

**S1 Table. *In silico* performance of bat-specific DNA mini-barcode primer pairs against all publically accessible Chiroptera COI barcode sequences.** In order to compare the potential of primers developed in current study to those previously utilized, we harvested sequences from the Barcode of Life Data Systems ([www.barcodinglife.org](http://www.barcodinglife.org)) without applying the strict taxonomic parameters used for primer design, dereplicated them at full length using USEARCH (derep\_fulllength), and then clustered to 100% similarity (cluster\_fast) to alleviate sequence overrepresentation. All tested primers were run through Primer Prospector's analyze\_primers.py script against all unaligned sequences using default parameters, which allows for some primer mismatch. We then generated *in silico* amplicons (get\_amplicon\_and\_reads.py), and visually inspected and removed all amplicon files for long N-characters and appropriate size. The total number of sequences for bats is in parentheses, with the number of hits for each primer pair below.

Primer	Author	Comparable <i>in silico</i> ?	Bat sequences (10523)
VF1	Ivanova et al. 2006	No*	38
BC1R	Ivanova et al. 2012	Yes	1182
BC2F	Ivanova et al. 2012	Yes	805
BC2R	Ivanova et al. 2012	Yes	0
BC3F	Ivanova et al. 2012	Yes	0
BC3R	Ivanova et al. 2012	Yes	0
BC4F	Ivanova et al. 2012	Yes	0
BC4R	Ivanova et al. 2012	Yes	0
BC5F	Ivanova et al. 2012	Yes	0
BC5R	Ivanova et al. 2012	Yes	0
BC6F	Ivanova et al. 2012	No*	5
VR1	Ward et al. 2005	No*	5
SFF_145f	Walker et al. 2016	Yes	6088
SFF_351r	Walker et al. 2016	Yes	6088
SFF_145f	Walker et al. 2016	Yes	6988
SFF_492r	Walker et al. 2016	Yes	6988
SFF_210f	Walker et al. 2016	Yes	5726
SFF_492r	Walker et al. 2016	Yes	5726
SFF_348f	Walker et al. 2016	Yes	4958
SFF_492r	Walker et al. 2016	Yes	4958
SFF_210f	Walker et al. 2016	Yes	5124
SFF_351r	Walker et al. 2016	Yes	5124
SFF_145f	Walker et al. 2016	Yes	9126
SFF_348r	Walker et al. 2016	Yes	9126
SFF_210f	Walker et al. 2016	Yes	5124
SFF_348r	Walker et al. 2016	Yes	5124
SFF_351f	Walker et al. 2016	Yes	5759
SFF_492r	Walker et al. 2016	Yes	5759
BEGLCOf	Walker et al. 2016	Yes	7618
BEGLCOr	Walker et al. 2016	Yes	7618

\*Primers hybridize outside of the standard barcode region, and thus are not strictly comparable using the downloaded sequence dataset.