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🌐 Identification of the different phylogroups from the *Klebsiella pneumoniae* species complex by real-time PCR

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Klebsiella Research and ...



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We use this protocol and it's working

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Abstract

KpSC phylogroup ID

We developed and optimized a real-time PCR for the identification of the different phylogroups (Kp1 to Kp6) belonging to *Klebsiella pneumoniae* species complex (KpSC) by amplification of five specific gene targets.

Primers and Probes

1

The **KpSC phylogroup ID** : Primers, Probes and Amplicon length

	A	B	C	D	E	F	G
	PhG - Target	Forward (5' – 3')	Reverse (5' – 3')	Probe	Quencher	Reporter	Amplicon length (bp)
	Kp1 - group_8970	CCTCAAA CACGCCA ATATGC	TACCGCG ACGAGTAA AGTGG	GATCCATTG ATTCCATTG GAACCGG	BHQ -1	JOE	85
	Kp2 - group_10724	TTGTTGA TTGGCAG GCCTTC	AAATGCTG TGACCAC CGTTG	CGCCTGGC GTCAGTGGC CCCGAGC	BHQ -1	JOE	70
	Kp3/Kp5 - tetR	GCGGGC CGGCTTT TC	CGCATCC CAGGGTAT ATTCG	AGGCCGAA GCCGCAAT GATATTACT CA	BHQ -1	FAM	71
	Kp4 - nanK	AAACAGC GGATGCT CTGAAC	ATGAGTCC GCCAAGA TTCTG	ATAGCCTCG ACCGGGATT ATTCTGTA	BHQ -2	TAMRA	145
	Kp6 - mdtG_1	GCGTTTC AATGATC CTGTCC	ATCTGCGT CTGGAAA ACAG	GTGCGCTG ATTGGTATT GGATATGGC	BHQ -2	TAMRA	76

PCR mix preparation

2 PCR mix preparation for each set of primers and probe

	A	B
	Mix Reagents	Volume per well (µL)



	A	B
	Takyon ROX Probe MasterMix dTTP blue	12.5
	Forward (500 nM)	1
	Reverse (500 nM)	1
	Probe (250 nM)	0.5
	DNA template	2.5
	PCR grade water	2.5

Master Mix: TakyonTM ROX Probe MasterMix dTTP blue(Ref.UF-RPMT-B0701, Eurogentec, Belgium)

Amplification program

3

	A	B	C
		Temperature (°C)	Time (minutes:seconds)
	Holding stage	95.0	03:00
	Cycling stage (x40)	95.0	00:15
	60.0	01:00	

Thermocycler: ABI StepOne Real-Time PCR system (Thermo Fischer Scientific)

Positive and negative controls

4

Negative control: PCR grade water

Positive controls:

- *Kp1* DNA (*Klebsiella pneumoniae* ATCC 13883^T) at concentration 2 ng/μl ; 2.5 μl used *i.e.*, 5 ng.



- Kp2 DNA (*Klebsiella quasipneumoniae* subsp. *quasipneumoniae* 01A030^T)
- Kp3 or Kp5 DNA (*Klebsiella variicola* subsp. *variicola* F2R9^T; *Klebsiella variicola* subsp. *tropica* 1266^T)
- Kp4 DNA (*Klebsiella pneumoniae* 07A044^T)
- Kp6 DNA (*Klebsiella quasivariicola* KPN1705^T)