

Bisulfite Sequencing

1) PCR

For each 25ul PCR reaction :

10x PCR buffer (below)	2.5ul
25mM dNTPs	1.5ul
20uM primer (S)	1.25ul
20uM primer (AS)	1.25ul
H ₂ 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.

2) Cloning

Take 1ul of PCR product and clone using TOPO-TA cloning kit (Invitrogen) according to the manufacturers directions.

Plate out bacteria on LB/AMP plates - you can use blue white selection to increase chances of picking colonies with inserts (optional) - grow O/N at 37°C

Pick ~10-12 colonies per plate, inoculate 3ml LB/AMP cultures, grow O/N at 37°C

Use your favorite miniprep protocol to isolate plasmid DNAs (whatever works for you - you will be sequencing down the line)

Digest minipreps with EcoRI and run on 1% agarose/1x TAE gel to check for inserts

Pick 8-10 minipreps for sequencing

10x PCR buffer

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