

MAPK10 - RT PCR and MSP

(cell lines)

Nov. → 6. 2010 (46)

primers:

RT-PCR: (for MAPK10) $\left\{ \begin{array}{l} F_3 - F_{11} \\ R_3 - F_{12} \end{array} \right\} 248 \text{ bp}$ GAPDH $\left\{ \begin{array}{l} F_{3334} - GAPDH_{33} \\ R_{335} - 3335 - GAPDH_{55} \end{array} \right\}$

MSP: $m \left\{ \begin{array}{l} m_1 - G_{03} \\ m_2 - G_{04} \end{array} \right\} 160 \text{ bp}$ $U \left\{ \begin{array}{l} U_1 - G_{05} \\ U_2 - G_{06} \end{array} \right\} 163 \text{ bp}$

Samples: ① Hep3B ② HepG2 ③ huiH₁ ④ huiH₄ ⑤ huiH₆
⑥ huiH₇ ⑦ Mahlavu ⑧ PLC/PRF/5 ⑨ SNU387
⑩ SNU398 ⑪ SNU423 ⑫ SNU449 ⑬ SNU475
⑭ normal control: normal liver for RT-PCR
YCC-B₁ for MSP
⑮ negative control (-ve)

RT-PCR

55°C 32 cycles (for GAPDH 23 cycles)

RT-PCR using Promega GoTaq

July 16, 2007/CUHK/Tao

	95°C/2', 32 cycles (95°C/30'', °C/30'', 72°C/30''), 72°C/3'	For 50 µl	For 12.5 µl	x 16	Final concentrat.
1:	5 x Flexi buffer	8 µl	2 µl	32	0.8x
2:	5' primer (10µM)	3 µl	0.75 µl	12	0.6 µM
3:	3' primer (10µM)	3 µl	0.75 µl	12	0.6 µM
4:	Promega GoTaq (5U/µl)	0.25 µl	0.0625 µl	1	1.25U/50 µl
5:	RT product	10 µl	2.5 µl	→ 2.5	about 0.5 µg/50 µl
6:	MgCl ₂ (25 mM)	2 µl	0.5 µl	8	2 mM
7:	dd H ₂ O	23.75 µl	5.9375 µl	95	
	Total volume	50 µl	12.5 µl		

(For GAPDH, use 23 cycles)

MSP

m: 58°C 40 cycles

U: 58°C 40 cycles

MSP for cell lines (using less Taq-Gold/1x) (since July 06')

July 26, 2006, Tao/CEL/CUHK

	95°C/10 min, 40 cycles (94°C/30'', °C/30'', 72°C/30''), 72°C/5 min	For 25 µl reaction	12.5 µl	16	20	Final Conc.
1:	10 x PCR buffer II (PE)	2.5 µl	1.25	20		1x
2:	25mM MgCl ₂ (PE)	2 µl	1	16		2 mM
3:	dNTP (2.5mM each)	2 µl	1	16		0.2 mM
4:	5' primer (10µM)	1.5 µl	0.75	12		0.6 µM
5:	3' primer (10µM)	1.5 µl	0.75	12		0.6 µM
6:	Taq-Gold (5U/µl) (PE)	0.125 µl	0.0625	1		1.25U / 50µl (1x)
7:	dd H ₂ O	14.375 µl	7.1875	115		
8:	*Bisulfited DNA*	1 µl	0.5	→ 0.5		0.1 µg / 100µl
	Total:	25 µl				

*(For DNA samples bisulfited before 3/2003', use 0.5ul/25ul; for DNA samples bisulfited after 3/2003', use 1ul/25ul)

28 Gel

① 150 V 1 min

② 130 V 18 min