

Figure S1. Median-joining network analysis for blocker designing. a) Median-joining network diagram of the TAReuk454FWD1 priming region at positions between 40 bp downstream and 1 bp upstream of the 3' end of the primer. The arrow indicates a single nucleotide mismatch at 24 bp downstream of the 3' end of the primer. b) Alignment of TAReuk454FWD1 and 18S rDNA sequences of the representative tick species. The left column in the table shows the primer ID or sequence accession number. The numbers at the top indicate the relative nucleotide position from the 3' end of the primer. c) Median-joining network diagram of the TAReukREV3 priming region at positions between 40 bp upstream and 1 bp downstream of the primer. The arrows indicate single nucleotide mismatch at 1 or 30 bp upstream of the primer. d) Alignment of TAReukREV3 and 18S rDNA sequences of the representative tick species. The left column in the table shows the primer ID or sequence accession number. The numbers at the top indicate the relative nucleotide positions from the 3' end of the primer.

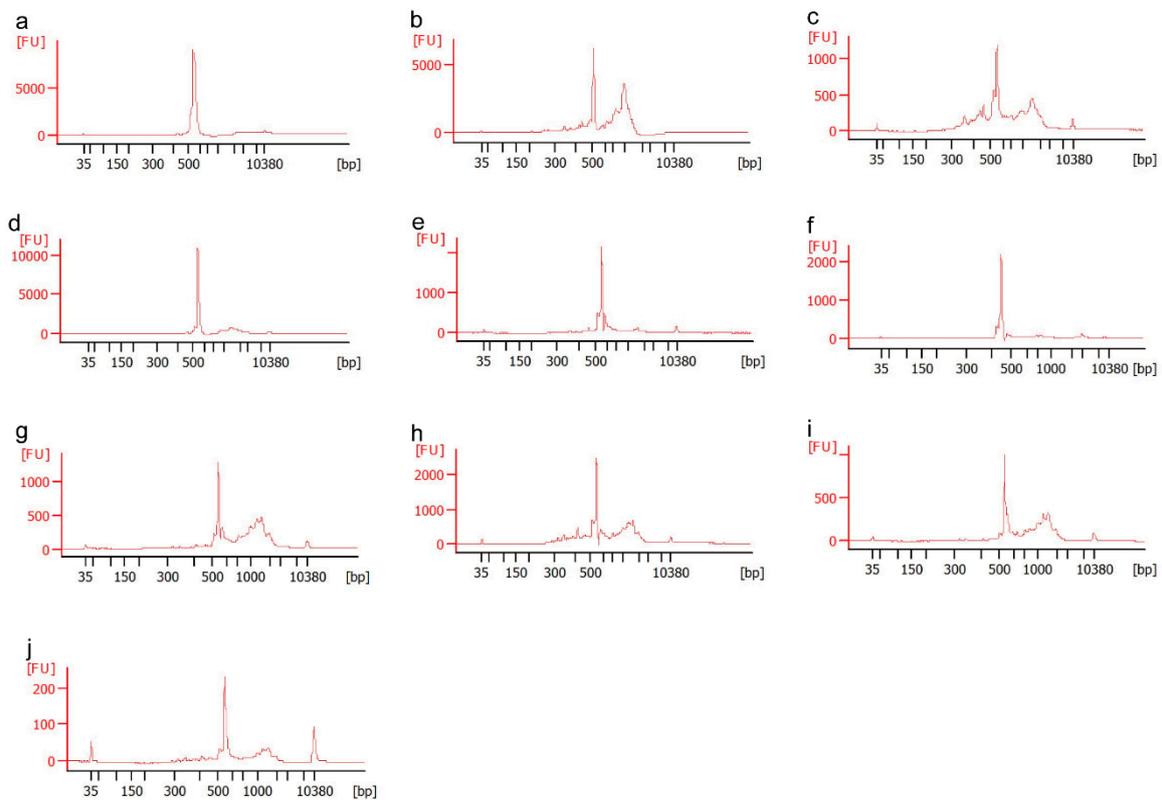


Figure S2. The fragment size distribution of the amplicon libraries using different PCR and purification methods. Amplicon libraries were prepared using *Ixodes persulcatus* DNA (tick ID: 933) (a-e) or *Amblyomma testudinarium* DNA (tick ID: 2874) (f-j). PCR and purification methods were either control with AMPure (a and f), TickB_PNA with AMPure (b and g), TickB_PNA with SizeSelect (c and h), TickB_LNA with AMPure (d and i), or TickB_LNA with SizeSelect (e and j).

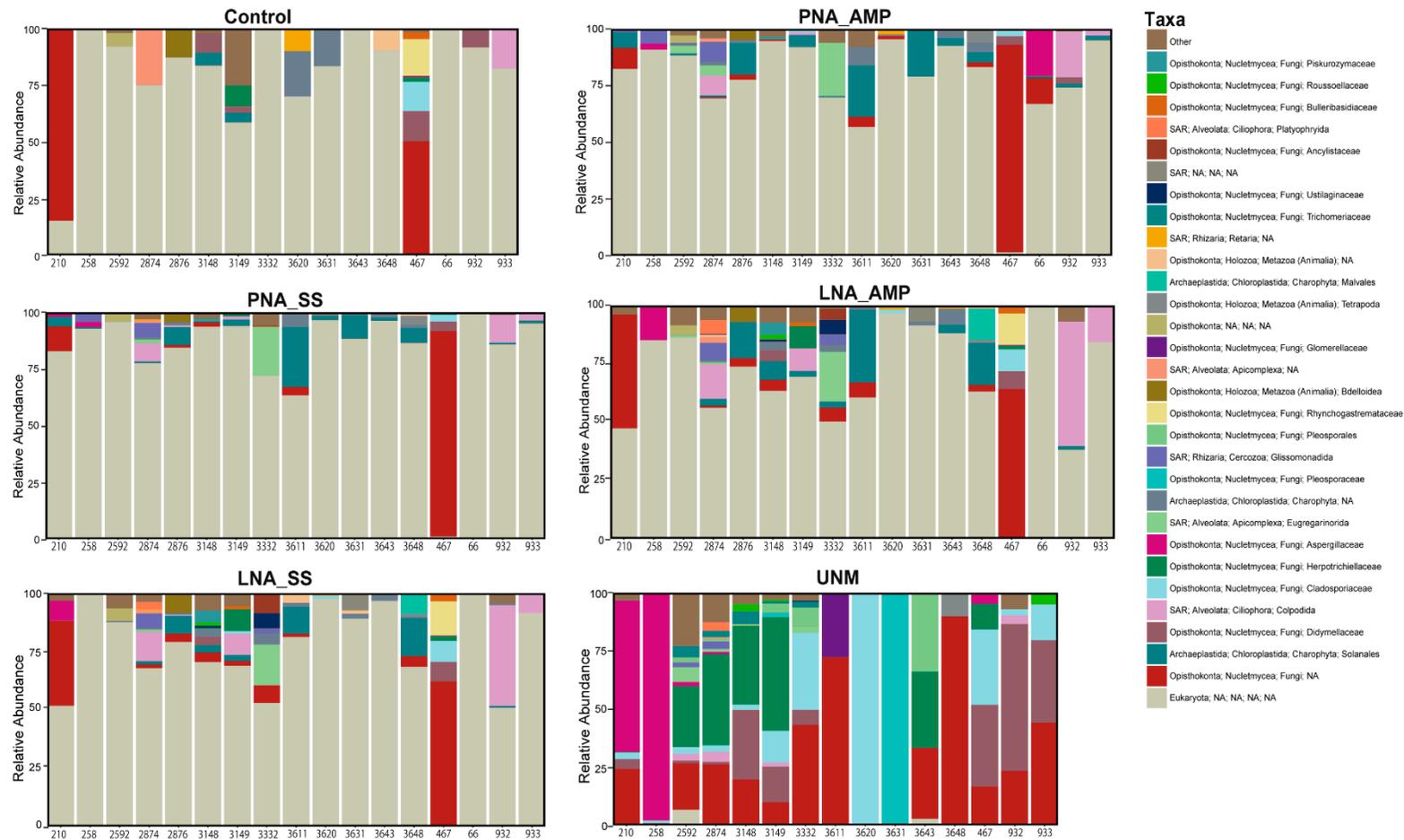


Figure S3. Relative abundance of eukaryotic taxa detected in 17 samples. PCR and purification methods for the amplicon analyses are provided on the top of bars. The PCRs were performed based on control, TickB_PNA (PNA), TickB_LNA (LNA), or UNOnMet-PCR (UNM). The purification method was either with AMPure (AMP) or SizeSelect (SS). Each bar represents the eukaryotic taxa detected in one tick sample. The sample ID is provided on the bottom of each bar. Tick ID 3631 for control and tick IDs 66 and 2876 for UNM were excluded since no non-tick reads was obtained.

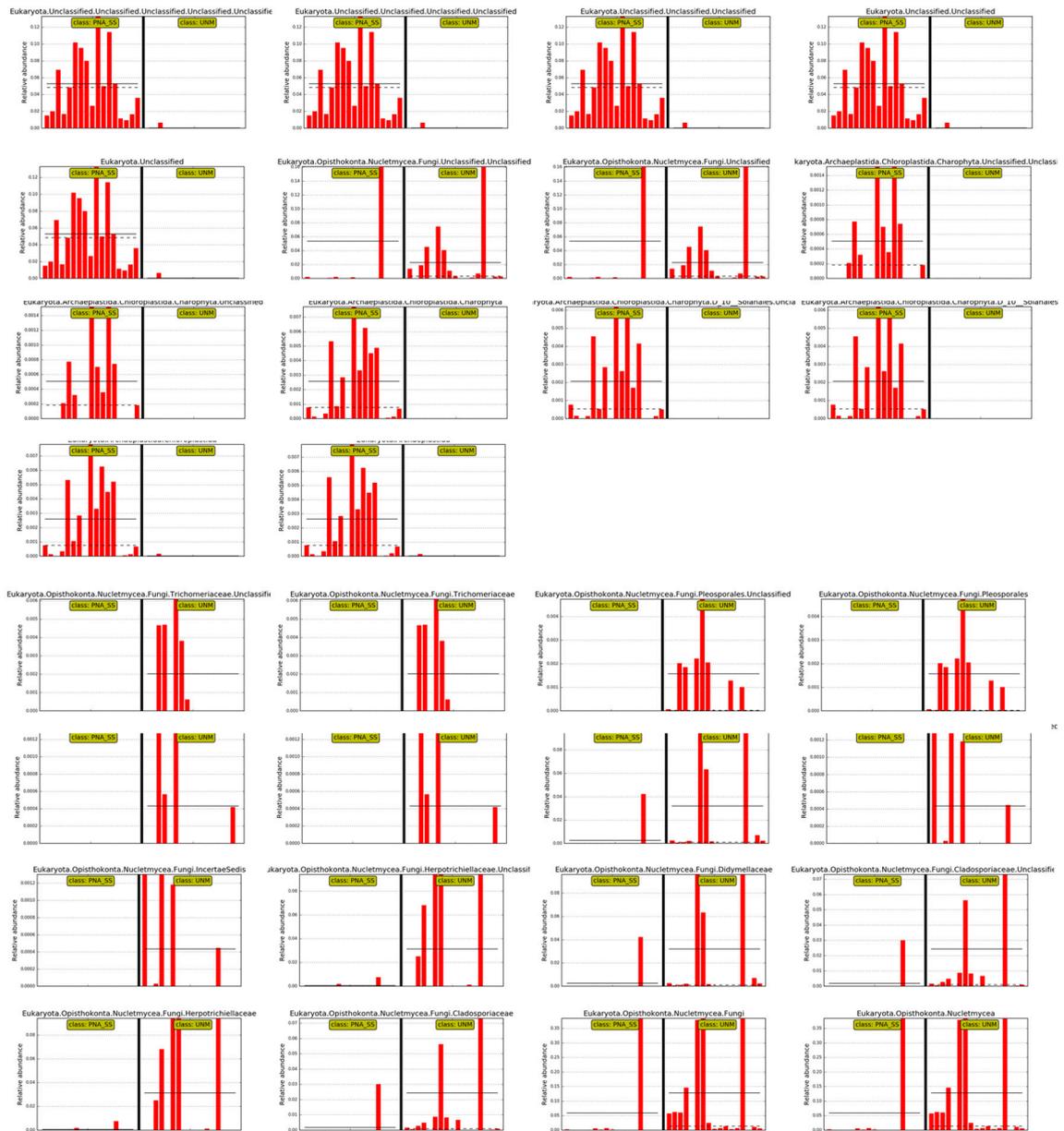


Figure S4. Significantly differential features between TickB_PNA and UNonMet-PCR based on LefSe analysis. Each graph indicates the differential features. The histogram indicates the relative abundance of each samples.

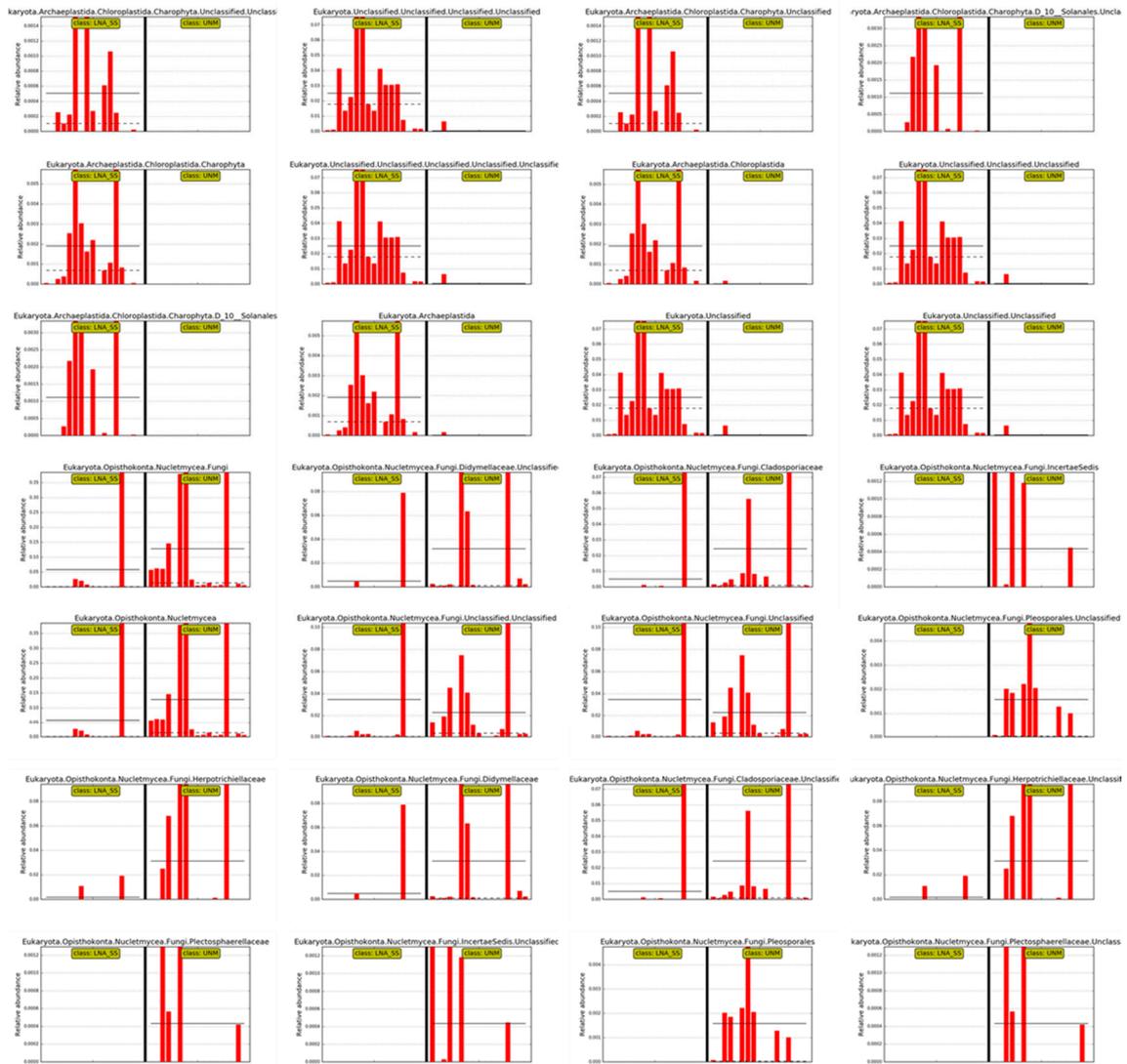


Figure S5. Significantly differential features between TickB_LNA and UNonMet-PCR based on LefSe analysis. Each graph indicates the differential features. The histogram indicates the relative abundance of each samples.

Table S2. The proportion of the non-tick reads and non-tick read enrichment value.

Tick ID	Proportion of non-tick reads						Non-tick read enrichment values*				
	Control	PNA_AMP	PNA_SS	LNA_AMP	LNA_SS	UNM	PNA_AMP	PNA_SS	LNA_AMP	LNA_SS	UNM
66	0.01%	0.75%	0.95%	0.01%	0.02%	0.00%	133.6	169.2	1.5	3.2	0.0
210	0.01%	1.57%	1.82%	0.09%	0.16%	5.74%	129.0	149.2	7.0	13.0	470.4
258	0.02%	1.52%	2.15%	0.09%	0.12%	6.23%	62.6	88.8	3.6	4.8	257.0
467	81.14%	99.14%	99.85%	94.39%	93.35%	99.56%	1.2	1.2	1.2	1.2	1.2
932	0.02%	1.01%	1.91%	0.25%	0.38%	1.11%	50.9	96.2	12.6	18.9	56.2
933	0.04%	1.56%	3.78%	0.13%	0.19%	0.63%	44.0	106.8	3.7	5.3	17.8
2592	0.21%	3.65%	7.22%	2.95%	4.72%	9.57%	17.4	34.5	14.1	22.6	45.8
2874	0.02%	1.49%	2.17%	1.32%	1.99%	17.44%	72.1	105.0	64.0	96.3	844.7
2876	0.06%	3.24%	5.75%	1.29%	2.83%	0.00%	58.0	102.7	23.0	50.6	0.0
3148	0.31%	5.41%	10.53%	8.64%	12.78%	37.90%	17.7	34.4	28.2	41.7	123.7
3149	0.49%	6.25%	10.14%	7.96%	11.71%	41.23%	12.8	20.8	16.3	24.0	84.6
3332	0.02%	8.59%	11.13%	2.36%	3.39%	2.52%	543.6	705.0	149.5	214.5	159.6
3611**	0.00%	2.70%	4.18%	0.62%	1.67%	0.45%	-	-	-	-	-
3620	0.03%	9.96%	13.75%	2.39%	4.21%	0.67%	309.2	427.0	74.2	130.8	20.8
3631	0.06%	2.99%	5.63%	1.84%	3.43%	1.37%	46.7	87.9	28.7	53.5	21.4
3643	0.03%	7.70%	11.87%	1.53%	3.18%	0.38%	230.4	355.0	45.7	95.0	11.3
3648	0.03%	3.96%	6.09%	3.36%	4.53%	0.78%	125.8	193.7	106.9	144.0	24.7
Median	0.03%	3.24%	5.75%	1.53%	3.18%	1.37%	60.3	103.8	19.6	32.9	35.2
Average	4.85%	9.50%	11.70%	7.60%	8.74%	13.27%	115.9	167.3	36.3	57.5	133.7
Max	81.14%	99.14%	99.85%	94.39%	93.35%	99.56%	543.6	705.0	149.5	214.5	844.7
Min	0.00%	0.75%	0.95%	0.01%	0.02%	0.00%	1.2	1.2	1.2	1.2	0.0

Proportion of non-tick reads = non-tick reads / eukaryotic reads

*Non-tick read enrichment values = proportion of non-tick reads (each method) / proportion of non-tick reads (control)

**Non-tick read enrichment value was not calculated for tick ID 3611 because there was no non-tick reads in the control.

Table S3. Significance of Shannon Diversity.

Contrast	Estimate	Standard Error	t-value	p-value
control - LNA_AMP	-0.4728	0.163221	-2.8967	0.052233
control - LNA_SS	-0.75577	0.163221	-4.63037	0.000178
control - PNA_AMP	-0.96824	0.163221	-5.93205	8.54E-07
control - PNA_SS	-1.16467	0.163221	-7.13555	4.23E-09
control - UNM	-1.8932	0.165926	-11.4099	3.56E-10
LNA_AMP - LNA_SS	-0.28297	0.163221	-1.73366	0.513642
LNA_AMP - PNA_AMP	-0.49543	0.163221	-3.03534	0.036107
LNA_AMP - PNA_SS	-0.69187	0.163221	-4.23885	0.000765
LNA_AMP - UNM	-1.4204	0.165926	-8.56042	3.61E-10
LNA_SS - PNA_AMP	-0.21246	0.163221	-1.30168	0.783363
LNA_SS - PNA_SS	-0.4089	0.163221	-2.50518	0.133795
LNA_SS - UNM	-1.13743	0.165926	-6.85502	1.44E-08
PNA_AMP - PNA_SS	-0.19644	0.163221	-1.2035	0.834043
PNA_AMP - UNM	-0.92496	0.165926	-5.57456	3.94E-06
PNA_SS - UNM	-0.72853	0.165926	-4.39068	0.000439

Table S4. Significance of Faith's phylogenetic diversity.

Contrast	Estimate	Standard Error	t-value	p-value
control - LNA_AMP	-1.29551	0.207578	-6.2411	2.21E-07
control - LNA_SS	-1.67406	0.207578	-8.06474	4.07E-10
control - PNA_AMP	-2.00497	0.207578	-9.65887	3.56E-10
control - PNA_SS	-2.30394	0.207578	-11.0992	3.56E-10
control - UNM	0.348529	0.211019	1.651644	0.567053
LNA_AMP - LNA_SS	-0.37855	0.207578	-1.82364	0.456171
LNA_AMP - PNA_AMP	-0.70945	0.207578	-3.41776	0.011942
LNA_AMP - PNA_SS	-1.00843	0.207578	-4.85808	7.36E-05
LNA_AMP - UNM	1.644043	0.211019	7.790965	5.41E-10
LNA_SS - PNA_AMP	-0.3309	0.207578	-1.59412	0.604588
LNA_SS - PNA_SS	-0.62988	0.207578	-3.03444	0.036196
LNA_SS - UNM	2.02259	0.211019	9.584864	3.56E-10
PNA_AMP - PNA_SS	-0.29898	0.207578	-1.44031	0.70246
PNA_AMP - UNM	2.353495	0.211019	11.15299	3.56E-10
PNA_SS - UNM	2.652472	0.211019	12.56981	3.56E-10

Table S5. Significance of observed ASVs.

Contrast	Estimate	Standard Error	t-value	p-value
control - LNA_AMP	-0.35771	0.124878	-2.86449	0.056764
control - LNA_SS	-0.65776	0.124878	-5.26724	1.42E-05
control - PNA_AMP	-0.43484	0.124878	-3.48208	0.009797
control - PNA_SS	-0.69337	0.124878	-5.55236	4.33E-06
control - UNM	-0.58827	0.126945	-4.63406	0.000176
LNA_AMP - LNA_SS	-0.30005	0.124878	-2.40274	0.166612
LNA_AMP - PNA_AMP	-0.07712	0.124878	-0.61759	0.989474
LNA_AMP - PNA_SS	-0.33566	0.124878	-2.68786	0.087966
LNA_AMP - UNM	-0.23056	0.126945	-1.81622	0.460841
LNA_SS - PNA_AMP	0.222927	0.124878	1.785156	0.480541
LNA_SS - PNA_SS	-0.03561	0.124878	-0.28512	0.999738
LNA_SS - UNM	0.069489	0.126945	0.547394	0.993967
PNA_AMP - PNA_SS	-0.25853	0.124878	-2.07028	0.312376
PNA_AMP - UNM	-0.15344	0.126945	-1.20869	0.831528
PNA_SS - UNM	0.105095	0.126945	0.827871	0.961572

Table S7. The proportion of the unclassified eukaryotic reads to all eukaryotic reads.

Tick ID	Control	LNA- AMPure	LNA- SizeSelect	PNA- AMPure	PNA- SizeSelect	UNM
66	0.01%	0.01%	0.02%	0.50%	0.95%	0.00%
210	0.00%	0.04%	0.08%	1.30%	1.51%	0.00%
258	0.02%	0.07%	0.12%	1.38%	2.00%	0.00%
467	0.26%	0.34%	0.75%	1.47%	1.16%	0.00%
932	0.02%	0.10%	0.19%	0.75%	1.64%	0.00%
933	0.03%	0.11%	0.17%	1.48%	3.60%	0.00%
2592	0.19%	2.55%	4.13%	3.22%	6.92%	0.64%
2874	0.02%	0.74%	1.35%	1.03%	1.68%	0.03%
2876	0.05%	0.95%	2.24%	2.52%	4.85%	0.00%
3148	0.26%	5.48%	9.01%	5.12%	9.84%	0.00%
3149	0.29%	5.52%	8.07%	5.75%	9.53%	0.00%
3332	0.02%	1.19%	1.79%	6.00%	8.00%	0.00%
3611	0.00%	0.37%	1.35%	1.53%	2.65%	0.00%
3620	0.02%	2.31%	4.11%	9.50%	13.27%	0.00%
3631	0.05%	1.68%	3.05%	2.36%	4.98%	0.00%
3643	0.03%	1.35%	3.07%	7.13%	11.42%	0.01%
3648	0.03%	2.12%	3.10%	3.30%	5.27%	0.00%
Average	0.08%	1.47%	2.51%	3.20%	5.25%	0.04%
Median	0.03%	0.95%	1.79%	2.36%	4.85%	0.00%
Min	0.00%	0.01%	0.02%	0.50%	0.95%	0.00%
Max	0.29%	5.52%	9.01%	9.50%	13.27%	0.64%