

## **Methylation Specific PCR**

For each 25ul reaction :

10x PCR buffer	2.5ul
25mM dNTPs	1.5ul
20uM primer (S)	1.25ul
20uM primer (AS)	1.25ul
H <sub>2</sub> O	15.5ul

Make up master mix of stock PCR reaction mix as above

Aliquot 22ul per sample

Add 1ul of bisulfite-treated DNA\* (see companion protocol)

Cover each sample with 2 drops of mineral oil.

MSP PCR program (MSP 1)

Hot start	95'C	5 mins
Hold	80'C	

Add 2ul of 0.25U/ul of Taq polymerase (1:20 dilution of 5U/ul)

Continue program-

95'C	30 secs
58'C	30 secs (manipulate as necessary for specific primer sets)
<u>72'C</u>	<u>30 secs</u>
X	35 cycles

Final extension	72'C	4 mins
Hold	4'C	

Run 10ul of sample on 6% acrylamide/1xTBE gel, for 1 hour at 180 V

Stain with ethidium bromide, and photo.

### **10x PCR buffer**

		<u>Final concentration (10x)</u>
1M (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	16.6 ml	166mM
2M Tris pH 8.8	33.5ml	670mM
1M MgCl <sub>2</sub>	6.7ml	67uM
14.4M mercaptoethanol	0.7ml	100mM