ICEme™
MX-ICP Mutation Enrichment Kit

Quick Start Guide for the ICEme Kit

This product is for Research Use Only.

Read the ICEme Kit User Guide before using Quick Guide Instructions.

Separate PCR Workstations or equivalent hoods (preferable in separate rooms) should be used for MX PCR and the MX-ICP.

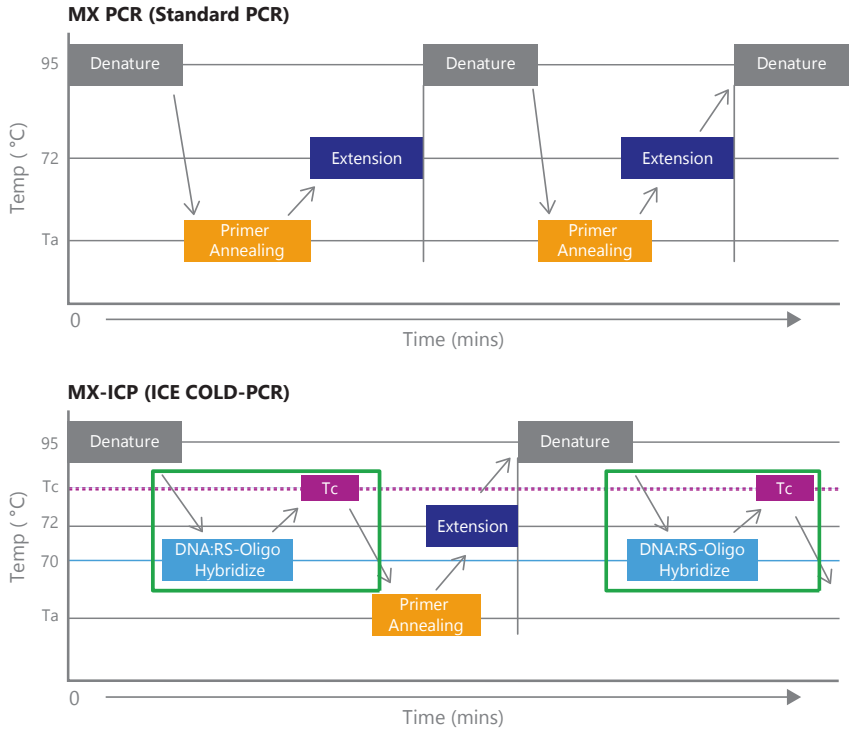
Additional information is available at transgenomic.com/iceme.

Description

Assays are composed of a two-step procedure: MX PCR followed by MX-ICP.

This procedure has only two differences to standard PCR methods: additional temperature stages to (1) allow sample DNA:RS-oligo hybridization and (2) denature mutant strand/RS-oligo heteroduplexes.

Figure 1. General comparison of the thermal cycling profiles for MX PCR (standard PCR) and MX-ICP (ICE COLD-PCR)



MX PCR Instructions

1. Thaw MX PCR Primer Mix, dNTPs, & 5X GC Buffer on ice. Remove DNA Polymerase from freezer. Vortex, centrifuge & place on ice.
2. Prepare MX PCR Master Mix on ice; volumes below for single reaction – multiply as required.
3. Pipette 35 µL aliquots into PCR tubes or 96-well plates
4. Add 15.0 µL template DNA or water for No Template Control, NTC1. Vortex & centrifuge.
5. Optional sample: MX-ICP Positive Control. 10 µL water & 5 µL MX-ICP Positive Control

Table 1. MX PCR Master Mix Guidance for 10 ng/µL DNA samples	
Water	18 µL
5X GC Buffer	10 µL
dNTPs	4 µL
MX PCR Primer Mix	2.5 µL
Phusion® HS II DNA Polymerase	0.5 µL
Total Volume	**35.0 µL**

Thermal Cycler Program

Ramp Rates: C1000: 1.5 °C/sec; Veriti: 38.5%; Tetrad: default (3.0 °C/sec)

Table 2. MX PCR Amplification Thermal Cycler Protocol			
	Cycles	Temp (°C)	Time
Initial Denaturation	1	98 °C	30 sec
Touchdown Amplification	15	98 °C	10 sec
		62 °C, -0.5 °C/cycle	20 sec
		72 °C	20 sec
Amplification	20	98 °C	10 sec
		55 °C	20 sec
		72 °C	20 sec
Final Extension	1	72 °C	5 min
Hold		12 °C	**Hold**

MX PCR products used as template for MX-ICP.

MX-ICP Instructions

Protocol for MX PCR & control products with Qubit values >7 ng/µL, dilute 1:200 in water (except KRAS 4B which would be 1:10 in water).

1. Thaw MX-ICP Primers, dNTPs, RS-oligo & Polymerase Buffer on ice. Remove DNA Polymerase from freezer. Briefly vortex all tubes, centrifuge & place on ice.

2. Prepare MX-ICP Master Mix on ice; volumes below for 1x reaction – multiply as required.

Table 3. MX-ICP Master Mix Preparation

Polymerase Selection	JumpStart™	Phusion (KRAS Exon 2 & BRAF Exon 15 only)
Water	36.0 µL	31.375 µL
Polymerase Buffer	5.0 µL	10.0 µL
dNTPs	4.0 µL	4.0 µL
MX-ICP Primers	1.0 µL	1.0 µL
RS-oligo	2.5 µL	2.5 µL
DNA Polymerase	0.5 µL	0.125 µL
Total Volume	**49.0 µL**	**49.0 µL**

3. Pipette 49 µL Master Mix aliquots into PCR tubes or 96-well plates.
4. To appropriate wells, add 1.0 µL of water to MX-ICP No Template Control, NTC2, 1.0 µL of diluted No Template Control from MX PCR (NTC1) and 1.0 µL of each diluted MX PCR sample.
5. If applicable, add 1.0 µL diluted MX PCR mutation positive control from MX PCR to the appropriate well.
6. Cap strips or tubes. Vortex and centrifuge.

Thermal Cycler Program for MX-ICP Enrichment Protocol

Ramp rates: C1000: 1.5°C/sec; Veriti: 38.5%; Tetrad: default (3.0°C/sec)

Store at ≤12 ºC until sequence analysis.

Table 4a. Thermal Cycler Protocols for MX-ICP

MX-ICP Reaction	Temperature										Time
	KRAS 2	KRAS 3	KRAS 4A	KRAS 4B	NRAS 2	NRAS 3	NRAS 4A	NRAS 4B	**BRAF EX 11**	**BRAF EX 15**	
Initial Denaturing	95 °C										5 min
30 cycles Amplification	95 °C										15 sec
	67 °C										2 min
	75.5 °C	76.4 °C	70.5 °C	70.3 °C	75.5 °C	69.0°C	69.5 °C	70.8°C	69.7°C	69.5°C	30 sec
	62 °C	52 °C	55 °C	55 °C	62 °C	60 °C	61 °C	55°C	55°C	58°C	30 sec
	72 °C										**30 sec**
5 cycles Amplification	95 °C										**15 sec**
	62 °C	**52 °C**	**55 °C**	**55 °C**	**62 °C**	**60 °C**	**61 °C**	**55°C**	**55°C**	**58°C**	**30 sec**
	72 °C										**30 sec**
Final Extension	72 °C										5 min

Table 4b. Thermal Cycler Protocols for MX-ICP

MX-ICP Reaction	Temperature							Time
	EGFR Ex 12	EGFR Ex 18	EGFR Ex 19	EGFR Ex 20	EGFR Ex 21	PIK3CA Ex 9	PIK3CA Ex 20	
Initial Denaturing	95 °C							5 min
30 cycles Amplification	95 °C							15 sec
	67 °C							2 min
	70.3 °C	76.9 °C	70 °C	71.0°C	78.2°C	71.5 °C	69.7 °C	30 sec
	55 °C	55 °C	55 °C	60 °C	55 °C	56 °C	61 °C	30 sec
	72 °C							**30 sec**
5 cycles Amplification	95 °C							**15 sec**
	55 °C	**55 °C**	**55 °C**	**60 °C**	**55°C**	**56 °C**	**61 °C**	**30 sec**
	72 °C							**30 sec**
Final Extension	72 °C							5 min

Quality Statement

ICEme Kit demonstrates preferential preferential enrichment of mutant DNA sequences in excess Wild-Type DNA through selective amplification of mutant DNA using kit control template and reagents.

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Transgenomic, Inc. | 12325 Emmet Street | Omaha, NE 68164 | USA | 888-813-7253 | info@transgenomic.com | transgenomic.com