

Genes OPRL (spp. Specific , 504 amplicon size) , OPRI (Genus specific , 249 bp):

OprI :	L	C (-ve)	1	2	16	17
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OprL:	L	C (-ve)	1	2	16	17
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Primers for molecular characterization of *P. aeruginosa*

Gene	Reverse Primer Sequence	Forward Primer Sequence	Amplicon size (b.p)
<i>OprI</i>	5'-CTTGCGGCTGGCTTTTTCCAG-3'	5'-ATGAACAACGTTCTGAAATTCTCTGCT-3'	249
<i>OprL</i>	5'-CTTCTTCAGCTCGACGCGACG-3'	5'-ATGGAAATGCTGAAATTCGGC-3'	504

Components of PCR Reaction

S.NO	Components of PCR Reaction	Concentration	Volume
1.	Taq Green PCR Master MIX	2X	12.5 µL
2.	Forward Primer	10 p mole	0.5 µL
3.	Reverse Primer	10 p mole	0.5 µL
4.	DNA	10 pg-1ug	1 µL
5.	Nuclease free Water	-	10.5 µL
Total volume			25 µL

Optimized Polymerase Chain Reaction Parameters

Gene	Initial denaturation		Denaturation		PCR Conditions Annealing		Extension		Final extension	
	T (°C)	T (min)	T (°C)	t (min)	T (°C)	T (min)	T (°C)	t (min)	T (°C)	t (min)
<i>OprI</i>	94	5	94	1	55	1	72	1	72	10
<i>OprL</i>	94	5	94	1	55	1	72	1	72	10