Supplementary Material For:

Optimization of 454 sequencing library preparation from small amounts of DNA permits sequence determination of both DNA strands

Tomislav Maricic and Svante Pääbo
Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany

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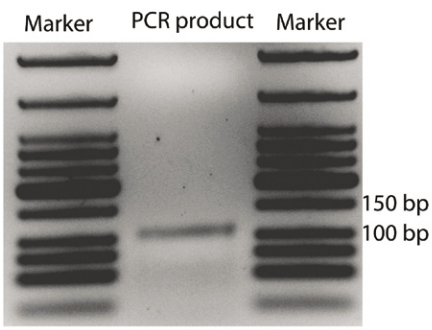
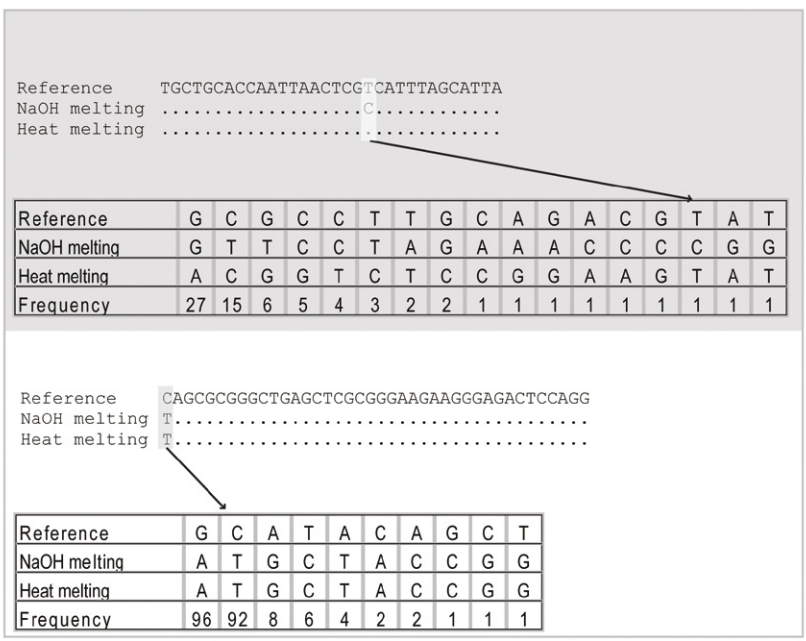
Supplementary Figure 1. Sequence of a 103-bp-long PCR product. Arrows represent annealing sites for the primers H221 (5′-CGGACTAAGTGAAATACATGCT-3′) and L164 (5′-GCATATAAGCATGTACATATTATGC-3′).



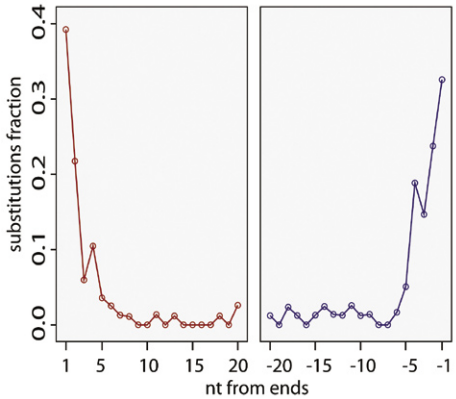
Supplementary Figure 2. Gel picture of the 103-bp-long non-radioactive PCR product.



Reference	G	C	G	C	C	T	T	G	C	A	G	A	C	G	T	A	T
NaOH melting	G	T	T	C	C	T	A	G	A	A	A	C	C	C	C	G	G
Heat melting	A	C	G	G	T	C	T	C	C	G	G	A	A	G	T	A	T
Frequency	27	15	6	5	4	3	2	2	1	1	1	1	1	1	1	1	1

Reference	G	C	A	T	A	C	A	G	C	T
NaOH melting	A	T	G	C	T	A	C	C	G	G
Heat melting	A	T	G	C	T	A	C	C	G	G
Frequency	96	92	8	6	4	2	2	1	1	1

Supplementary Figure 3. Numbers of substitutions in NaOH- and heat-treated DNA strands. (A) An example where the NaOH-treated sequence has a substitution to both the reference and the heat-treated sequence. (B) Table showing the numbers of substitutions where either the NaOH- or heat-treated sequences (but not both) differ from the reference. (C) An example where both NaOH- and heat-treated sequences have substitutions to the reference sequence. (D) Table showing the numbers of observations where both sequences differ from the reference. Dots in the alignments indicate that the base matches the reference sequence.



Supplementary Figure 4. Frequencies of C-to-T (red, left) and G-to-A (blue, right) substitutions as a function of distance from 5′ and 3′ ends of Neanderthal DNA molecules, respectively. Only substitutions occurring on both strands are depicted. C-to-T and G-to-A substitution fractions were calculated by dividing the number of substitutions at every position by the number of Cs and Gs in the reference sequence at the position, respectively.

Supplementary Table 1. Materials Used in 454 Library Production

Fragment end polishing	Volume	Initial conc.	Final conc.	Manufacturer
Template	23			
10× Polishing buffer	5	10×	1×	NEBuffer2; New England Biolabs (NEB), Ipswich, MA, USA
BSA	5	1 mg/mL	0.1 mg/mL	NEB
ATP	5	10 mM	1 mM	Amersham Biosciences, Piscataway, NJ, USA
dNTPs	2	10 mM	400 µM	NEB
T4 PNK	5	10 U/µL	1 U/µl	NEB
T4 DNA Polymerase	5	3 U/µL	0.3 U/µl	NEB
Final volume	50			
Adaptor ligation				
Polished DNA	15			
2× Ligase buffer	20	2×	1×	2× Quick Ligation Reaction Buffer; NEB
Adaptors	1	20 µM	0.5 µM	Original from 454 kit (454 Life Sciences, Branford, CT, USA) or oligos ordered from Sigma Aldrich (Taufkirchen, Germany)
Quick ligase	4	n/a	n/a	Quick T4 DNA Ligase; NEB
Final volume	40			
Library immobilization				
2× Library binding buffer				Self-made 2× B&W buffer
1× Library binding buffer				Self-made 1× B&W buffer
Library immobilization beads				M-270 streptavidin beads; Dynal, Oslo, Norway
Fill-in reaction				
Water	40			
10× Fill-in buffer	5	10×	1×	10× ThermoPol Reaction Buffer; NEB
dNTPs	2	10 mM	0.5 mM	NEB
Fill-in polymerase	3	8 U/µL	0.6 U/µL	NEB
Final volume	50			

Materials used are based on a detailed description of the materials in the 454 library production described in Margulies et al. 2005. Genome sequencing in microfabricated high-density picolitre reactors. Nature *437*:376–380.

Supplementary Table 2. Scintillation Measurements of the First Four Library Preparation Steps

I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
Library number	Library preparation step	Component	V_component (µL)	V_ aliquot (µL)	cpm (aliquot)	cpm (fraction)	100% RA of the step	% of RA entering each step	Average	STDEV	SEM	Average_n	STDEV_n	SEM_n
1	End polishing	End polishing reaction	50	2	97,912	2,447,800	2,349,888							
2	Figure 2A				96,400	2,410,000	2,313,600							
3					92,538	2,313,450	2,220,912							
4					72,040	1,801,000	1,728,960							
5					97,127	2,428,175	2,331,048							
6					94,233	2,355,825	2,261,592							
1		Binding buffer	297.5	50	77,025	458,299		19.5%	20.8%	2.4%	1.0%	22.9%	2.6%	1.1%
2					71,514	425,508		18.4%						
3					74,737	444,685		20.0%						
4					72,732	432,755		25.0%						
5					77,369	460,346		19.7%						
6					83,653	497,735		22.0%						
1		Wash buffer	750	50	219	3,285		0.1%	0.2%	0.1%	0.0%	0.2%	0.1%	0.0%
2					325	4,875		0.2%						
3					159	2,385		0.1%						
4					336	5,040		0.3%						
5					376	5,640		0.2%						
6					141	2,115		0.1%						
1		Column	NA	NA	327,048	327,048		13.9%	15.7%	2.9%	1.2%	17.2%	3.2%	1.3%
2					396,380	396,380		17.1%						
3					269,979	269,979		12.2%						
4					343,853	343,853		19.9%						
5					402,425	402,425		17.3%						
6					307,757	307,757		13.6%						
1	Adapter ligation	Ligation reaction	40	2.5	65,147	1,042,352	977,205	44.4%	54.2%	10.7%	4.4%	59.7%	11.8%	4.8%
2	Figure 2B				65,599	1,049,584	983,985	45.4%						
3					73,492	1,175,872	1,102,380	52.9%						
4					79,875	1,278,000	1,198,125	73.9%						
5					76,317	1,221,072	1,144,755	52.4%						
6					79,746	1,275,936	1,196,190	56.4%						
1		Binding buffer	237.5	50	6,801	32,305		3.3%	3.2%	0.6%	0.2%	3.0%	0.5%	0.2%
2					6,041	28,695		2.9%						
3					9,285	44,104		4.0%						
4					6,468	30,723		2.6%						
5					6,554	31,132		2.7%						
6					9,250	43,938		3.7%						
1		Wash buffer	750	50	32	480		0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
2					17	255		0.0%						
3					34	510		0.0%						
4					25	375		0.0%						
5					29	435		0.0%						
6					45	675		0.1%						

Supplementary Table 2. Scintillation Measurements of the First Four Library Preparation Steps

1		Column	NA	NA	144,259	144,259		14.8%	14.1%	1.2%	0.5%	13.4%	1.2%	0.4%
2					131,418	131,418		13.4%						
3					134,490	134,490		12.2%						
4					168,637	168,637		14.1%						
5					180,419	180,419		15.8%						
6					172,428	172,428		14.4%						
1		Eluate	25	2.5	106,304	1,063,040		108.8%	88.1%	12.4%	5.1%	83.6%	11.7%	4.8%
2					90,746	907,460		92.2%						
3					95,472	954,720		86.6%						
4					98,406	984,060		82.1%						
5					81,602	816,020		71.3%						
6					104,435	1,044,350		87.3%						
1	Library immobili-zation	Beads	47.5	2.5	45,444	863,436	817,992							
2	Figure 2C				39,526	750,994	711,468							
3					43,626	828,894	785,268							
4					43,927	834,613	790,686							
5					38,118	724,242	686,124							
6					43,434	825,246	781,812							
1		Supernatant	45	40	451,012	507,389		62.0%	60.1%	3.5%	1.4%	58.7%	3.4%	1.4%
2					381,316	428,981		60.3%						
3					434,967	489,338		62.3%						
4					384,041	432,046		54.6%						
5					350,177	393,949		57.4%						
6					444,806	500,407		64.0%						
1		First wash	100	90	6,613	7,348		0.9%	1.2%	0.4%	0.2%	1.2%	0.4%	0.2%
2					4,383	4,870		0.7%						
3					10,950	12,167		1.5%						
4					8,913	9,903		1.3%						
5					6,903	7,670		1.1%						
6					12,106	13,451		1.7%						
1		Second wash	100	90	1,339	1,488		0.2%	0.3%	0.1%	0.1%	0.3%	0.1%	0.1%
2					1,180	1,311		0.2%						
3					2,344	2,604		0.3%						
4					1,645	1,828		0.2%						
5					1,623	1,803		0.3%						
6					3,966	4,407		0.6%						
1	Fill-in reaction	Fill-in reaction	50	2.5	15,862	317,240	301,378	38.8%	40.8%	3.0%	1.2%	39.9%	2.9%	1.2%
2	Figure 2D				14,581	291,620	277,039	41.0%						
3					15,094	301,880	286,786	38.4%						
4					18,006	360,120	342,114	45.5%						
5					14,799	295,980	281,181	43.1%						
6					14,867	297,340	282,473	38.0%						
1		Supernatant	47.5	30	6,235	9,872		3.3%	3.3%	0.4%	0.2%			
2					5,121	8,108		2.9%						
3					5,920	9,373		3.3%						
4					6,694	10,599		3.1%						

Supplementary Table 2. Scintillation Measurements of the First Four Library Preparation Steps

5				5,979	9,467	3.4%				
6				7,387	11,696	4.1%				
1	First wash	100	90	338	376	0.1%	0.2%	0.0%	0.0%	
2				464	516	0.2%				
3				499	554	0.2%				
4				380	422	0.1%	0.1%	0.1%	0.0%	
5				447	497	0.2%				
6				608	676	0.2%				
1	Second wash	100	90	192	213	0.1%				
2				111	123	0.0%				
3				160	178	0.1%				
4				188	209	0.1%				
5				135	150	0.1%				
6				587	652	0.2%				
1	Stayed on the beads	NA	NA	NA	290,917	96.5%	96.4%	0.5%	0.2%	
2				NA	268,292	96.8%				
3				NA	276,680	96.5%				
4				NA	330,884	96.7%				
5				NA	271,068	96.4%				
6				NA	269,449	95.4%				

The first four steps are (step 1) end polishing, (step 2) adaptor ligation, (step 3) library immobilization, and (step 4) fill-in reaction. Column I indicates the number of the six libraries made in parallel. Column II shows the step in the library preparation process and the corresponding position of that step in Figure 2. Each step of the library preparation process comprises several fractions that are described in column III. The volumes of the fractions are shown in column IV. The volumes of the aliquots that were taken from each component for scintillation measurements are given in column V, and the values of the scintillation measurements are shown in column VI as counts per minute (cpm). From this, cpms were inferred for the entire fraction from which the aliquot had been taken (column VII). The first fraction in each of the four steps (column VIII) represents 100% of the radioactivity (RA) of that step. Column IX shows each fraction's share of the radioactivity present in each step. From the six percentages in each step in column IX, the average (column X), standard deviation (STDEV, column XI) and standard error of the mean (SEM, column XII) were calculated. In order to depict them in Figure 1, the average (Average_n), standard deviation (STDEV_n), and standard error of the mean (SEM_n) were normalized (columns XIII, XIV, XV, respectively) so that the averages of each step total 100%. In the fill-in reaction step, normalization was omitted; instead, "Stayed on the beads" cpm (fraction) was calculated by subtracting the cpm (fraction) values of the supernatant, first wash and second wash from the fill-in reaction cpm (fraction).

Supplementary Table 3. Scintillation Measurements of the NaOH Treatment Step

I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
Library number	Library preparation step	Fraction	V_fraction (µL)	V_aliquot (µL)	cpm (aliquot)	cpm (fraction)	100% RA of the step	% of RA entering each step	Average	STDEV	SEM	Average_n	STDEV_n	SEM_n
1	NaOH treatemnt	Stayed on the beads					290,917							
2	Figure 2E						268,292							
3							276,680							
1		First incubation	50	2.5	120	133		0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
2					104	116		0.0%						
3					76	84		0.0%						
1		Second incubation	50	2.5	39	43		0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%
2					160	178		0.1%						
3					43	48		0.0%						

Columns I–XV are in the same order as in Supplementary Table 2. Library-containing beads that are described in Supplementary Table 2 (library numbers 1–3) were taken after the fill-in step, and two NaOH treatments were performed.

Supplementary Table 4. Scintillation Measurements of the Heat Treatment Step

I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
Library number	Library preparation step	Fraction	V_fraction (µL)	V_ aliquot (µL)	cpm (aliquot)	cpm (fraction)	100% RA of the step	% of RA entering each step	Average	STDEV	SEM	Average_n	STDEV_n	SEM_n
4	Heat treatment	Stayed on the beads						330,884						
5	Figure 2F							271,068						
6								269,449						
4		First heat treatment	47.5	40	243,439	289,084	91.6%		89.9%	3.0%	1.8%	93.6%	3.6%	2.1%
5					200,061	237,572	91.7%							
6					185,671	220,484	86.4%							
4		Second heat treatment	50	40	9,379	11,724	3.7%		4.6%	1.2%	0.7%	4.8%	1.2%	0.7%
5					8,618	10,773	4.2%							
6					12,288	15,360	6.0%							
4		Remained on the beads	110	110	1,013	1,013	0.3%		0.5%	0.2%	0.1%	0.0%	0.0%	0.0%
5					1,368	1,368	0.5%							
6					2,016	2,016	0.8%							
4		Tubes	NA	NA	851	851	0.3%		1.0%	1.1%	0.6%	1.0%	1.1%	0.6%
5					1,411	1,411	0.5%							
6					5,650	5,650	2.2%							

Column I–XV are in the same as in Supplementary Table 2. Library-containing beads that are described in Supplementary Table 2 (library numbers 4–6) were taken after the fill-in step and two heat treatments were performed.