

PCR PROTOCOL for Mtm1

PCR REACTION:

10X PCR buffer	3ul
MgCL ₂ 25 mM	3ul
dNTP'S 2.5 mM	3ul
Primer Mtm1_F	5 pmol per reaction (3ul)
Primer Mtm1_R	5 pmol per reaction (3ul)
Taq	0,6ul
DNA	3ul
DMSO	3ul (10%)
H ₂ O	Up to volume 30λ (8.4λ)

Mtm1_F: 5'-AGACGGAATGGGAGGTGGT-3'

Mtm1_R: 5'-GGCTTCATTACACTGCTCTTGA-3'

PCR CONDITIONS:

1. 94°C for 5 min.
2. 94°C for 45 sec.
3. 60°C for 30 sec.
4. 72°C for 45 sec.
5. Steps 2-4 for 35 more cycles.
6. 72°C for 5 min.
7. 4°C for infinite

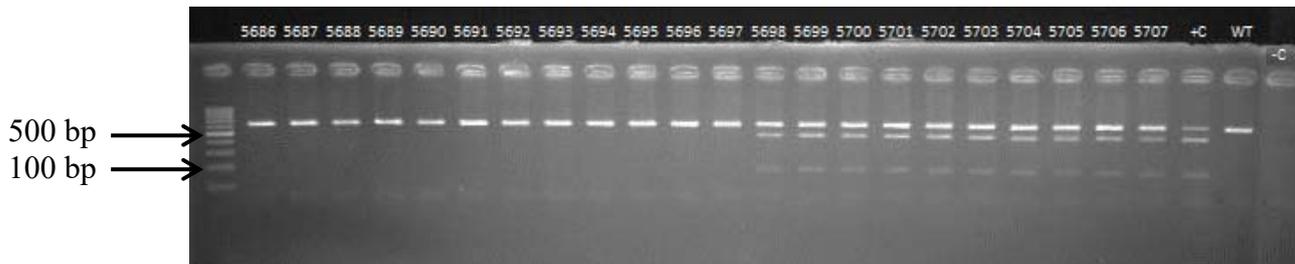
PCR PRODUCT SIZE: 631 bp



DIGESTION REACTION OF PCR PRODUCTS

XhoI enzyme*	0.5 λ
10x CutSmartBuffer	1,5 λ
DNA	5 λ
H ₂ O	Up to volume 15 λ (8 λ)

-37°C for 3h
-65°C for 20min



PRODUCTS:

wt → 631 bp

mut → 461 bp & 170 bp

* XhoI enzyme: Cat# R0146, New England BioLabs.