

TITLE:

TickPath Layerplex: adaptation of a real-time PCR methodology for the simultaneous detection and molecular surveillance of tick-borne pathogens

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(C) Organism	qPCR	Bp.flaB.406F (5' → 3')	Bp.flaB.443P (5' → 3')	Bp.flaB.522R (5' → 3')
<i>B. parkeri</i>	+	T T G T C C A A T A A G T C A G C T G C T C A G	C T G A A G A G C T T G G A A T G C A A C C T G C A	T C T T A A T G T C C A T G A A G C T T G T G C
<i>B. turicatae</i>	-C.....AA
<i>B. hermsii</i> GGI	-C.....AC.....A
<i>B. hermsii</i> GGII	-C.....AC.....A
<i>B. anserina</i>	-T.....C.....AG.....A
<i>B. coriacea</i>	-T.....A.....G.....C.....A
<i>B. miyamotoi</i>	-A.....T.....AC.....A
<i>B. recurrentis</i>	-A.....T.....G A.....T.....G.....AT.....A
<i>B. crocidurae</i>	-A.....T.....G A.....T.....G.....AT.....A
<i>B. burgdorferi</i>	-A.....A.....C.....A.....T.....T.....AG.....A.....A.....C.....A.....A
<i>B. afzelii</i>	-A.....A.....C.....A.....T.....T.....C.....AG.....A.....A.....A.....A.....A
<i>B. garinii</i>	-A.....C.....A.....T.....T.....C.....AA.....G.....A.....A.....A.....A

(D) Organism	qPCR	Bt.bipA.728F / Bt.bipA.731F (5' → 3')	Bt.bipA.817P (5' → 3')	Bt.bipA.869R (5' → 3')
<i>B. turicatae</i> 1	+	A G A C C G G T A C A C A G G A T T C T A A A G C	A G T T T T G G G A A G T G T T G T T G G T G G C G T	G T T C C T G C T C C C T G A A T A A C A T T A T C
<i>B. turicatae</i> 2	+C C G G C A C A C A G G A T T C T A A A A C
<i>B. parkeri</i>	-T A A A.....AA.....A
<i>B. hermsii</i> GGI	-T T C.....C.....G T C.....T.....G.....A.....A G T.....AA.....G A.....T A T G.....A C.....A G A A.....G A.....C T.....T G C.....G.....G G.....C.....T
<i>B. hermsii</i> GGII	-T T T.....C.....G T T T T.....G.....A.....A G T.....AA.....G A.....M A T G.....A C.....A G A A.....G A CC.....C T.....C.....G.....G G C C.....T
<i>B. miyamotoi</i>	-
<i>B. coriacea</i>	-
<i>B. anserina</i>	-
<i>B. crocidurae</i>	-
<i>B. recurrentis</i>	-
<i>B. burgdorferi</i>	-
<i>B. afzelii</i>	-
<i>B. garinii</i>	-

(E) Organism	qPCR	Ee.16S.40F (5' → 3')	Ehr-spp.16S.83P (5' → 3')	Ech-ec.16S.174R (5' → 3')
<i>E. ewingii</i>	+	C G A A C G A A C A A T T C C T A A A T A G T C T C T	A G A T T C C T A C G C A T T A C T C A C C C G T C T G C	C C A T C A T T T C T A A T G G C T A T T C C A T A C T
<i>E. canis</i>	-G.....A T.....T.....T A G C C T C T GT.....C.....G.....
<i>E. muris</i>	-G.....T.....G C.....A.....C C.....T A G C.....T.....T
<i>E. ruminantium</i>	-G.....G.....A T.....T.....T A G C.....T C G GT.....A.....
<i>E. chaffeensis</i>	-G.....G.....T.....T.....T A.....C C T.....T
<i>A. phagocytophilum</i>	-G.....T T.....T.....T.....T A G C.....T G.....T.....C.....G.....C.....
<i>A. marginale</i>	-G.....C G.....A T A C G C A G C.....T G.....T.....C.....G.....C.....
<i>A. ovis</i>	-G.....C G.....A.....G C G C A G C.....T G.....T.....C.....G.....C.....T.....
<i>A. centrale</i>	-G.....C.....A.....G C G C A G C.....T G.....T.....C.....G.....C.....T.....
<i>A. bovis</i>	-T.....C.....T A G C.....W G.....T.....C.....C.....G.....C.....T.....
<i>R. rickettsii</i>	-A.....G.....A C G C.....A T C G G T A T.....C T.....A AC.....T G.....T T.....T.....A A.....T.....G.....
<i>R. typhi</i>	-A G.....A C G.....A C G C.....A T C G G T A T.....C T.....A AC.....T G.....T T.....T.....A A.....T.....G.....

(F) Organism	qPCR	Ech.16S.64F (5' → 3')	Ehr-spp.16S.83P (5' → 3')	Ech-ec.16S.174R (5' → 3')
<i>E. chaffeensis</i>	+	G A A C G G A C A A T T G C T T A T A A C C T T T T	A G A T T C C T A C G C A T T A C T C A C C C G T C T G C	C C A T C A T T T C T A A T G G C T A T T C C A T A C T
<i>E. canis</i>	-A T.....G.....C.....GT.....C.....G.....
<i>E. muris</i>	-T.....G C.....A.....C C.....G.....T.....
<i>E. ruminantium</i>	-G.....A T.....T.....G.....T.....C G GT.....A.....
<i>E. ewingii</i>	-A.....C.....A.....A T.....G T C.....C
<i>A. phagocytophilum</i>	-T T.....C T.....G.....T.....G C.....T.....C.....G.....C.....
<i>A. marginale</i>	-C G.....A T A C G C.....G.....T.....G C.....T.....C.....G.....C.....
<i>A. ovis</i>	-C G.....A.....G C G C.....G.....T.....G C.....T.....C.....G.....C.....T.....
<i>A. centrale</i>	-C.....A.....G C G C.....G.....T.....G C.....T.....C.....G.....C.....T.....
<i>A. bovis</i>	-T.....C.....C.....G.....W.....G C.....T.....C.....C.....G.....C.....T.....
<i>R. rickettsii</i>	-A.....A C G C.....A T C G G.....T G.....A AC.....T G.....T T.....T.....A A.....T.....G.....
<i>R. typhi</i>	-A G.....A C.....A C G C.....A T C G G.....T G.....A AC.....T G.....T T.....T.....A A.....T.....G.....

(J) Organism	qPCR	Babsp.18S.65-1F / Babsp.18S.67-2F (5' → 3')	Babsp.18S.228P (5' → 3')	Babsp.18S.289R (5' → 3')
<i>B. canis vogeli</i>	+	C G C A - - - T T T A - - - G C G A T G G A C C A	C A T C A G C T T - - G A C G G T A G G G T A T T G G C C	C C T A A T T C C C C G T T A C C C G T T
<i>B. canis rossii</i>	+	G C - - - T T T T A - - - G C G A T G G A C C A T T C A		
<i>B. canis canis</i>	+			
<i>B. gibsoni</i>	+			
<i>B. caballi</i>	+			
<i>B. odocollei</i>	+			
<i>B. divergens</i>	+			
<i>B. bigemina</i>	+			
<i>B. bovis</i>	-			
<i>B. microti</i>	-			
<i>B. duncani</i>	-			
<i>B. conradae</i>	-			
<i>T. equi</i>	-			
<i>C. felis</i>	-			