

## Additional file 1.

Table: melting temperatures for amplicons obtained from questing ticks DNA extracted using the KingFisher Cell and Tissue kit. **T<sub>m</sub>1**. melting temperature peak 1; **T<sub>m</sub>2**. melting temperature peak 2 (if present). In bold are newly added hosts species for the primer set, identified by sequencing because of deviating melting pattern.

Host-group primer set	Amplified host species	<b>T<sub>m</sub>1</b>			<b>T<sub>m</sub>2</b>		
		min	max	mean	min	max	mean
<b>Canidae</b>	<i>C. l. familiaris</i>	80.86	81.56	81.4	-	-	-
	<i>V. vulpes</i>	81.34	82.04	81.68	83.54	84.2	83.74
<b>Cervidae</b>	<i>C. capreolus</i>	81.36	81.76	81.59	83	83.5	83.21
	<i>C. elaphus</i>	81.5	82.44	81.86	83.74	84.2	84
	<b><i>Ovis</i> sp.</b>	81.96	82.16	82.05	83.4	83.9	83.75
	<b><i>D. dama</i></b>	na	na	81.66	na	na	83.64
	<b><i>R. rupicapra</i></b>	81.8	81.9	81.85	83.5	83.64	83.57
	<b><i>T. merula</i></b>	84.2	85.34	84.9	-	-	-
	<i>T. philomelus/ E. rubecula</i>	84.00	84.50	84.24	-	-	-
	<b><i>Turdus</i> spp.</b>	84.10	84.24	84.17	-	-	-
<b>Soricidae</b>	<i>Sorex</i> sp.	81.16 (80.5*)	81.44	81.32°	-	-	-
	<i>C. leucodon</i>	na	na	82.70	-	-	-
	<i>C. suaveolens</i>	81.10	81.20	81.15	-	-	-
	<b><i>Apodemus</i> sp.<sup>#</sup></b>	82.2	82.84	82.37	-	-	-
	<b><i>C. griseus</i></b>	na	na	82.40			
	<b><i>O. aries</i></b>	na	na	77	na	na	82.9
	<b><i>R. rupicapra</i></b>	na	na	77.1	na	na	81.1
	<b><i>C. hircus</i></b>	77.9	78	77.95	-80.86	-80.9	80.99

\* a single amplicon showed this low T<sub>m</sub>.

° the lowest Tm was not used to compute the mean T<sub>m</sub>.

<sup>#</sup> deviating melting temperature for *Apodemus* amplicons with deletions and mutated positions are not included in that calculation of T<sub>m</sub> (see Additional file 3).