

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

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Written by: Elodie Barbier, Pôle BiomE, INRA, Dijon; Carla Rodrigues, BEBP, Institut Pasteur, Paris

Validated by: Pascal Piveteau, Pôle BiomE, INRAE, Rennes; Sylvain Brisse, BEBP, Institut Pasteur, Paris

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.

Name of PCR: **KpSC phylogroup ID**

## 1. Primers, probes and amplicon length

PhG -Target	Forward (5' – 3')	Reverse (5' – 3')	Probe	Quencher	Reporter	Amplicon length (bp)
<b>Kp1 - group_8970</b>	CCTCAAAACACGC	TACCGCGACGAGT	GATCCATTGATTCCA	BHQ-1	JOE	85
	CAATATGC	AAAGTGG	TTCGAACCGG			
<b>Kp2 - group_10724</b>	TTGTTGATTGGC	AAATGCTGTGACC	CGCCTGGCGTCAGTG	BHQ-1	JOE	70
	AGGCCTTC	ACCGTTG	GCCCCGAGC			
<b>Kp3/Kp5 - tetR</b>	GCGGGCCGGCTT	CGCATCCCAGGGT	AGGCCGAAGCCGCAA	BHQ-1	FAM	71
	TTC	ATATTCTG	TGATATTACTCA			
<b>Kp4 - nanK</b>	AAACAGCGGATG	ATGAGTCCGCCAA	ATAGCCTCGACCGGG	BHQ-2	TAMRA	145
	CTCTGAAC	GATTCTG	ATTATTCGTGA			
<b>Kp6 - mdtG_1</b>	GCGTTTCAATGA	ATCTGCGTCTGGA	GTGCGCTGATTGGTA	BHQ-2	TAMRA	76
	TCCTGTCC	AAACAG	TTGGATATGGC			

## 2. PCR mix

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

Mix Reagents	Volume per well (µL)
Takyon™ Rox PROBE MasterMix 2x	12.5
Forward (500 nM)	1
Reverse (500 nM)	1
Probe (250 nM)	0.5
DNA template	2.5
PCR grade water	2.5

### 3. Amplification programme

	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 thermocycler (Thermo Fischer Scientific)

**DNA template:** For environmental matrixes such as soil, 500 µl of 24h-enrichments (e.g., in LB with 10 mg/L ampicillin) are centrifuged (5 min., 5 800 x g) and washed with sterile water before boiling for 10 min. Boiled suspensions are then diluted 1/10 and 1/100 for qPCR.

#### **Positive and negative controls:**

Negative control: PCR grade water

Positive controls:

- Kp1 DNA (*Klebsiella pneumoniae* ATCC 13883<sup>T</sup>) at concentration 2 ng/µl ; 2.5 µl used *i.e.*, 5 ng.
- Kp2 DNA (*Klebsiella quasipneumoniae* subsp. *quasipneumoniae* 01A030<sup>T</sup>)
- Kp3 or Kp5 DNA (*Klebsiella variicola* subsp. *variicola* F2R9<sup>T</sup>; *Klebsiella variicola* subsp. *tropica* 1266<sup>T</sup>)
- Kp4 DNA (*Klebsiella quasipneumoniae* subsp. *similipneumoniae* 07A044<sup>T</sup>)
- Kp6 DNA ('*Klebsiella quasivariicola*' KPN1705<sup>T</sup>)