterations

Analysis of Samples using ICEme Kit

The ICEme Kit should only be used in the context of the workflow indicated in Figure 4.

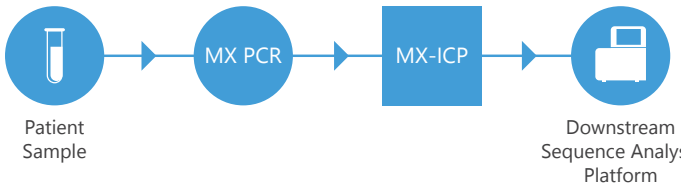


Figure 4. ICEme Kit workflow for Mutation Analysis with any Downstream Sequencing Platform

Analysis of Samples using ICEme Kit

All components necessary for downstream sequencing platforms must be supplied by the laboratory using this kit.

Note:

- Due to the genetic heterogeneity of tumors and the carryover of normal tissue cells associated with tumors, biopsy samples may contain Wild-Type and mutant tumor cells as well as normal cells.
- The Limit of Detection (LOD) of any mutations present in the sample DNA following MX PCR is dependent of the sensitivity of the downstream sequence detection platform.
- Only the DNA Polymerases supplied with this kit should be used for the MX PCR and the MX-ICP portion of assays (these may be different polymerases).
- Please follow the specific instructions for your laboratory's downstream sequence detection platform by consulting the instruction manual.

To use this kit successfully, we strongly recommend that you read the User Guide thoroughly and carefully to follow the instructions and guidelines provided. First time users should perform the control experiments outlined in Positive Control (transgenomic.com/iceme).

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