

Table S1: Oligonucleotides used in this protocol. All cytosines in RAD adapters are methylated (indicated by C^m). Phos indicates a phosphate group.

Type	Name	Sequence
Barcoded methylated RAD adapters	GCATG_P1.1m	AC ^m AC ^m TC ^m TTTC ^m C ^m C ^m TAC ^m AC ^m GAC ^m GC ^m TC ^m TTC ^m C ^m GATC ^m TGC ^m ATGC ^m ATG
	AACCA_P1.1m	AC ^m AC ^m TC ^m TTTC ^m C ^m C ^m TAC ^m AC ^m GAC ^m GC ^m TC ^m TTC ^m C ^m GATC ^m TAAC ^m C ^m AC ^m ATG
	CGATC_P1.1m	AC ^m AC ^m TC ^m TTTC ^m C ^m C ^m TAC ^m AC ^m GAC ^m GC ^m TC ^m TTC ^m C ^m GATC ^m TC ^m GATC ^m C ^m ATG
	TCGAT_P1.1m	AC ^m AC ^m TC ^m TTTC ^m C ^m C ^m TAC ^m AC ^m GAC ^m GC ^m TC ^m TTC ^m C ^m GATC ^m TTC ^m GATC ^m ATG
	TGCAT_P1.1m	AC ^m AC ^m TC ^m TTTC ^m C ^m C ^m TAC ^m AC ^m GAC ^m GC ^m TC ^m TTC ^m C ^m GATC ^m TTGC ^m ATC ^m ATG
	CAACC_P1.1m	AC ^m AC ^m TC ^m TTTC ^m C ^m C ^m TAC ^m AC ^m GAC ^m GC ^m TC ^m TTC ^m C ^m GATC ^m TC ^m AAC ^m C ^m C ^m ATG
	GGTTG_P1.1m	AC ^m AC ^m TC ^m TTTC ^m C ^m C ^m TAC ^m AC ^m GAC ^m GC ^m TC ^m TTC ^m C ^m GATC ^m TGGTTGC ^m ATG
	AAGGA_P1.1m	AC ^m AC ^m TC ^m TTTC ^m C ^m C ^m TAC ^m AC ^m GAC ^m GC ^m TC ^m TTC ^m C ^m GATC ^m TAAGGAC ^m ATG
	AGCTA_P1.1m	AC ^m AC ^m TC ^m TTTC ^m C ^m C ^m TAC ^m AC ^m GAC ^m GC ^m TC ^m TTC ^m C ^m GATC ^m TAGC ^m TAC ^m ATG
	ACACA_P1.1m	AC ^m AC ^m TC ^m TTTC ^m C ^m C ^m TAC ^m AC ^m GAC ^m GC ^m TC ^m TTC ^m C ^m GATC ^m TAC ^m AC ^m AC ^m ATG
	GCATG_P1.2m	[Phos]C ^m ATGC ^m AGATC ^m GGAAGAGC ^m GTC ^m GTGTAGGGAAAGAGTGT
	AACCA_P1.2m	[Phos]TGGTTAGATC ^m GGAAGAGC ^m GTC ^m GTGTAGGGAAAGAGTGT
	CGATC_P1.2m	[Phos]GATC ^m GAGATC ^m GGAAGAGC ^m GTC ^m GTGTAGGGAAAGAGTGT
	TCGAT_P1.2m	[Phos]ATC ^m GAAGATC ^m GGAAGAGC ^m GTC ^m GTGTAGGGAAAGAGTGT
	TGCAT_P1.2m	[Phos]ATGC ^m AAGATC ^m GGAAGAGC ^m GTC ^m GTGTAGGGAAAGAGTGT
	CAACC_P1.2m	[Phos]GGTTGAGATC ^m GGAAGAGC ^m GTC ^m GTGTAGGGAAAGAGTGT
	GGTTG_P1.2m	[Phos]C ^m AAC ^m C ^m AGATC ^m GGAAGAGC ^m GTC ^m GTGTAGGGAAAGAGTGT
	AAGGA_P1.2m	[Phos]TC ^m C ^m TTAGATC ^m GGAAGAGC ^m GTC ^m GTGTAGGGAAAGAGTGT
	AGCTA_P1.2m	[Phos]TAGC ^m TAGATC ^m GGAAGAGC ^m GTC ^m GTGTAGGGAAAGAGTGT
	ACACA_P1.2m	[Phos]TGTGTAGATC ^m GGAAGAGC ^m GTC ^m GTGTAGGGAAAGAGTGT
flex_P2.1m	GTGAC ^m TGGAGTTC ^m AGAC ^m GTGTGC ^m TC ^m TTC ^m C ^m GATC ^m T	
flex_P2.2m	[Phos]AATTAGATC ^m GGAAGAGC ^m GAGAAC ^m AA	
Indexed PCR primers	PCR1	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACG
	PCR2_ATCACG	CAAGCAGAAGACGGCATAACGAGATCGTGATGTGACTGGAGTTCAGACGTGTGC
	PCR2_CGATGT	CAAGCAGAAGACGGCATAACGAGATACATCGGTGACTGGAGTTCAGACGTGTGC
	PCR2_TTAGGC	CAAGCAGAAGACGGCATAACGAGATGCCTAAGTGACTGGAGTTCAGACGTGTGC
	PCR2_TGACCA	CAAGCAGAAGACGGCATAACGAGATTGGTCAGTGACTGGAGTTCAGACGTGTGC
	PCR2_ACAGTG	CAAGCAGAAGACGGCATAACGAGATCACTGTGTGACTGGAGTTCAGACGTGTGC
	PCR2_GCCAAT	CAAGCAGAAGACGGCATAACGAGATATTGGCGTGACTGGAGTTCAGACGTGTGC
	PCR2_CAGATC	CAAGCAGAAGACGGCATAACGAGATGATCTGGTGACTGGAGTTCAGACGTGTGC
	PCR2_ACTTGA	CAAGCAGAAGACGGCATAACGAGATTCAAGTGTGACTGGAGTTCAGACGTGTGC
	PCR2_GATCAG	CAAGCAGAAGACGGCATAACGAGATCTGATCGTGACTGGAGTTCAGACGTGTGC
PCR2_TAGCTT	CAAGCAGAAGACGGCATAACGAGATAAGCTAGTGACTGGAGTTCAGACGTGTGC	

Table S2: Specimen information and sequencing statistics for all specimens used in this study. Specimens beginning with MZ were sampled from the University of Michigan Museum of Zoology collection. The Age column indicates the specimen age at the time of DNA extraction (years old). Sequencing statistics indicate the concentration of DNA after extraction (Ext DNA conc), the starting amount of DNA used for library preparation (Starting DNA amt), the sample-specific bisulfite conversion rate measured using a spike-in of fully unmethylated phage DNA (Bisulfite conv rate), the number of read pairs retained after demultiplexing and cleaning (Demult read pairs), the number of read pairs retained after alignment (Aligned read pairs), the mapping efficiency (Mapping eff), the total number of unique CpG positions sequenced at a read depth of 1X and 10X, and the total number of unique loci sequenced at a read depth of 10X. The starred specimens were sequenced in two separate libraries and the reads from the two libraries were combined prior to methylation calling; therefore, the number of CpG positions reported in the last two columns represent this combined value.

Specimen	Species	Sex	Date collected	Age (yo)	County	Location	Ext DNA conc (ng/ μ L)	Starting DNA amt (ng)	Bisulfite conv rate	Demult read pairs	Aligned read pairs	Mapping eff (%)	CpG pos (1X)	CpG pos (10X)	Unique loci (10X)
13MN007	<i>P. leucopus</i>	M	8/6/13	3	Menominee	45.52,-87.41	33.90	350	0.99	1205740	606793	50.30	111150	14258	5875
13MN031	<i>P. leucopus</i>	M	8/7/13	3	Menominee	45.48,-87.42	14.40	350	1.00	59625	22876	38.40	15672	NA	NA
13MN033	<i>P. leucopus</i>	F	8/7/13	3	Menominee	45.48,-87.42	15.40	350	1.00	56337	20381	36.20	14416	NA	NA
13MN035	<i>P. leucopus</i>	M	8/7/13	3	Menominee	45.48,-87.42	35.00	350	0.99	189141	100893	53.30	26793	529	216
13MN040	<i>P. leucopus</i>	F	8/8/13	3	Menominee	45.48,-87.42	34.20	350	0.99	716689	395173	55.10	50551	18155	7806
13MN045	<i>P. leucopus</i>	F	8/8/13	3	Menominee	45.48,-87.42	23.60	350	0.99	25412	7627	30.00	2381	39	17
14MN003	<i>P. leucopus</i>	M	8/12/14	2	Menominee	45.52,-87.41	26.80	350	1.00	35995	15056	41.80	8884	33	5
14MN005	<i>P. leucopus</i>	F	8/12/14	2	Menominee	45.48,-87.42	28.10	350	1.00	512745	247837	48.30	74927	6087	2386
14MN010	<i>P. leucopus</i>	M	8/12/14	2	Menominee	45.48,-87.42	4.14	350	1.00	58085	19408	33.40	11171	8	5
14MN024	<i>P. leucopus</i>	F	8/13/14	2	Menominee	45.48,-87.42	34.50	350	0.99	29078	10629	36.60	4464	12	4
15MN001	<i>P. leucopus</i>	F	7/24/15	1	Menominee	45.48,-87.42	10.60	350	0.99	63300	20337	32.10	5754	258	116
15MN002	<i>P. leucopus</i>	M	7/24/15	1	Menominee	45.48,-87.42	49.20	350	0.99	601948	211803	35.20	52939	8451	2631
15MN003	<i>P. leucopus</i>	M	7/24/15	1	Menominee	45.48,-87.42	18.90	350	0.99	1136172	552200	48.60	119224	28042	10330
MZ11320	<i>P. maniculatus</i>	M	10/22/16	0	Menominee	45.5,-87.41	350.00	350	1.00	3252521	1467424	45.10	278344	91530	34220
MZ11321	<i>P. maniculatus</i>	M	10/22/16	0	Menominee	45.5,-87.41	54.00	350	1.00	167673	73602	43.90	34418	204	79
MZ11322	<i>P. maniculatus</i>	F	10/22/16	0	Menominee	45.5,-87.41	40.30	350	1.00	221935	97250	43.80	43589	867	313
MZ11323	<i>P. maniculatus</i>	M	10/22/16	0	Menominee	45.5,-87.41	41.50	350	1.00	338886	149205	44.00	58905	5336	2006
MZ11324	<i>P. maniculatus</i>	M	10/22/16	0	Menominee	45.5,-87.41	53.00	350	1.00	119650	48721	40.70	29456	160	49
MZ11325	<i>P. maniculatus</i>	F	10/23/16	0	Menominee	45.5,-87.41	42.90	350	1.00	269511	108061	40.10	46106	1688	636
MZ11326	<i>P. maniculatus</i>	F	10/23/16	0	Menominee	45.5,-87.41	44.60	350	1.00	79037	24894	31.50	17904	4	2
MZ11327	<i>P. maniculatus</i>	M	10/23/16	0	Menominee	45.5,-87.41	27.90	350	1.00	89295	31376	35.10	19566	103	28
MZ11328	<i>P. maniculatus</i>	M	10/23/16	0	Menominee	45.5,-87.41	154.00	350	1.00	2608601	980606	37.60	175557	73036	28086
MZ11329	<i>P. maniculatus</i>	M	10/23/16	0	Menominee	45.5,-87.41	304.00	350	0.98	1508072	637781	42.30	94246	43490	17659
MZ11330	<i>P. maniculatus</i>	M	10/23/16	0	Menominee	45.5,-87.41	58.00	350	0.99	114101	51363	45.00	19948	359	144
MZ11331	<i>P. maniculatus</i>	M	10/23/16	0	Menominee	45.5,-87.41	34.10	350	0.99	154619	78622	50.80	22912	4190	1231
MZ11332	<i>P. maniculatus</i>	F	10/23/16	0	Menominee	45.5,-87.41	326.00	350	1.00	1867830	714744	38.30	150854	51548	21115
MZ11333	<i>P. maniculatus</i>	M	10/23/16	0	Menominee	45.5,-87.41	31.10	350	0.99	170140	88778	52.20	33793	4935	1204
MZ176017	<i>P. leucopus</i>	F	10/15/03	13	Menominee	45.51,-87.43	21.60	150	0.99	89863	22644	25.20	3162	NA	NA
MZ176018	<i>P. leucopus</i>	F	10/15/03	13	Menominee	45.51,-87.43	11.70	150	0.98	192419	51019	26.50	10455	NA	NA
MZ176020	<i>P. leucopus</i>	F	10/15/03	13	Menominee	45.5,-87.4	6.54	150	0.97	64924	24147	37.20	5603	NA	NA

Table S2: Specimen information and sequencing statistics for all specimens used in this study. Specimens beginning with MZ were sampled from the University of Michigan Museum of Zoology collection. The Age column indicates the specimen age at the time of DNA extraction (years old). Sequencing statistics indicate the concentration of DNA after extraction (Ext DNA conc), the starting amount of DNA used for library preparation (Starting DNA amt), the sample-specific bisulfite conversion rate measured using a spike-in of fully unmethylated phage DNA (Bisulfite conv rate), the number of read pairs retained after demultiplexing and cleaning (Demult read pairs), the number of read pairs retained after alignment (Aligned read pairs), the mapping efficiency (Mapping eff), the total number of unique CpG positions sequenced at a read depth of 1X and 10X, and the total number of unique loci sequenced at a read depth of 10X. The starred specimens were sequenced in two separate libraries and the reads from the two libraries were combined prior to methylation calling; therefore, the number of CpG positions reported in the last two columns represent this combined value.

Specimen	Species	Sex	Date collected	Age (yo)	County	Location	Ext DNA conc (ng/ μ L)	Starting DNA amt (ng)	Bisulfite conv rate	Demult read pairs	Aligned read pairs	Mapping eff (%)	CpG pos (1X)	CpG pos (10X)	Unique loci (10X)
MZ176021	<i>P. leucopus</i>	M	10/15/03	13	Menominee	45.5,-87.4	12.70	150	0.97	134520	32733	24.30	7323	NA	NA
MZ176022	<i>P. leucopus</i>	M	10/27/03	13	Menominee	45.5,-87.39	7.36	150	0.99	44797	7410	16.50	1565	2	1
MZ176023	<i>P. leucopus</i>	M	10/27/03	13	Menominee	45.5,-87.39	1.72	40	0.99	191600	35374	18.50	4441	2676	1141
MZ176024	<i>P. leucopus</i>	F	10/27/03	13	Menominee	45.5,-87.39	13.40	150	0.99	954988	108678	11.40	14047	7	4
MZ176026	<i>P. leucopus</i>	F	10/28/03	13	Menominee	45.5,-87.39	40.80	150	0.99	325649	65356	20.10	10833	6	3
MZ176027	<i>P. leucopus</i>	M	10/28/03	13	Menominee	45.5,-87.39	4.64	150	1.00	83411	22934	27.50	3644	NA	NA
MZ176028	<i>P. leucopus</i>	F	10/28/03	13	Menominee	45.5,-87.39	0.83	40	0.99	530725	351089	66.20	33067	26074	10928
MZ176029	<i>P. leucopus</i>	M	10/29/03	13	Menominee	45.55,-87.33	5.28	150	0.98	132091	45778	34.70	7730	NA	NA
MZ176047	<i>P. leucopus</i>	M	10/28/03	13	Menominee	45.5,-87.39	5.80	150	0.97	13742	649	4.70	183	NA	NA
MZ176048	<i>P. leucopus</i>	F	10/28/03	13	Menominee	45.5,-87.39	21.60	150	0.98	213002	55793	26.20	7932	NA	NA
MZ176050	<i>P. leucopus</i>	F	10/27/03	13	Menominee	45.5,-87.39	19.10	150	0.99	668606	134782	20.20	15395	4	3
MZ176101	<i>P. maniculatus</i>	M	10/27/03	13	Menominee	45.5,-87.39	7.41	150	0.98	35139	6399	18.20	1303	36	18
MZ176107	<i>P. maniculatus</i>	M	10/27/03	13	Menominee	45.5,-87.39	3.52	150	0.98	23853	5493	23.00	1013	3	2
MZ176109	<i>P. maniculatus</i>	F	10/27/03	13	Menominee	45.5,-87.39	1.75	40	0.99	59100	15227	25.80	1570	879	371
MZ176111	<i>P. maniculatus</i>	F	10/27/03	13	Menominee	45.5,-87.39	26.00	150	0.99	95420	20439	21.40	5174	NA	NA
MZ176115	<i>P. maniculatus</i>	M	10/27/03	13	Menominee	45.5,-87.39	0.87	40	1.00	13468	94	0.70	24	NA	NA
MZ176116	<i>P. maniculatus</i>	F	10/27/03	13	Menominee	45.5,-87.39	6.33	150	0.99	98053	7178	7.30	1846	18	7
MZ176117	<i>P. maniculatus</i>	F	10/27/03	13	Menominee	45.5,-87.39	1.27	40	0.99	13189	419	3.20	175	11	4
MZ176123	<i>P. maniculatus</i>	M	10/27/03	13	Menominee	45.5,-87.39	31.80	150	1.00	47125	12431	26.40	2726	2	1
MZ176125	<i>P. maniculatus</i>	M	10/27/03	13	Menominee	45.5,-87.39	37.50	150	0.98	90963	11079	12.20	2830	13	7
MZ176127	<i>P. maniculatus</i>	M	10/27/03	13	Menominee	45.5,-87.39	10.90	150	1.00	381001	70084	18.40	1663	NA	NA
MZ176129	<i>P. maniculatus</i>	F	10/27/03	13	Menominee	45.5,-87.39	40.40	150	0.98	128604	16942	13.20	4338	2	2
MZ176136	<i>P. maniculatus</i>	M	10/27/03	13	Menominee	45.5,-87.39	1.74	40	0.99	7880	3804	48.30	246	171	92
MZ176140	<i>P. maniculatus</i>	F	10/27/03	13	Menominee	45.5,-87.39	15.60	150	0.98	49075	11947	24.30	1963	19	9
MZ84532	<i>P. maniculatus</i>	M	8/19/40	76	Menominee	45.41,-87.46	1.09	40	0.99	20561	3277	15.90	360	236	91
MZ84533	<i>P. maniculatus</i>	M	8/20/40	76	Menominee	45.41,-87.46	1.86	40	0.99	7083	68	1.00	39	NA	NA
MZ84534	<i>P. maniculatus</i>	F	8/21/40	76	Menominee	45.41,-87.46	1.88	40	0.99	47104	175	0.40	74	NA	NA
MZ84535	<i>P. maniculatus</i>	F	8/21/40	76	Menominee	45.41,-87.46	1.08	40	0.99	171764	20054	11.70	1231	787	444
MZ84536	<i>P. maniculatus</i>	M	8/21/40	76	Menominee	45.41,-87.46	2.90	40	0.99	52603	7738	14.70	3775	235	102
MZ84537	<i>P. maniculatus</i>	M	8/21/40	76	Menominee	45.41,-87.46	4.74	40	0.97	8746	1071	12.20	60	30	18

Table S2: Specimen information and sequencing statistics for all specimens used in this study. Specimens beginning with MZ were sampled from the University of Michigan Museum of Zoology collection. The Age column indicates the specimen age at the time of DNA extraction (years old). Sequencing statistics indicate the concentration of DNA after extraction (Ext DNA conc), the starting amount of DNA used for library preparation (Starting DNA amt), the sample-specific bisulfite conversion rate measured using a spike-in of fully unmethylated phage DNA (Bisulfite conv rate), the number of read pairs retained after demultiplexing and cleaning (Demult read pairs), the number of read pairs retained after alignment (Aligned read pairs), the mapping efficiency (Mapping eff), the total number of unique CpG positions sequenced at a read depth of 1X and 10X, and the total number of unique loci sequenced at a read depth of 10X. The starred specimens were sequenced in two separate libraries and the reads from the two libraries were combined prior to methylation calling; therefore, the number of CpG positions reported in the last two columns represent this combined value.

Specimen	Species	Sex	Date collected	Age (yo)	County	Location	Ext DNA conc (ng/ μ L)	Starting DNA amt (ng)	Bisulfite conv rate	Demult read pairs	Aligned read pairs	Mapping eff (%)	CpG pos (1X)	CpG pos (10X)	Unique loci (10X)
MZ84540	<i>P. maniculatus</i>	M	8/22/40	76	Menominee	45.41,-87.46	4.84	40	1.00	9081	736	8.10	130	18	8
MZ84541	<i>P. maniculatus</i>	F	8/22/40	76	Menominee	45.41,-87.46	2.38	40	0.98	1863	324	17.40	92	6	3
MZ84625	<i>P. leucopus</i>	M	8/17/40	76	Menominee	45.18,-87.62	3.96	40	0.99	17191	352	2.00	122	7	4
MZ84627	<i>P. leucopus</i>	F	8/18/40	76	Menominee	45.41,-87.46	1.42	40	0.96	13109	657	5.00	11	9	6
MZ84628	<i>P. leucopus</i>	F	8/19/40	76	Menominee	45.41,-87.46	1.96	40	0.99	28681	972	3.40	304	12	5
MZ84629	<i>P. leucopus</i>	M	8/19/40	76	Menominee	45.41,-87.46	1.94	40	0.99	4150	36	0.90	17	NA	NA
MZ84631	<i>P. leucopus</i>	F	8/19/40	76	Menominee	45.41,-87.46	3.06	40	0.99	7106	176	2.50	48	4	3
MZ84634	<i>P. leucopus</i>	M	8/19/40	76	Menominee	45.41,-87.46	1.29	40	0.99	49897	1016	2.00	248	14	9
MZ84636	<i>P. leucopus</i>	F	8/20/40	76	Menominee	45.41,-87.46	1.20	40	0.94	3063	598	19.50	365	NA	NA
MZ84637	<i>P. leucopus</i>	F	8/21/40	76	Menominee	45.41,-87.46	1.43	40	0.99	16009	2132	13.30	152	99	43
MZ84639	<i>P. leucopus</i>	M	8/21/40	76	Menominee	45.41,-87.46	1.33	40	0.99	7673	76	1.00	36	NA	NA
MZ84641HI*	<i>P. leucopus</i>	F	8/21/40	76	Menominee	45.41,-87.46	9.64	150	0.99	6388	707	11.10	6729	5005	2262
MZ84641LO*	see above	-	-	-	-	-	-	40	0.98	281116	87695	31.20	-	-	-
MZ84642	<i>P. leucopus</i>	F	8/21/40	76	Menominee	45.41,-87.46	3.24	40	0.98	8917	198	2.20	54	2	2
MZ84645	<i>P. leucopus</i>	M	8/21/40	76	Menominee	45.41,-87.46	0.68	40	0.99	15964	2443	15.30	310	99	52
MZ84657HI*	<i>P. leucopus</i>	M	8/26/40	76	Menominee	45.41,-87.46	48.60	150	0.99	582375	189843	46.20	18318	212	139
MZ84657LO*	see above	-	-	-	-	-	-	40	0.99	11410	5267	32.60	-	-	-