

MT328 linear heart tubeTAATTG(**G>T**)ATTT(**_>T**)CCAGAAA**PCR**

	name	seq
primer(fw):	MT328_fw	TTCAGGCTATAGGCGCTACC
primer(Rv):	MT328_rv	CAAACGTTGGGTCCAGTTCT
amplicon size: WT: 473 bp		

PCR reaction:

	1X (μl)	20 X
MilliQ	5.75	115.0
5X PCR Buffer	2.0	40.0
25 mM MgCl ₂	1.0	20.0
10 mM dNTPs	0.2	4.0
Primer Fw (10uM)	0.5	10.0
Primer Rv (10uM)	0.5	10.0
KAPA Taq (5U/μl)	0.05	1.0
DNA from lysis	1.0	
Total	11.0	200.0

<-change this number only!
The rest is automatic.

PCR condition:

Step	Temp	Time
1	94 °C	2 min
2	94 °C	15 sec
3	57 °C	15 sec
4	72 °C	30 sec
*go to step 2		total 30 cycles
5	72 °C	30 sec
6	4 °C	∞

ExoSAP-IT Express PCR Cleanup Reagents**ExoSAP-IT Express reaction:**

	1X (μl)	20 X
MilliQ	5.0	100.0
ExoSAP-IT	0.1	2.0
PCR product	5.0	
Total	10.1	102.0

<-change this number only!
The rest is automatic.

ExoSAP-IT Express condition:

Step	Temp	Time
1	37 °C	4 min
2	80 °C	1 min
3	4 °C	∞

Cycle Sequencing

Sequencing reaction:

	1X (μl)	20 X
MilliQ	6.0	120.0
10X Seq Buffer	1.0	20.0
Primer (10uM)	1.0	20.0
Big Dye ver3.1	1.0	20.0
template	1.0	
Total	10.0	180.0

<-change this number only!
The rest is automatic.

Sequencing condition:

Step	Temp	Time
1	96 °C	1 min
2	96 °C	10 sec
3	50 °C	5 sec
4	60 °C	2 min
*go to step 2		total 30 cycles
5	4 °C	∞

*ethanol precipitation → FA 10 μl/well → denature → ABI3130xl run

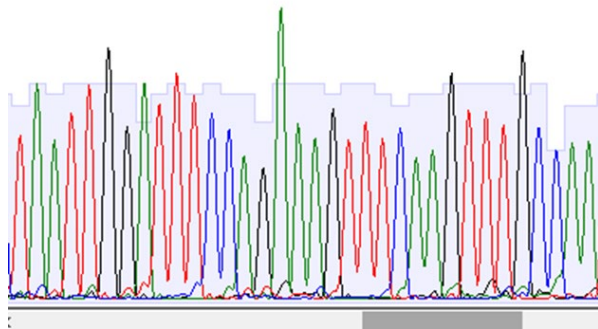
sequencing primer : MT328_fw

WT : TAATTG(G)ATTTCCAG(A)AAGTT(T)CAAGTTTGCCAA

Cab_G12_014.ab1

File Edit Window Help

240 250 260 2
T A A T T G G A T T T C C A G A A A G T T T C A A G T T T G C C A A



MT_hetero : TAATTG(G>T)ATTT(_>T)CCAG(A)AAGT(T)CAAGTTTGCCAA

MT328-het_1_A01_001.ab1

File Edit Window Help

240 250 260
: T A A T T G G A T T T T C C A A A G G T T C C A G T T T G C C A A

