PCR amplification of *dsr*AB genes

The polymerase chain reaction (PCR) is an *in vitro* method for enzymatically Synthesizing defined sequences of DNA. The reaction uses two oligonucleotide primers that hybridize to opposite strands and flank the target DNA sequence that is to be amplified. The elongation of the primers that is catalyzed by *Taq* DNA Polymerase, a heat-stable DNA polymerase that is isolated from thermophilic eubacterium *Thermus aquaticus.* A repetitive series of cycles involving three steps: (1) template denaturation, (2) primer annealing, and (3) extension of annealing primers by *Taq* DNA Polymerase results in exponential accumulation of specific DNA fragment. The ends of the fragment are defined by the 5" ends of the primers. Because the primer extension products synthesized in a given cycle can serve as a template in the next cycle, the number of target DNA copies approximately doubles every cycle: thus 20 cycles of PCR yield about a million copies of the target DNA.

Equipment:
Thermocycler
PCR reaction tubes (200 µ l)

Reagents
Taq Polymerase
PCR buffer (10X con.) Primers
(forward and reverse)

Primers specific for dsrAB
Forward: dsr-1F (5' AC[C/G]CACTGGAAGCACG '3)
Reverse :dsr4R (5'GTG TAG CAG TTA CCG CA-3')

Reference:
Leloup J., Quillet L., Oger C., Boust D. and Petit F. (2003) Molecular quantification of sulfate-reducing microorganisms (carrying *dsrAB* genes) by competitive PCR in estuarine sediments. FEMS Microbiol. Ecol. 1605, 1-8.

PCR nucleotide mix (contains dATP, dCTP, dGTP, and dTTP)
$MgCl_2$
BSA (bovine serum albumin)
DNA template (genomic DNA from pure culture; 50 ng μl^{-1})

Protocol

Vortex and briefly centrifuge all reagents *except* BSA and *Taq* Polymerase before beginning the procedure. Keep *Taq* Polymerase at -20°C until needed and return immediately when finsihed.

1. Prepare an amplification mixture (master mix) by adding reagents to a sterile microcentrifuge tube in the following order:

Volume 20 µL Reaction

Reagents	**Volume 1 X reaction**	**Volume 5 X reaction**	**Volume 10 X reaction**
dH_2O	14.5µl	72.5µl	145µl
10X Reaction buffer (100mM Tris,	2µl	10µl	20µl
25 mM $MgCl_2$	1µl	5µl	10µl
10 mM PCR nucleotide mix	0.4µl	2µl	4µl
50 µM F primer	0.1µl	0.5µl	1µl
50 µM R primer	0.1µl	0.5µl	1µl
BSA	0.2µl	1µl	2µl
DMSO	0.3µl	1.5µl	3µl
5U μ^{1-1} *Taq* Polymerase	0.4µl	2µl	4µl
Template DNA	1µl	5µl	10µl
Total *	20µ l	100µ l	200µ l

*Note: PCR Recipe for the 20*µl *reaction*

2. Combine the above components in a sterile microcentrifuge. The reaction volume can be scaled as long as the final concentration remains constant.

3. Vortex the mixture, and centrifuge briefly to collect the sample at the bottom of the tube.

4. Place the PCR tubes d in a thermal cycle

5. Start the thermal cycling program.

Thermal cycling protocol for 16S rDNA:

STEP	**TEMPERATURE**	**TIME**	**NUMBER OF CYCLES**
Hot start	80°C	30 seconds	1 cycle
Initial denaturation	94ºC	2 minutes	1 cycle
Denaturation	94ºC	30 seconds	30 cycles
Annealing	65ºC	1 minute	
Extension	72ºC	30 seconds	
Final extension	72ºC	7 minutes	1 cycle
Soak	4ºC	Indefinite	1 cycle

6. Store PCR products at -20ºC.