

MAPK10 — RT PCR and MSP 24/11/2010 (45)

primers:

RT-PCR: $\left\{ \begin{array}{l} F3 - F11 \\ R3 - F12 \end{array} \right.$ $\left\{ \begin{array}{l} GAPDH33 - 3334 \\ GAPDH55 - 3335 \end{array} \right.$

MSP: $\left\{ \begin{array}{l} m_1 - G03 \\ m_2 - G04 \end{array} \right.$ $\left\{ \begin{array}{l} U_1 - G05 \\ U_2 - G06 \end{array} \right.$

samples:

- ① Hep3B
- ② Hep3B (A+T)
- ③ HepG2
- ④ HepG2 (A+T)
- ⑤ SNU387
- ⑥ SNU387 (A+T)
- ⑦ positive (normal liver for RT PCR. Ycc-B₁ for msp)
- ⑧ -ve

RT-PCR:

55°C 32 cycles (GAPDH: 55°C 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 | | Final concentrat. |
|----|---|--------------|----------------|-------|--------------------|
| | | | | x 8 | |
| 1: | 5 x Flexi buffer | 8 µl | 2 µl | 16 | 0.8x |
| 2: | 5' primer (10µM) | 3 µl | 0.75 µl | 6 | 0.6 µM |
| 3: | 3' primer (10µM) | 3 µl | 0.75 µl | 6 | 0.6 µM |
| 4: | Promega GoTaq (5U/µl) | 0.25 µl | 0.0625 µl | 0.5 | 1.25U/50 µl |
| 5: | RT product | 10 µl | 2.5 µl | → 0.5 | about 0.5 µg/50 µl |
| 6: | MgCl ₂ (25 mM) | 2 µl | 0.5 µl | 4 | 2 mM |
| 7: | dd H ₂ O | 23.75 µl | 5.9375 µl | 47.5 | |
| | Total volume | 50 µl | 12.5 µl | | |

MSP: m: 60°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 | x 8 | Final Conc. |
|----|---|-----------------------|---------|-------|-------------------|
| 1: | 10 x PCR buffer II (PE) | 2.5 µl | 1.25 | 10 | 1x |
| 2: | 25mM MgCl ₂ (PE) | 2 µl | 1 | 8 | 2 mM |
| 3: | dNTP (2.5mM each) | 2 µl | 1 | 8 | 0.2 mM |
| 4: | 5' primer (10µM) | 1.5 µl | 0.75 | 6 | 0.6 µM |
| 5: | 3' primer (10µM) | 1.5 µl | 0.75 | 6 | 0.6 µM |
| 6: | Taq-Gold (5U/µl) (PE) | 0.125 µl | 0.0625 | 0.5 | 1.25U / 50µl (1x) |
| 7: | dd H ₂ O | 14.375 µl | 7.1875 | 57.5 | |
| 8: | *Bisulfited DNA* | 1 µl | 0.5 | → 0.5 | 0.1µg/100µl |
| | Total: | 25 µl | | | |