

## PCR amplification of *dsrAB* 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  $\mu$  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 :*dsr*4R (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<sub>2</sub>

BSA (bovine serum albumin)

DNA template (genomic DNA from pure culture; 50 ng  $\mu$ l<sup>-1</sup>)

## 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 finished.

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

### Volume 20 $\mu$ L Reaction

Reagents	Volume 1 X reaction	Volume 5 X reaction	Volume 10 X reaction
dH <sub>2</sub> O	14.5 $\mu$ l	72.5 $\mu$ l	145 $\mu$ l
10X Reaction buffer (100mM Tris,	2 $\mu$ l	10 $\mu$ l	20 $\mu$ l
25 mM MgCl <sub>2</sub>	1 $\mu$ l	5 $\mu$ l	10 $\mu$ l
10 mM PCR nucleotide mix	0.4 $\mu$ l	2 $\mu$ l	4 $\mu$ l
50 $\mu$ M F primer	0.1 $\mu$ l	0.5 $\mu$ l	1 $\mu$ l
50 $\mu$ M R primer	0.1 $\mu$ l	0.5 $\mu$ l	1 $\mu$ l
BSA	0.2 $\mu$ l	1 $\mu$ l	2 $\mu$ l
DMSO	0.3 $\mu$ l	1.5 $\mu$ l	3 $\mu$ l
5U $\mu$ <sup>-1</sup> <i>Taq</i> Polymerase	0.4 $\mu$ l	2 $\mu$ l	4 $\mu$ l
Template DNA	1 $\mu$ l	5 $\mu$ l	10 $\mu$ l
*Total	20 $\mu$ l	100 $\mu$ l	200 $\mu$ l

\* Template DNA should be around 20-50 ng/ml

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 dsrAB**

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

6. Store PCR products at -20°C.