

Table S2: PCR primers and protocols

## multiplex real-time PCR

<b>OspA(Outer membrane Protein A)</b>	± 139bp	
F-primer :	5'-AAT ATT TAT TGG GAA TAG GTC TAA-3'	reference: Heylen, D.J.A.; Tijssse, E.; Fonville, M.; Matthysen, E.; Sprong, H. Transmission dynamics of <i>Borrelia burgdorferi</i> s.l. in a bird tick community. <i>Environ. Microbiol</i> <b>2013</b> , <i>15</i> , 663–673.
R-primer:	5'-CTT TGT CTT TTT CTT TRC TTA CA-3'	
Probe (Atto520):	5'- <b>Atto520</b> -AAG CAA AAT GTT AGC AGC CTT GA- <b>BHQ1</b> -3'	
<b>FlaB(Flagelin B)</b>	± 89bp	
F-primer :	5'-CAG AIA GAG GTT CTA TAC AIA TTG AIA TAG A-3'	reference: Heylen, D.J.A.; Tijssse, E.; Fonville, M.; Matthysen, E.; Sprong, H. Transmission dynamics of <i>Borrelia burgdorferi</i> s.l. in a bird tick community. <i>Environ. Microbiol</i> <b>2013</b> , <i>15</i> , 663–673.
R-primer2:	5'-GTG CAT TTG GTT AIA TTG CGC-3'	
R-primer:	5'-GTG CAT TTG GTT AIA TTG TGC-3'	
Probe (Atto425):	5'- <b>Atto425</b> -CAA CTI ACA GAI GAA AXT AAI AGA ATT GCT GAI CA- <b>Pho</b> -3'	
	X = BHQ-1-dT	
<b>FlaB B. miyamotoi</b>	± 155bp	reference: Hovius, J.W.R.; de Wever, B.; Sohne, M.; Brouwer, M.C.; Coumou, J.; Wagemakers, A.; Oei, A.; Knol, H.; Narasimhan, S.; Hodiamont, C.J.; Jahfari, S.; Pals, S.T.; Horlings, H.M.; Fikrig, E.; Sprong, H.; van Oers, M.H.J. A case of meningoencephalitis by the relapsing fever spirochaete <i>Borrelia miyamotoi</i> in Europe. <i>Lancet</i> <b>2013</b> , <i>382</i> , 658
F-primer :	5'-AGA AGG TGC TCA AGC AG-3'	
R-primer:	5'-TCG ATC TTT GAA AGT GAC ATA T-3'	
Probe (Atto647N):	5'- <b>ATTO647N</b> -AGC ACA ACA GGA GGG AGT TCA AGC- <b>BHQ2</b> -3'	

## IGS conventional PCR

<b>IGS primers</b>	± 390-420bp	
F-primer :	5'-GAGTTCGCGGGAGAGTAGGTTATTGCC-3'	reference: Heylen, D.J.A.; Tijssse, E.; Fonville, M.; Matthysen, E.; Sprong, H. Transmission dynamics of <i>Borrelia burgdorferi</i> s.l. in a bird tick community. <i>Environ. Microbiol</i> <b>2013</b> , <i>15</i> , 663–673.
R-primer:	5'-TCAGGGTACTTAGATGGTTCACTTCC-3'	
<b>IGS sequencing - short primers</b>		
F-primer :	5'-GCGGGAGAGGTTATTGCC-3'	
R-primer:	5'-GTACTTAGATGGTTCACTTCC-3'	

<b>IGS PCR protocol</b>	temperature (°C)	Time (hh:mm:ss)	Cycles
Taq activation	94	0:15:00	1
Denaturation	94	0:00:20	10
Annealing	70	0:00:30	(lowering 1°C per cycle)
Elongation	72	0:00:30	
Denaturation	94	0:00:20	40
Annealing	60	0:00:30	
Elongation	72	0:00:30	
Extension	72	0:07:00	1
Hold	12	forever	

## FlaB conventional PCR

<b>FlaB primers</b>	± 497 bp	
F-primer :	5'-AARGAATTGGCAGTTCAATC-3'	reference: Clark, K.; Hendricks, A.; Burge, D. Molecular identification and analysis of <i>Borrelia burgdorferi</i> sensu lato in lizards in the southeastern United States. <i>Appl Environ Microbiol</i> . <b>2005</b> , <i>71</i> , 2616-2625
R-primer:	5'-GCATTTCWATTTTAGCAAGTGATG-3'	

<b>FlaB PCR protocol</b>	temperature (°C)	Time (hh:mm:ss)	Cycles
Taq activation	96	0:05:00	1
Denaturation	95	0:00:30	30
Annealing	52	0:00:30	
Elongation	72	0:01:00	
Extension	72	0:10:00	1
Hold	12	forever	

## ospC conventional nested PCR

<b>ospC outer primers</b>	± 657 bp	
F-primer :	5'-ATGAAAAAGAATACATTAAGTGC-3'	reference: Bunikis, J.; Garpmo, U.; Tsao, J.; Berglund, J.; Fish, D.; Barbour, AG. Sequence typing reveals extensive strain diversity of the Lyme borreliosis agents <i>Borrelia burgdorferi</i> in North America and <i>Borrelia afzelii</i> in Europe. <i>Microbiology</i> <b>2004</b> , <i>150</i> , 1741–1755
R-primer:	5'-ATTAATCTTATAATATTGATTTTAAATGAAG-3'	
<b>ospC inner primers</b>	± 617 bp	
F-primer :	5'-TATTAATGACTTTATTTTATTTATATCT-3'	
R-primer:	5'-TTGATTTTAATTAAGGTTTTTTTGG-3'	

<b>ospC 1st PCR protocol</b>	temperature (°C)	Time (hh:mm:ss)	Cycles
Taq activation	95	0:05:00	1
Denaturation	94	0:00:30	35
Annealing	52	0:00:30	
Elongation	74	0:00:30	
Extension	72	0:10:00	1
Hold	12	forever	
<b>ospC nested PCR protocol</b>	temperature (°C)	Time (hh:mm:ss)	Cycles
Taq activation	95	0:05:00	1
Denaturation	94	0:00:30	25
Annealing	52	0:00:30	
Elongation	74	0:00:30	
Extension	72	0:10:00	1
Hold	12	forever	

<b>ospA species specific PCR</b>		
<b>G1 primers set</b>	± 543 bp	reference: Demaerschalck, I.; Ben Messaoud, A.; De Kesel, M.; Hoyois, B.; Lobet, Y.; Hoet, P.; Bigaignon, G.; Bollen, A.; Godfroid, E. Simultaneous presence of different <i>Borrelia burgdorferi</i> genospecies in biological fluids of Lyme disease patients. <i>J Clin Microbiol</i> . <b>1995</b> , <i>33</i> , 602–608.
F-primer :	5'-AACAAAGACGGCAAGTACGATCTAATT-3'	
R-primer:	5'-TTACAGTAATTGTAAAGTTGAAGTGCC-3'	
<b>GII primers set</b>	± 344 bp	
F-primer :	5'-TGATAAAACAACGGTCTGGAAC-3'	
R-primer:	5'-GTAACITTCATGTTGTTGTTGCCG-3'	
<b>GIII primers set</b>	± 189 bp	
F-primer :	5'-TAAAGACAAAACATCAACAGATGAAATG-3'	
R-primer:	5'-TTCCAATGTTACTTTATCATTAGCTACTT-3'	

<b>ospA PCR protocol</b>	temperature (°C)	Time (hh:mm:ss)	Cycles
Taq activation	95	0:05:00	1
Denaturation	95	0:00:30	35
Annealing	55	0:00:30	
Elongation	72	0:01:00	
Extension	72	0:10:00	1
Hold	12	forever	