

PCR conditions for amplifying *Pneumocystis* mtLSUrRNA gene:

Name	Sequence (5'-3')	Sense	Reference
pH207	ACA AAT CGG ACT AGG ATA TAG CTG GT	Forward	Chabé <i>et al.</i> Med Mycol, 2014
pAZ102-E	GAT GGC TGT TTC CAA GCC CA	Reverse	Wakefield <i>et al.</i> Lancet, 1990

Program: 95°C 5 min, 45 cycles of (94°C 15 s, 57°C 15 s, 72°C 25 s), 4°C for ever

	Final concentration
10 X PCR Buffer (containing 15mM MgCl ₂)	1 X
dNTP mix	250 µM of each
MgCl ₂	2 mM
Forward primer pH207	400 nM
Reverse primerse pAZ102-E	400 nM
HotStar®Taq Plus DNA Polymerase (Qiagen, France)	2.5 U
DNA (<50 ng per reaction)	2 µl
H ₂ O	50 µl final volume

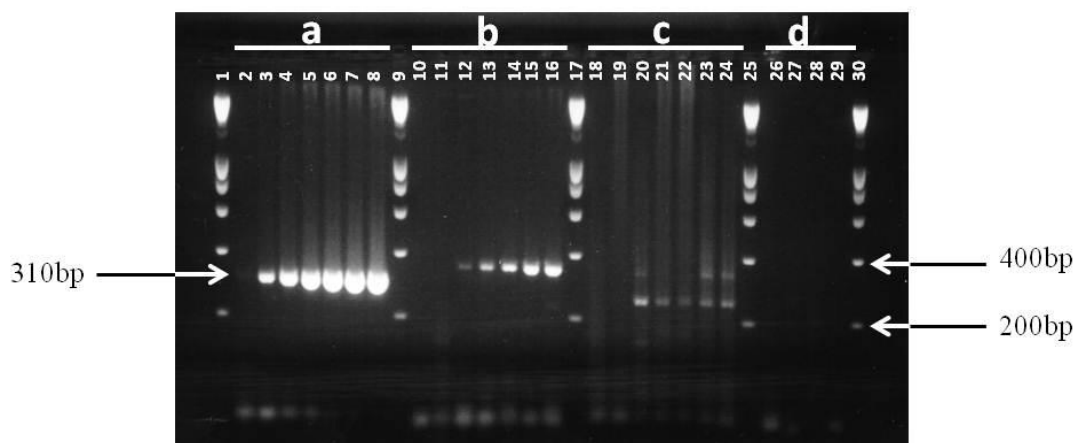


Fig.1. Ethidium bromide fluorescence image showing electrophoresis of *Pneumocystis carinii* mtLSUrRNA PCR amplification products obtained by single-round new PCR (a) and by our previous in-house single-round (b) and nested PCR (c). Lanes 1, 9, 17, 25 and 30, molecular weight ladder. Lanes 2-8 (a), 10-fold serial dilutions of *P. carinii* mtLSUrRNA plasmid DNA ($2 \cdot 10^0$ to $2 \cdot 10^6$ copies, respectively) in 25 ng of rat lung DNA amplified with new PCR protocol. Lanes 10-16 (b), 10-fold serial dilutions of *P. carinii* mtLSUrRNA plasmid DNA ($2 \cdot 10^0$ to $2 \cdot 10^6$ copies, respectively) in 25 ng of rat DNA amplified with an in-house first-round PCR. Lanes 18-24 (c), mtLSUrRNA PCR products of in-house first-round PCR (corresponding to lanes 10-16) amplified with a previous in-house nested PCR. Lanes 26, 27, 28 and 29 (d): negative controls for single and nested PCRs. (from Chabé *et al.*, 2014).