Supplementary material, Carøe *et al.* "Single-tube library preparation for degraded DNA"

Overview of oligonucleotides

#	Sequence 5'-3'	Name	Use	Ref.
1	G*A*T*C*GGAA*G*A*G*C[C3spacer]	IS3_ATDC3	For ATDC3 adapter	Present paper
2	A*G*A*T*CGGAA*G*A*G*C[C3spacer]	IS3_BEDC3	For BEDC3 adapter	Present paper
3	A*G*A*T*CGGAA*G*A*G*C	IS3	For BEMC adapter	Meyer and Kircher (2010)
4	A*C*A*C*TCTTTCCCTACACGACGCTCTTCCG*A*T*C*T	IS1	For all adapters	Meyer and Kircher (2010)
5	G*T*G*A*CTGGAGTTCAGACGTGTGCTCTTCCG*A*T*C*T	IS2	For all adapters	Meyer and Kircher (2010)
6	ACACTCTTTCCCTACACGAC	IS7	qPCR	Meyer and Kircher (2010)
7	GTGACTGGAGTTCAGACGTGT	IS8	qPCR	Meyer and Kircher (2010)
8	CAAGCAGAAGACGGCATACGAGATNNNNNNGTGACTGGAGTTCAGACGTGT GCTCTTCCGATCT	P7 index primer	Index PCR	Present paper
9	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCC GATC*T	P5 common primer	Index PCR	Present paper
10	A*C*ACTCTTTCCCTACACGACGCTCTTCCGAT*C*T	Illumina short Y-shaped P5	For Y-shaped adapter	Present paper
11	G*A*TCGGAAGAGCACACGTCTGAACTCCAGTC*A*C	Illumina short Y-shaped P7	For Y-shaped adapter	Present paper
12	5'OMedT*AGATCGGAAGAG*C [C3spacer]	IS3_BEDC3_V1	For BEDC3_V1 adapter	Present paper
13	5'OMedT*ACACTCTTTCCCTACACGACGCTCTTCCGATCT*A	IS1_BEDC3_V1	For BEDC3_V1 adapter	Present paper
14	5'OMedT*GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT*A	IS2_BEDC3_V1	For BEDC3_V1 adapter	Present paper
15	AGATCGGAAGAGC[C3spacer]	IS3_BEDC3_V2	For BEDC3_V2 adapter	Present paper

Table S1. Synthetic DNA oligos used in this study.*=Phosphothioate linkage. N=index. All oligos were synthesized at Biomers, Germany, HPLC purified, shipped dry and resuspended in 10 mM Tris-HCl

Sample overview

Sample#	Sample ID	Species	Collected Year A.D.	Age determination	Origin	Tissue type	Average length DNA (bp)
1	CN2047	Wolf	1923	Collected	Arctic	Hide	117
2	CN2045	Wolf	1923	Collected	Arctic	Hide	132
3	CN1766	Wolf	1922	Collected	Arctic	Hide	184
4	CN1239	Wolf	1908	Collected	Arctic	Hide	129
5	CN3134	Wolf	1939	Collected	Arctic	Hide	69
6	CN1921	Wolf	1925	Collected	Arctic	Hide	58
7	CN214	Wolf	Before 1869	Registered	Arctic	Hide	87
8	CN2044	Wolf	1922/ 23	Collected	Arctic	Hide	80

Table S2. Samples collected at the Natural History Museum of Denmark. Average length of DNA was measured on an Agilent 2100 Bioanalyzer in the interval 35-1000bp. As seen from Figure S1 (below), many samples had fragments below the lower marker of the Bioanalyzer. However, when measuring the average length, this was left out not to include the lower marker in the measurement. The true average fragment length might therefore be lower for some samples than the value indicated in the present table.

DNA extraction

DNA extraction was performed by pre-digesting samples for one hour at 56°C in one mL extraction buffer (Gilbert *et al.* 2007) and subsequently digesting overnight in fresh buffer. The digest was purified with 1× volume phenol (pH 8.0), followed by 1× volume chloroform:isoamylalcohol (24:1). The supernatant was mixed with 10× volume of customized PB buffer (Allentoft *et al.* 2015), and purified with MinElute columns (Qiagen, Hilden, Germany) with Zymo-spin extension reservoirs (Zymo Research, California, USA) (Dabney *et al.* 2013). DNA was eluted twice with 25 µL EB buffer and fragment length estimations of DNA extracts were performed with an Agilent 2100 Bioanalyzer (Agilent Technologies, CA, USA).

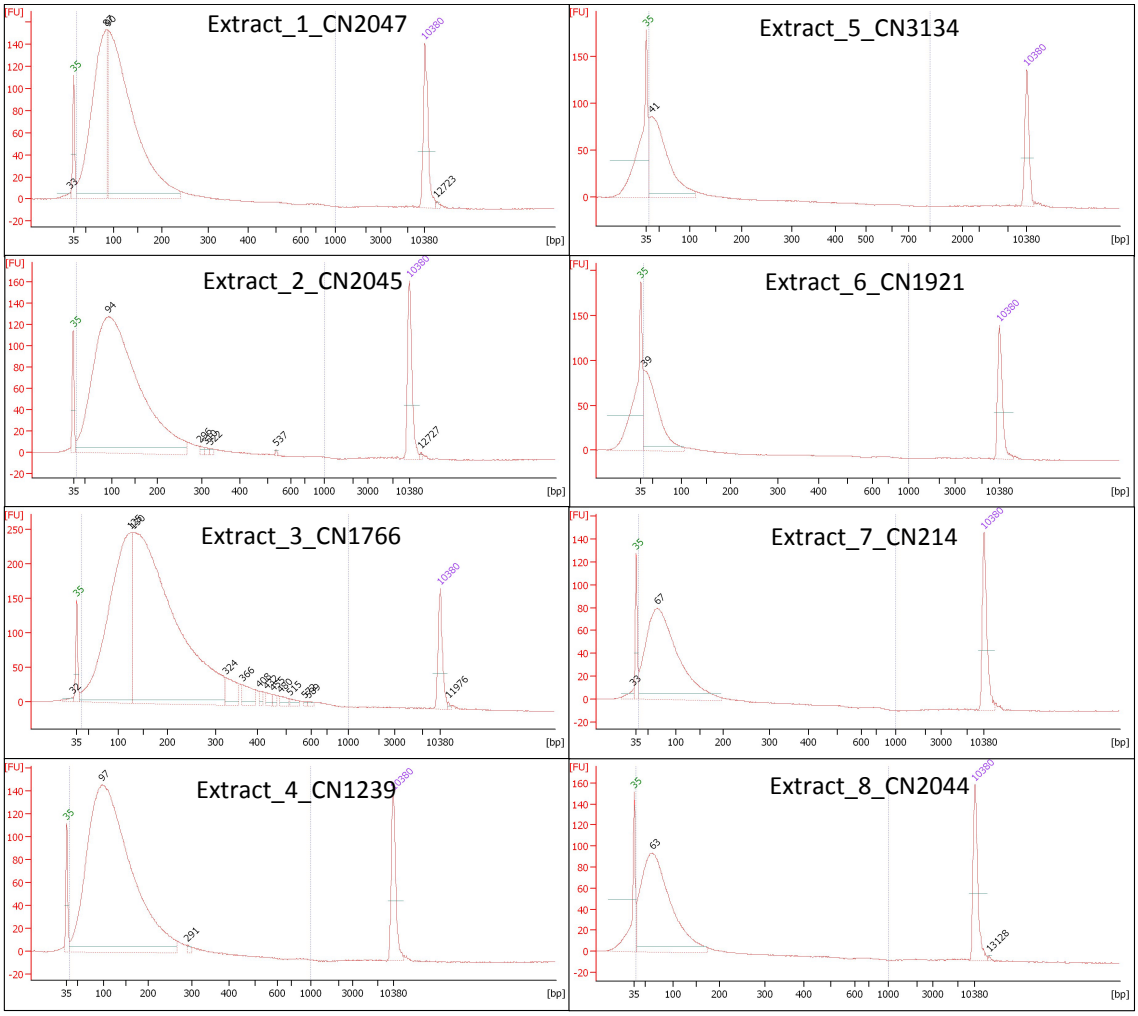

Figure S1. Size profiles from extracts of all samples, produced with an Agilent 2100 Bioanalyzer using HS chip and reagents and with appropriate dilution of the samples prior to loading to avoid overloading of the chip.

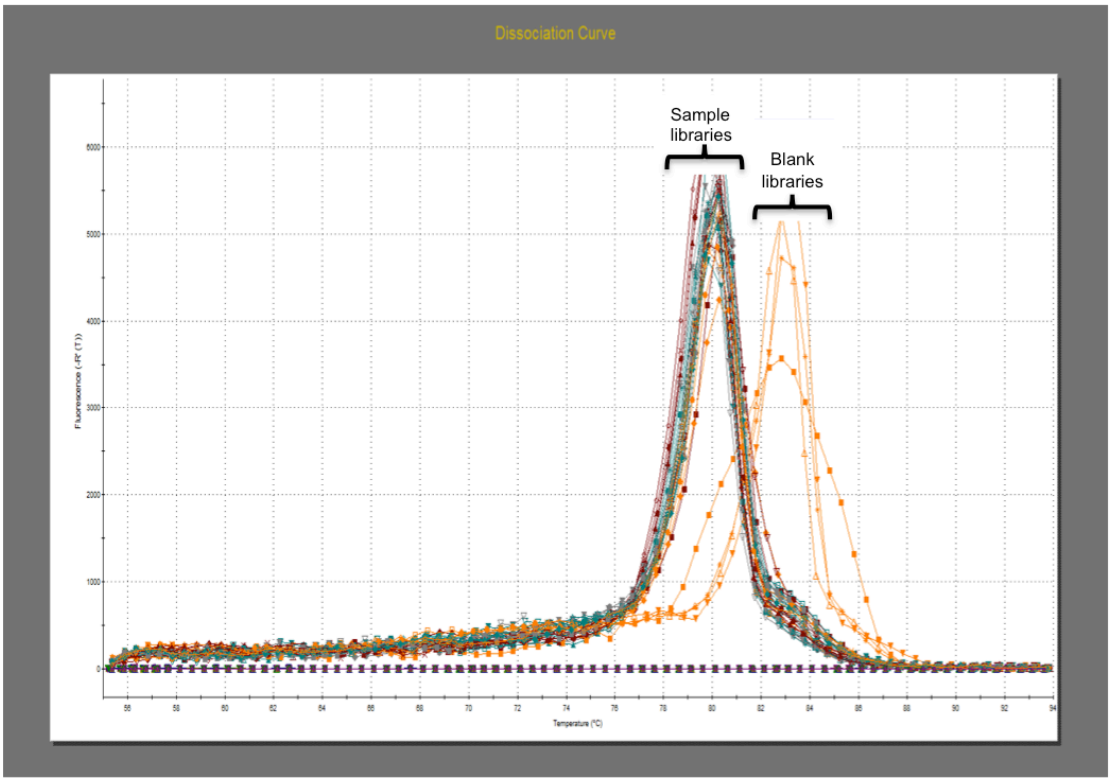

Figure S2. Dissociation curves obtained after qPCR. Sample libraries and blank libraries are indicated with brackets and show two distinct products. X-axis shows temperature and Y-axis shows fluorescence signal. It is noteworthy that the blank samples have a higher melting point, which often is interpreted as longer fragment length. However, we consistently observe this phenomenon using our qPCR setup, even if blanks are subsequently measured to be shorter using gel electrophoresis.

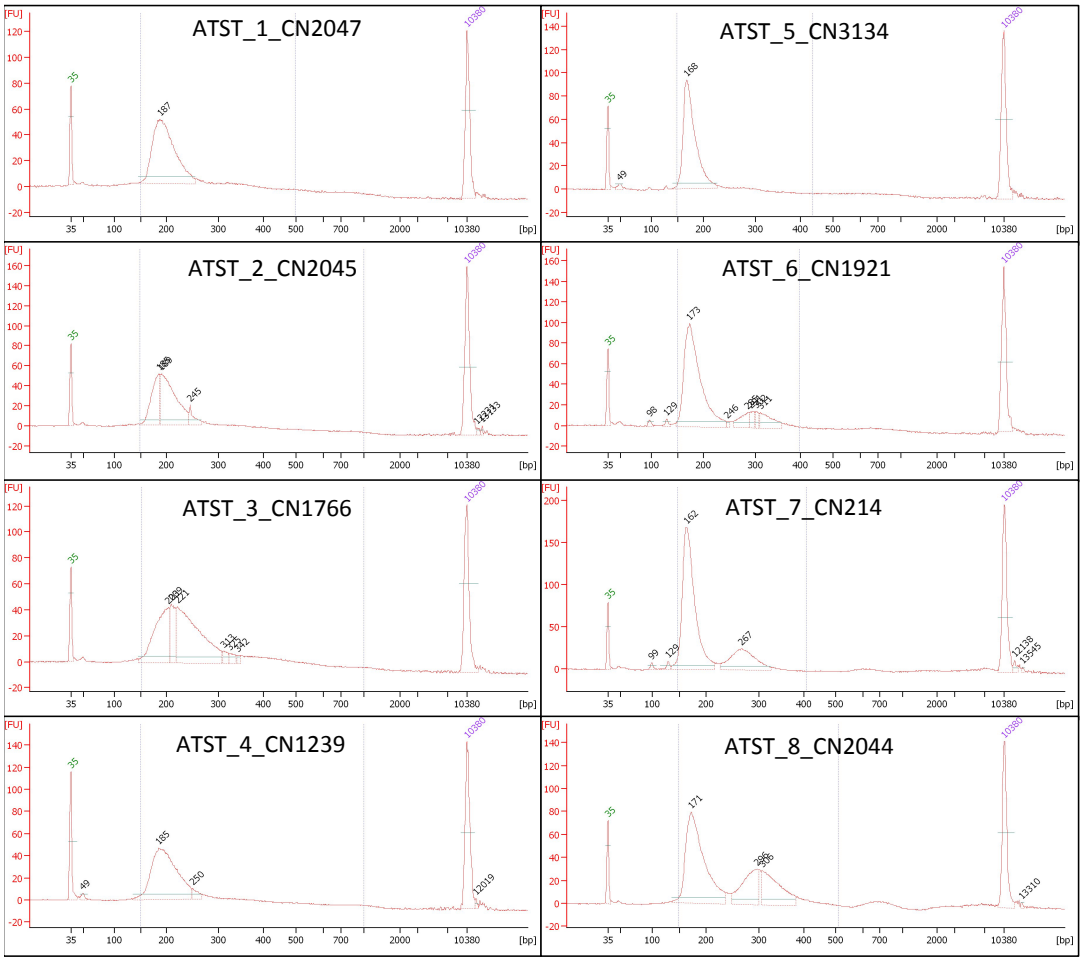

Figure S3. Size profiles of index-amplified libraries from all samples produced with the ATST method. Produced on an Agilent 2100 Bioanalyzer using HS chip and reagents and with appropriate dilution of the samples prior to loading to avoid overloading of the chip.

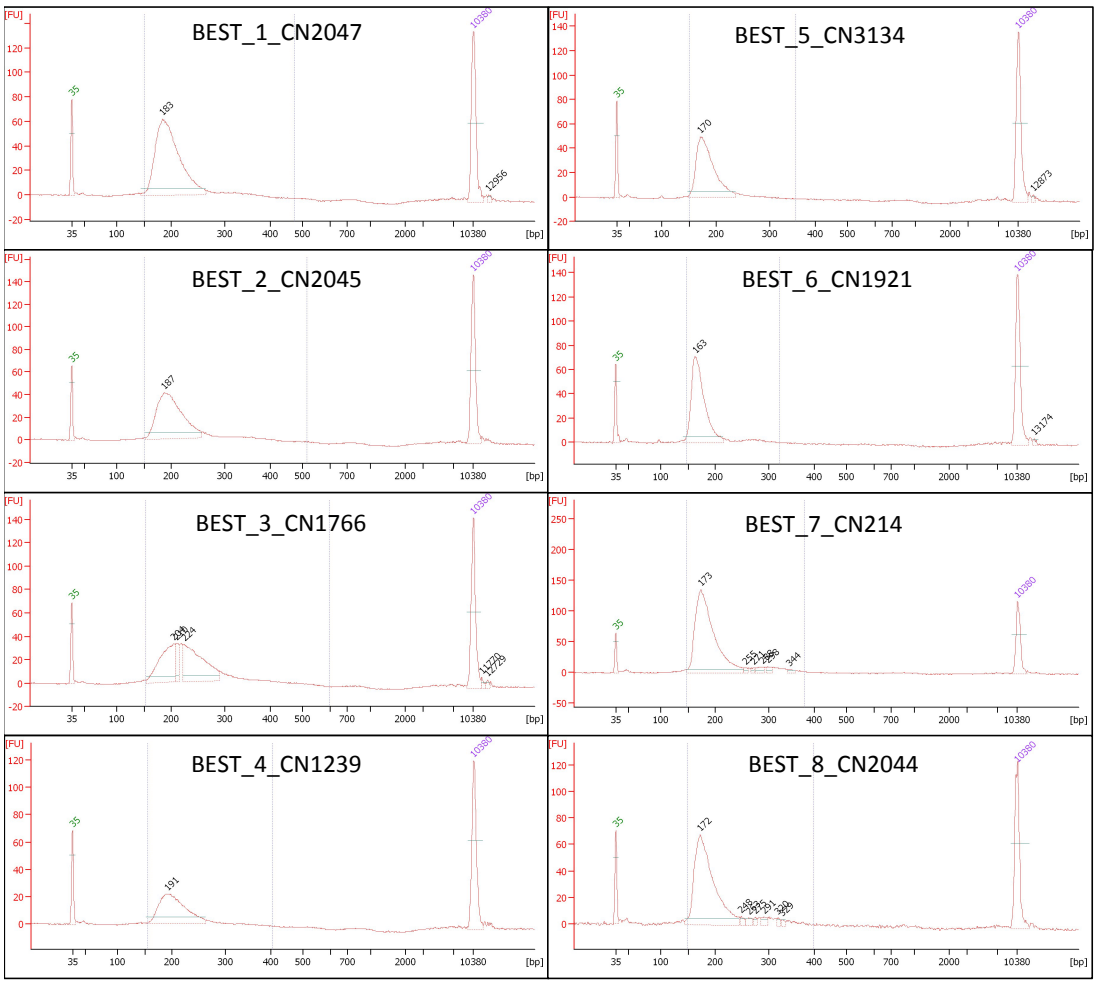

Figure S4. Size profiles of index-amplified libraries from all samples produced with the BEST method. Produced on an Agilent 2100 Bioanalyzer using HS chip and reagents and with appropriate dilution of the samples prior to loading to avoid overloading of the chip.

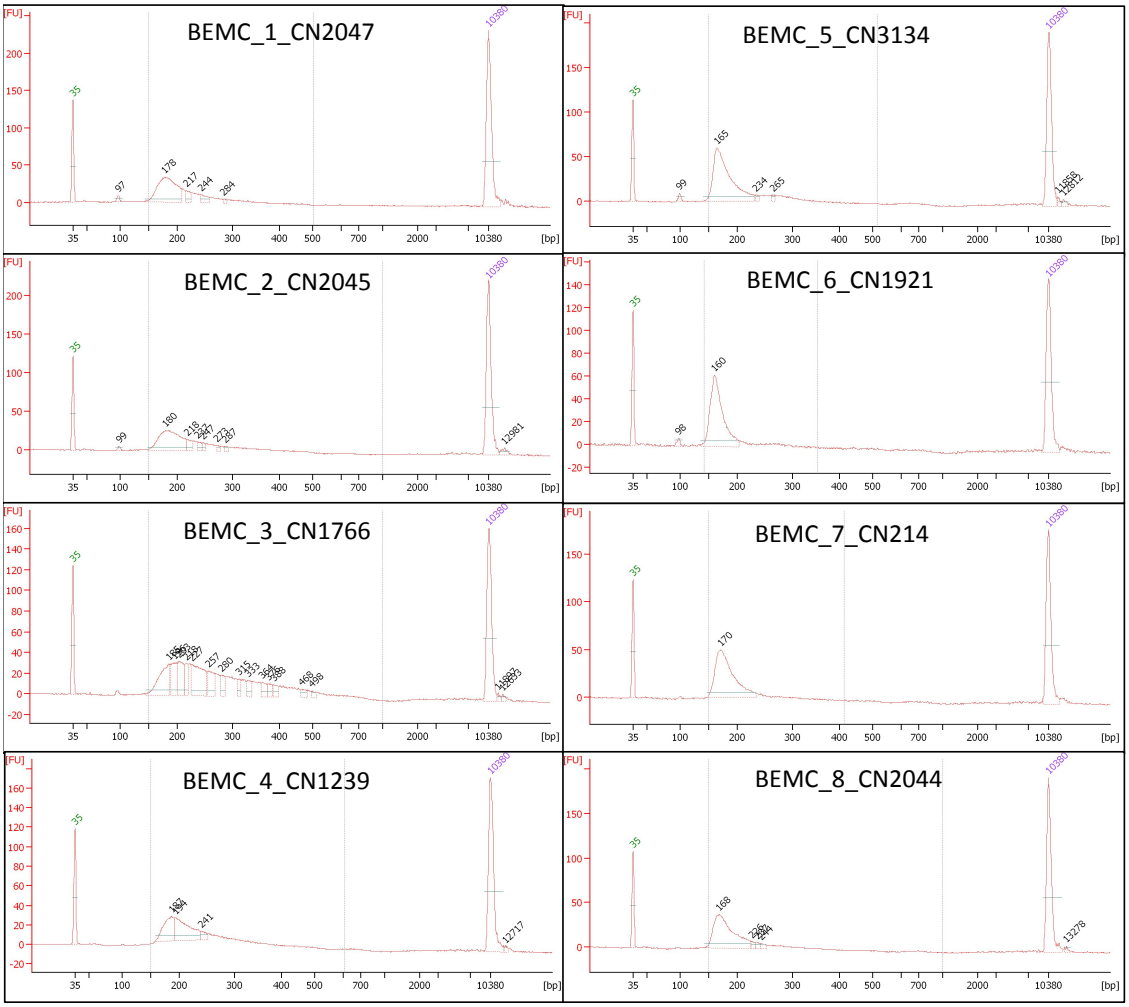

Figure S5. Size profiles of index amplified libraries from all samples produced with the BEMC method. Produced on an Agilent 2100 Bioanalyzer using HS chip and reagents and with appropriate dilution of the samples prior to loading to avoid overloading of the chip.

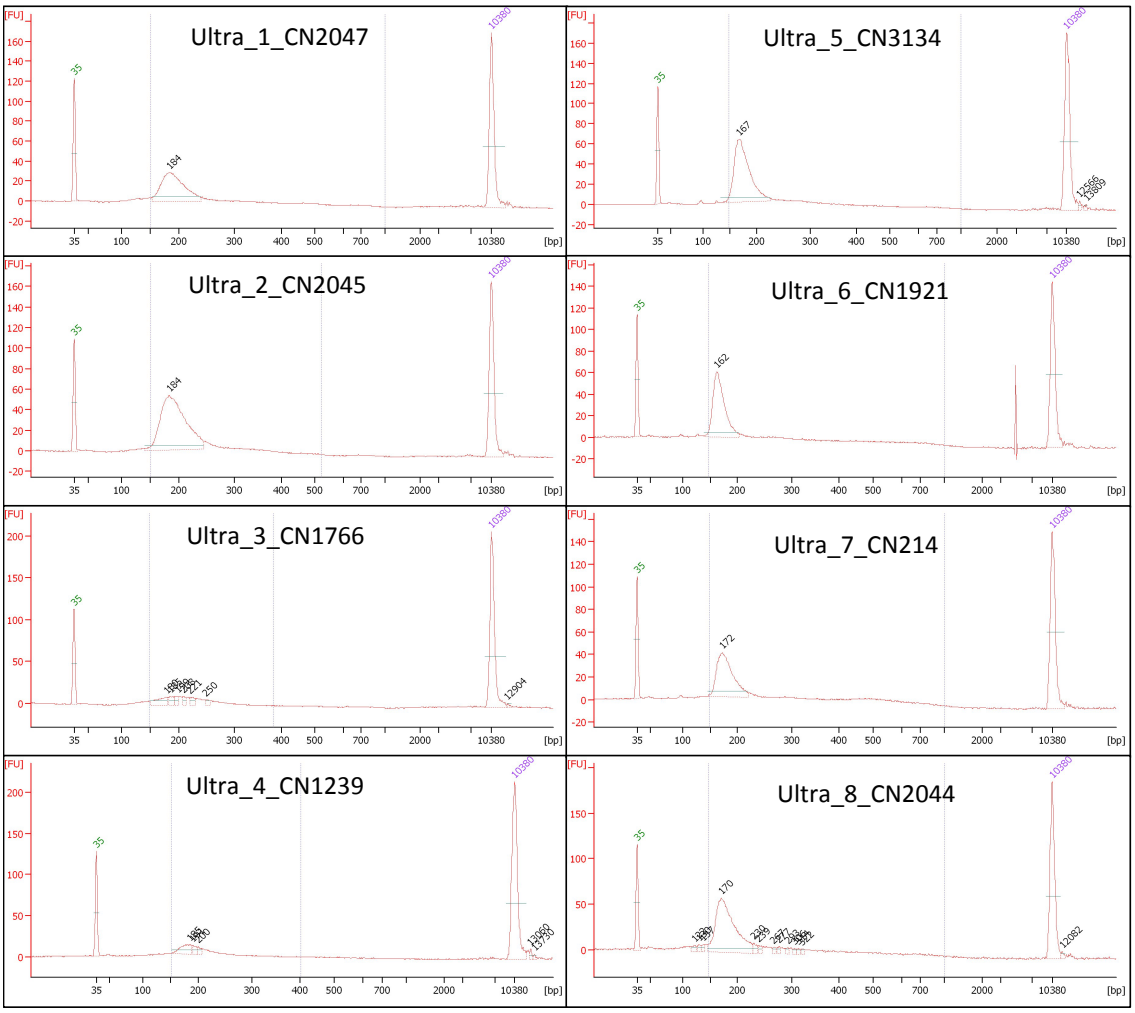

Figure S6. Size profiles of index amplified libraries from all samples produced with the Ultra method. Produced on an Agilent 2100 Bioanalyzer using HS chip and reagents and with appropriate dilution of the samples prior to loading to avoid overloading of the chip.

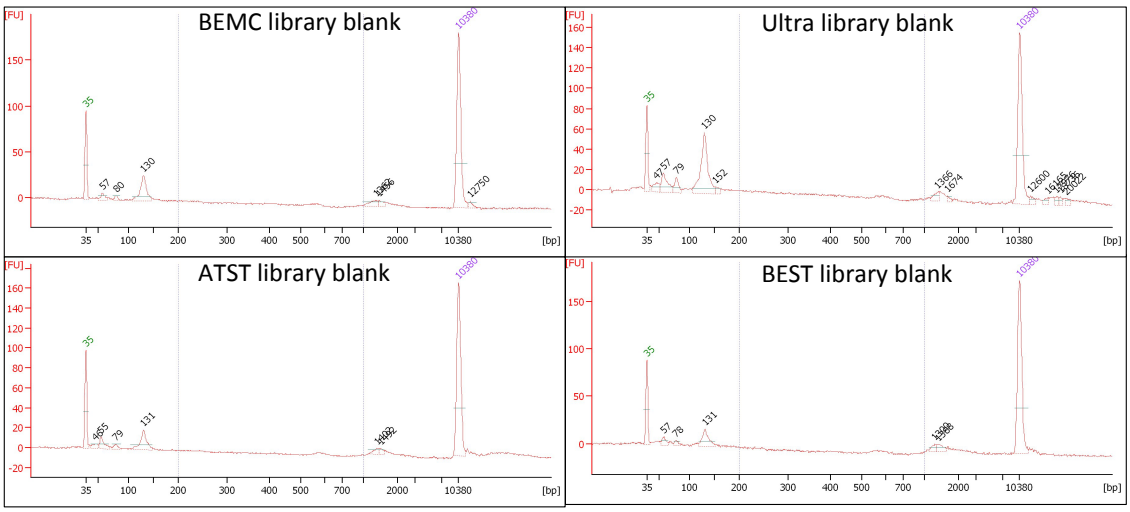

Figure S7. Size profiles of index amplified blank libraries. Produced on an Agilent 2100 Bioanalyzer using HS chip and reagents and with appropriate dilution of the samples prior to loading to avoid overloading of the chip. We interpret the peaks at ~130 bp as adapter-dimer and smaller peaks as various primer-dimer background.

Overview of pre-sequencing parameters and statistics

Sample	Input ng	qPCR Ct	Index cycles	Ct - index	PCR yield nM
ATST - 1	77.5	13.9	11	2.9	5.4
ATST - 2	65.0	14.6	12	2.6	3.2
ATST - 3	80.0	13.5	11	2.5	5.6
ATST - 4	80.0	14.2	11	3.2	4.1
ATST - 5	2.4	18.1	15	3.1	4.3
ATST - 6	4.2	19.5	16	3.5	5.8
ATST - 7	6.0	16.5	14	2.5	6.6
ATST - 8	8.28	14.8	12	2.8	6.7
Average	**40.42**	**15.64**	**12.75**	**2.89**	**5.21**
STDEV	**37.96**	**2.18**	**1.98**	**0.36**	**1.24**
BEST - 1	77.5	12.8	10	2.8	4.8
BEST - 2	65.0	12.9	10	2.9	3.6
BEST - 3	80.0	11.5	10	1.5	4.2
BEST - 4	80.0	12.7	10	2.7	2.1
BEST - 5	2.4	18.1	15	3.1	3
BEST - 6	4.2	20.3	18	2.3	3.4
BEST - 7	6.0	16.8	15	1.8	10.4
BEST - 8	8.28	15.3	14	1.3	5.2
Average	**40.42**	**15.05**	**12.75**	**2.30**	**4.59**
STDEV	**37.96**	**3.11**	**3.15**	**0.69**	**2.55**
BEMC-1	77.5	18.6	16	2.6	2.1
BEMC-2	65.0	18.8	16	2.8	1.9
BEMC-3	80.0	17.2	15	2.2	4.6
BEMC-4	80.0	18.1	16	2.1	2.7
BEMC-5	2.4	20.2	18	2.2	3.1
BEMC-6	4.2	21.6	18	3.6	2.8
BEMC-7	6.0	19.1	16	3.1	2.7
BEMC-8	8.28	16.7	14	2.7	2.2
Average	**40.42**	**18.79**	**16.13**	**2.66**	**2.76**
STDEV	**37.96**	**1.58**	**1.36**	**0.51**	**0.85**
Ultra - 1	77.5	13.9	11	2.9	2.2
Ultra - 2	65.0	14.3	11	3.3	4
Ultra - 3	80.0	9.1	6	3.1	0.9
Ultra - 4	80.0	12.7	10	2.7	1
Ultra - 5	2.4	18.6	16	2.6	3.3
Ultra - 6	4.2	18.4	15	3.4	3.3
Ultra - 7	6.0	15.9	14	1.9	3
Ultra - 8	8.28	13.9	11	2.9	3.3
Average	**40.42**	**14.60**	**11.75**	**2.85**	**2.63**
STDEV	**37.96**	**3.10**	**3.20**	**0.47**	**1.15**

Table S3. Overview of pre-sequencing parameters with DNA input in nanograms, Ct values from qPCR, number of PCR cycles given in index amplification, the Ct value minus the number of index cycles given and yield of index amplification as measured on a Bionanalyzer 2100 using HS chip and reagents in the 150-1000bp interval.

Sequencing statistics

Method	Total reads	Reads retained	Read length	% Clonality	% Uniq mapped	Est.Cov.5M	GC content
ATST - 1	8,671,425	8,605,093	53.0	2.3	**56.1**	0.06	0.42
ATST - 2	18,947,111	18,866,134	70.5	1.7	**78.4**	0.12	0.44
ATST - 3	11,280,946	11,235,451	81.3	1.2	**77.2**	0.14	0.50
ATST - 4	9,775,914	9,356,406	42.9	6.6	34.6	0.03	0.45
ATST - 5	15,482,036	15,376,847	54.9	1.7	**17.4**	0.02	0.43
ATST - 6	13,832,231	13,769,418	66.0	1.4	**77.3**	0.11	0.44
ATST - 7	27,048,757	26,933,236	69.0	2.2	**74.1**	0.11	0.48
ATST - 8	8,904,037	8,759,865	54.8	9.5	15.2	0.02	0.41
Average	**14,242,807**	**14,112,806**	**61.5**	**3.3**	**53.8**	**0.08**	**0.45**
STDEV	**6,280,305**	**6,308,324**	**12.3**	**3.0**	**27.5**	**0.05**	**0.03**
BEST - 1	23,489,375	22,967,243	51.1	3.4	54.7	0.06	0.53
BEST - 2	18,306,760	18,213,690	69.3	1.4	**77.8**	0.12	0.46
BEST - 3	18,918,434	18,822,347	80.4	1.2	**76.7**	0.14	0.44
BEST - 4	23,554,072	22,039,356	40.7	12.2	32.5	0.03	0.44
BEST - 5	27,107,807	26,928,686	54.2	1.8	**17.1**	0.02	0.50
BEST - 6	15,002,348	14,922,217	65.1	1.3	75.9	0.11	0.48
BEST - 7	21,226,275	21,078,457	67.7	1.6	**73.7**	0.11	0.44
BEST - 8	5,398,637	5,259,643	47.7	5.1	**17.4**	0.02	0.45
Average	**19,125,464**	**18,778,955**	**59.5**	**3.5**	**53.2**	**0.08**	**0.47**
STDEV	**6,686,089**	**6,522,986**	**13.2**	**3.8**	**27.0**	**0.05**	**0.03**
BEMC-1	14,170,498	13,946,825	53.2	8.5	46.7	0.05	0.44
BEMC-2	13,476,033	13,370,066	74.0	5.4	59.3	0.09	0.45
BEMC-3	11,703,451	11,625,881	82.5	2.8	62.0	0.11	0.50
BEMC-4	12,965,492	11,807,500	41.1	20.5	26.4	0.02	0.45
BEMC-5	15,605,937	15,363,841	57.2	4.2	11.9	0.01	0.44
BEMC-6	9,729,921	9,636,896	68.2	8.4	57.9	0.08	0.46
BEMC-7	10,579,969	10,493,548	71.2	10.0	53.8	0.08	0.54
BEMC-8	17,964,387	17,445,717	49.2	17.4	9.4	0.01	0.47
Average	**13,274,461**	**12,961,284**	**62.1**	**9.7**	**40.9**	**0.06**	**0.47**
STDEV	**2,690,255**	**2,598,808**	**14.1**	**6.3**	**21.8**	**0.04**	**0.03**
Ultra - 1	14,283,373	14,118,133	51.0	3.5	54.2	0.06	0.50
Ultra - 2	14,581,467	14,507,838	66.8	1.4	76.9	0.11	0.44
Ultra - 3	8,900,350	8,861,237	79.0	1.0	75.2	0.13	0.54
Ultra - 4	10,668,854	10,006,211	40.7	4.7	**35.6**	0.03	0.47
Ultra - 5	12,608,571	12,498,586	51.9	1.4	16.4	0.02	0.44
Ultra - 6	12,172,268	12,090,368	63.1	1.3	75.7	0.11	0.45
Ultra - 7	16,196,457	16,110,102	65.5	1.3	73.1	0.11	0.50
Ultra - 8	12,245,621	11,819,820	50.6	10.6	14.8	0.02	0.44
Average	**12,707,120**	**12,501,537**	**58.6**	**3.2**	**52.7**	**0.07**	**0.47**
STDEV	**2,309,236**	**2,383,653**	**12.2**	**3.3**	**27.0**	**0.05**	**0.04**

Table S4. Sequencing statistics with total reads obtained from HiSeq sequencing, reads retained after trimming and filtering, average read length of all retained reads, clonality as percentage of retained reads mapped to reference genome, percentage of reads mapping uniquely to reference genome and estimated coverage from 5 million reads.

Statistics - t-test

	BEMC vs BEST	BEST vs ATST	ATST vs Ultra	BEMC vs ATST	BEMC vs Ultra	BEST vs Ultra
RT-PCR	0.0016*	0.1768	0.0911	0.0001*	0.0006*	0.3971
corr.	0.0097*	1.0608	0.5463	0.0005*	0.0034*	2.3826
Conc.	0.102	0.402	0.0038*	0.0016*	0.8342	0.0687
corr.	0.612	2.412	0.0228*	0.0093*	5.0052	0.412

Clonality	0.0017*	0.8664	0.6205	0.0025*	0.0041*	0.7857
corr.	0.0087*	4.332	3.1025	0.0123*	0.0206*	3.9285
Mapping	0.0007*	0.2581	0.0205*	0.0007*	0.0008*	0.4465
corr.	0.0045*	1.5486	0.1229	0.0042*	0.0049*	2.679
GC%	0.4163	0.0047*	0.0335*	0.0035*	0.5878	0.6982
corr.	2.4978	0.0282*	0.2009	0.0212*	3.5268	4.1892
Read length	0.001035*	0.03185*	0.0000151*	0.6152	0.01136*	0.1747
corr.	0.00621*	0.1911	0.0000906*	3.6912	0.06816	1.0482

Table S5. Based on values presented in Table S3 and S4. Paired t-test with and without Bonferroni correction (corr.). Bonferroni correction was done by multiplying *p*-values with the number of comparisons (6). Numbers with an asterisk indicate significance at the 0.05 level. GC% is for all reads in the sample. Clonality is measured for mapped reads. Mapping refers to percentage of reads mapping uniquely to the reference genome. qPCR refers to Ct values measured from RT-PCR. "Conc." refers to nM of amplified libraries measured on an Agilent 2100 Bioanalyzer.

Sequencing statistics - normalized read numbers

Method	Reads retained	Read length	% Clonality	% Uniq mapped	Est.Cov.5M	GC content
ATST - 1	5,374,238	66.0	0.7	77.9	0.12	0.42
ATST - 2	5,375,474	69.0	0.6	75.2	0.12	0.44
ATST - 3	5,376,782	81.3	0.7	77.6	0.15	0.50
ATST - 4	5,375,501	70.5	0.6	79.3	0.13	0.45
ATST - 5	5,310,914	54.8	6.6	15.7	0.02	0.43
ATST - 6	5,167,220	42.9	3.9	35.6	0.03	0.43
ATST - 7	5,357,333	53.0	1.6	56.5	0.07	0.48
ATST - 8	5,361,901	54.9	0.9	17.5	0.02	0.41
Average	**5,337,420**	**61.5**	**2.0**	**54.4**	**0.08**	**0.44**
STDEV	**72,180**	**12.3**	**2.2**	**27.7**	**0.05**	**0.03**
BEST - 1	5,369,771	65.1	0.6	76.4	0.12	0.43

BEST - 2	5,361,082	67.7	0.6	74.5	0.12	0.45
BEST - 3	5,371,133	80.4	0.5	77.2	0.15	0.53
BEST - 4	5,371,318	69.3	0.5	78.5	0.13	0.46
BEST - 5	5,259,643	47.7	5.1	17.4	0.02	0.44
BEST - 6	5,051,443	40.7	3.3	35.7	0.03	0.44
BEST - 7	5,278,318	51.1	1.2	55.9	0.07	0.50
BEST - 8	5,362,855	54.2	0.8	17.3	0.02	0.48
Average	**5,303,195**	**59.5**	**1.6**	**54.1**	**0.08**	**0.47**
STDEV	**111,159**	**13.2**	**1.7**	**27.0**	**0.05**	**0.03**
BEMC-1	5,347,140	68.3	4.9	60.2	0.09	0.44
BEMC-2	5,354,500	71.2	5.4	56.6	0.09	0.45
BEMC-3	5,362,571	82.5	1.4	63.0	0.12	0.53
BEMC-4	5,356,330	74.0	2.3	61.3	0.1	0.47
BEMC-5	5,242,772	49.2	6.8	10.7	0.01	0.44
BEMC-6	4,917,567	41.1	9.7	30.0	0.02	0.45
BEMC-7	5,313,146	53.2	3.7	49.2	0.06	0.50
BEMC-8	5,314,789	57.2	1.7	12.2	0.02	0.45
Average	**5,276,102**	**62.1**	**4.5**	**42.9**	**0.06**	**0.47**
STDEV	**150,063**	**14.1**	**2.8**	**22.1**	**0.04**	**0.03**
Ultra - 1	5,362,271	63.1	0.7	76.2	0.11	0.44
Ultra - 2	5,369,833	65.5	0.6	73.7	0.11	0.46
Ultra - 3	5,374,957	79.0	0.7	75.4	0.14	0.54
Ultra - 4	5,371,633	66.8	0.6	77.5	0.12	0.47
Ultra - 5	5,210,835	50.6	5.8	15.6	0.02	0.44
Ultra - 6	5,063,283	40.7	2.6	36.4	0.03	0.45
Ultra - 7	5,336,330	51.0	1.6	55.3	0.07	0.50
Ultra - 8	5,351,513	51.9	0.8	16.5	0.02	0.44
Average	**5,305,082**	**58.6**	**1.7**	**53.3**	**0.08**	**0.47**
STDEV	**111,648**	**12.2**	**1.8**	**26.9**	**0.05**	**0.03**

Table S6. Sequencing statistics for all samples and all library preparation methods, with reads subsampled to match the sample with the lowest number of reads (5,398,637 reads for each sample/library method combination). It is noteworthy that the second column contains retained reads after filtering, explaining the minor differences between samples.

Production and removal of adapter-dimers

In a separate round of library building and in complete absence of sample DNA, additional blank libraries were made in duplicate for each sample to test the production of adapter-dimer using various adapters. This was also done using a Y-shaped adapter and an adapter similar to the one used for the BEST method but with blocked 5' ends (5'OMe-dT) to prevent phosphorylation and ligation of this end (Supplementary, Table S1). In addition, we tested whether addition of PEG4000 (1 μL, 50% stock) and BSA (1 μL, 20mg/mL stock) added to the end-repair reaction would affect adapter-dimer formation by stabilizing the T4 polynucleotide kinase and preventing proper inactivation (Figure S8). 1 μL of finished library was

amplified by qPCR as described in methods and materials, melting curve confirmed similar PCR product size for all libraries.
As expected the Y-adapter has a much lower Ct value due to the formation of a large amount of adapter. Because the adapters are precisely the expected length (Figure S9) it is likely that a ligation is the cause of the formation of the dimer, implying a phosphorylation of the otherwise phosphate-free adapter. Since the use of 5' blocking residues does not inhibit adapter-dimer formation, it is possible that the synthesis of the blocking agent was ineffective, the modification easily is lost or adapter-dimers arise in another context such as the fill-in reaction or PCR. We have subsequently produced and sequenced libraries using this adapter with both amount of library molecules and sequencing data turning out as expected (results not shown). Addition of PEG and BSA did not seem to affect adapter-dimer formation (Figure S8).

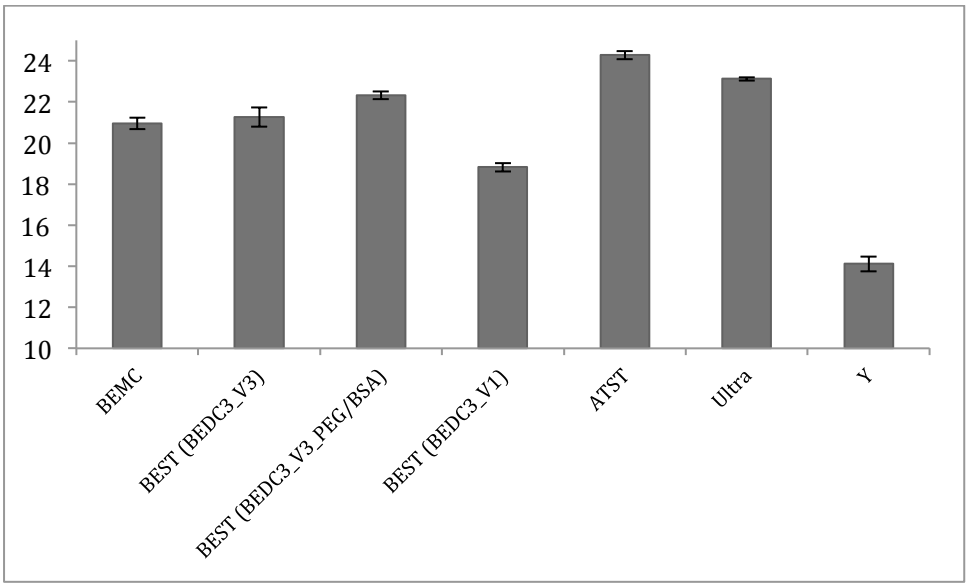

Figure S8. Plot from qPCR values on blank controls. Y-axis shows Ct value. N=2, bars represent average values with standard deviations. ANOVA was performed in R giving $F_{(6,7)}$=282.2, p-value: 5.508e-08. A Tukey test subsequently showed no significant difference (p>0.01) comparing Ultra to ATST and comparing BEDC3_V3_PEG+BSA to BEDC3_V3, BEMC and Ultra. All other comparisons were statistically significant (p<0.01).

The setup demonstrated in this paper, using the displacement adapter, was specifically chosen to overcome the problem of adapter-dimers, as known from Illumina's Y-shaped adapter (Bennett *et al.* 2014). However, adapter-dimers seem to occur in some cases and to our experience it varies considerably within the same method, and we have observed it in all 4 methods described in this paper (Figure S7), although as shown in Figure

S8, at much lower levels than the Y-shaped adapter (given that they amplify later in qPCR).

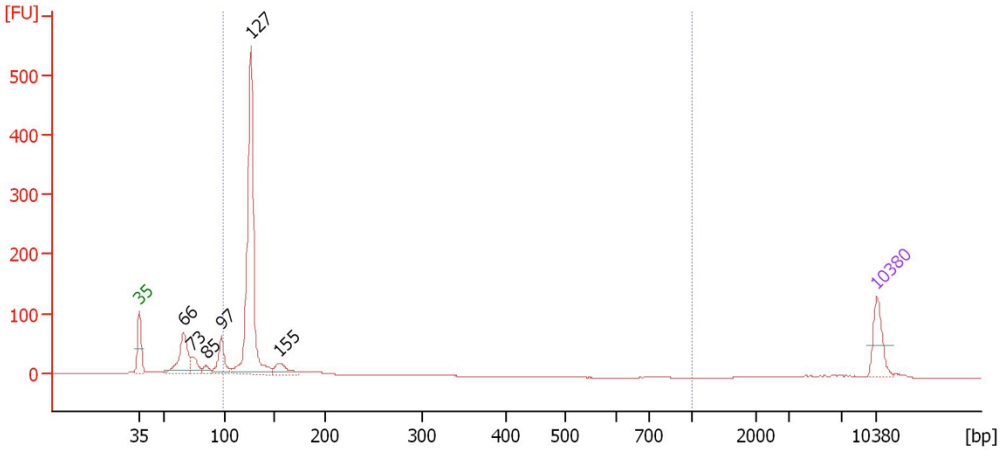

Figure S9. Example of adapter-dimer at 127 bp. The exact length of the expected dimer is 128, but due to variation of the Bioanalyzer 2100, the actual peak is often within ± 3 bases. This library was produced using the Nebnext E6070 (BEMC method) kit with 20% of the library amplified 20 cycles.

To enable a library build almost completely free of adapter-dimers, we tested whether it was possible to prevent or remove adapter-dimers from the library reaction. Normally this would be done using a purification method that separates the sequencing library from the adapter-dimer by size selection. However, this procedure is not suitable for very short fragments because the size gap between library and dimer is very small and removing adapter-dimer therefore also removes a considerable part of the library. As mentioned earlier, it can be speculated that the adapter-dimer in our setup, likely arises as a result of phosphorylation of the adapter, followed by ligation. This should create a dimer that is only double stranded at the middle with 12-24 bases (depending if one or both adapters are phosphorylated), and needs fill-in to work as a true library dimer and be amplified in PCR. If this holds true, one should be able to either block the 5' end of the adapter to prevent phosphorylation or to heat-denature the adapter-dimer once formed and remove the ligated oligos with a single-strand exonuclease.

In general, we have observed adapter-dimer formation for displacement adapters to vary greatly, and overall the BEMC seems to produce very few adapter-dimers although this is difficult to estimate due to the inefficiency of the method and the production of other background noise at similar levels, e.g. primer-dimers. In some cases this method has also produced high amounts of dimer even after few cycles (Figure S9).

We have produced libraries on samples down to a few picograms of DNA (approximated quantification from qPCR on finished libraries) with inserts in the size order of ~70 bp *without* adapter-dimers in the final indexed amplification using the BEST method and with both 5'OMe and non-5'OMe adapters (data not shown).

In another attempt to remove adapter-dimers, an exonuclease (ExoI from NEB) was tested as previously done (Bennett *et al.* 2014). In our setup, we made an adapter free of PTO bonds in the 3' end of the IS1 and IS2 molecules to facilitate the enzymatic hydrolysis of the backbone. We did not find this to be very effective given that dimers in many cases still occurred after index amplification and that the yield of library seemed to decrease when adding exonuclease (Figure S10).

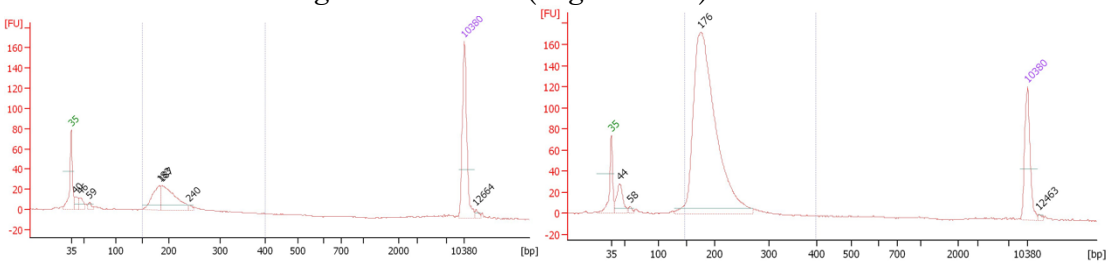

Figure S10. Size profiles of index amplified libraries measured with a Bioanalyzer 2100, demonstrating the effect of exonuclease treatment using Exonuclease I (NEB). Left electropherogram shows a library *with* exonuclease treatment, while right electropherogram shows a replicate *without* exonuclease treatment.

Sequence end bias – individual patterns

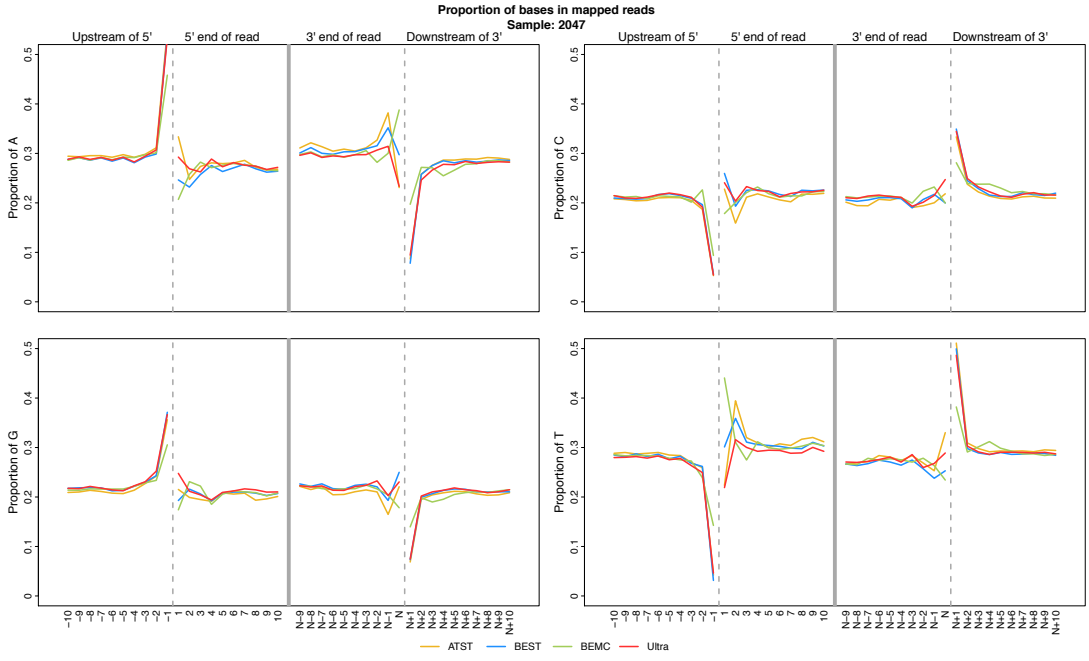

Figure S11. Sequence nucleotide composition analysis for sample 1_CN2047. Proportions of each base for the first and last 10 bases of each read as well as the flanking 10 bases in the reference genome.

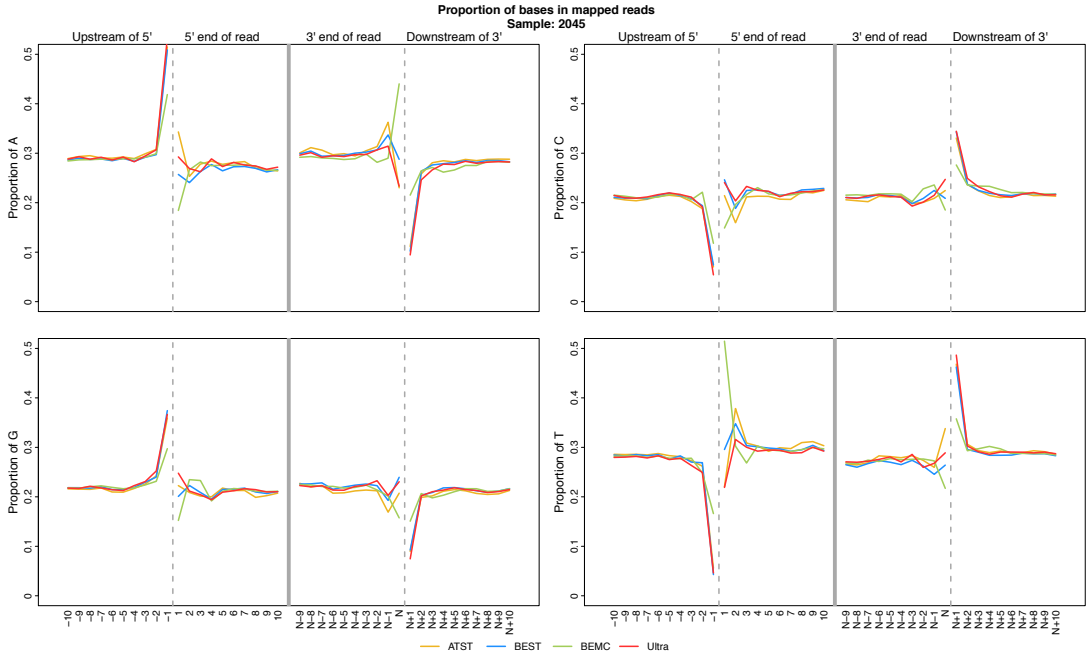

Figure S12. Sequence nucleotide composition analysis for sample 2_CN2045. Proportions of each base for the first and last 10 bases of each read as well as the flanking 10 bases in the reference genome.

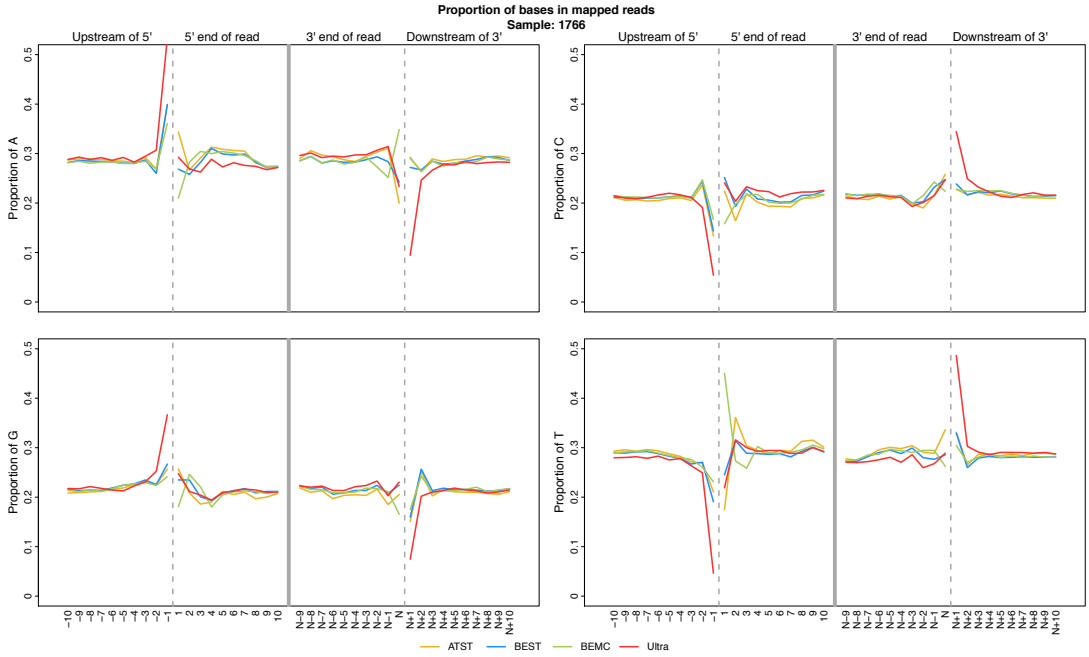

Figure S13. Sequence nucleotide composition analysis for sample 3_CN1766. Proportions of each base for the first and last 10 bases of each read as well as the flanking 10 bases in the reference genome.

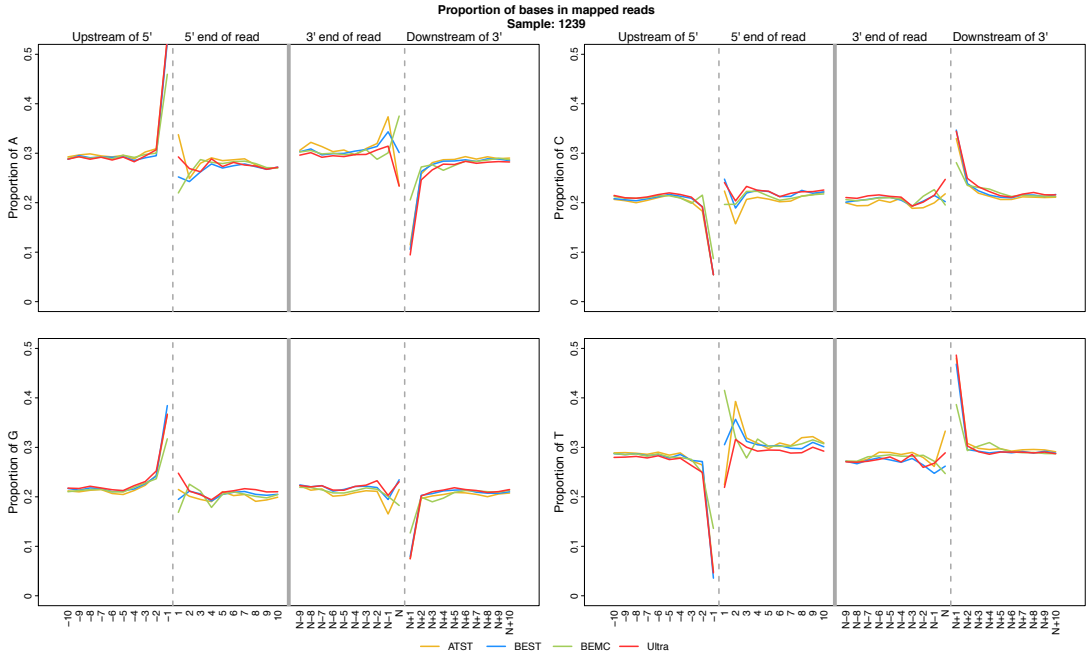

Figure S14. Sequence nucleotide composition analysis for sample 4_CN1239. Proportions of each base for the first and last 10 bases of each read as well as the flanking 10 bases in the reference genome.

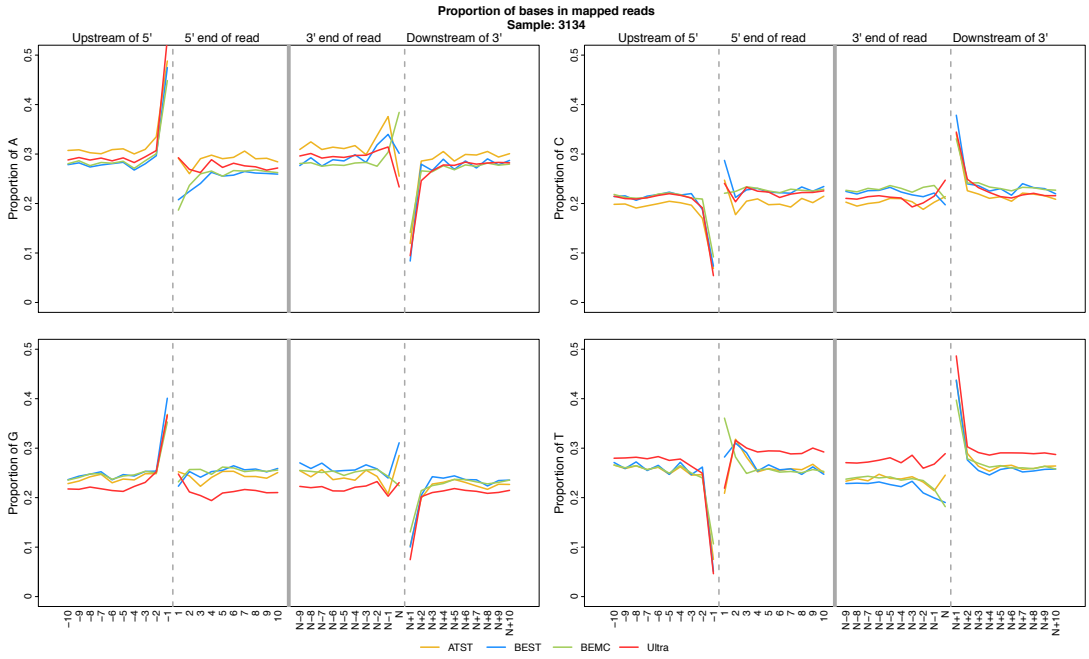

Figure S15. Sequence nucleotide composition analysis for sample 5_CN3134. Proportions of each base for the first and last 10 bases of each read as well as the flanking 10 bases in the reference genome.

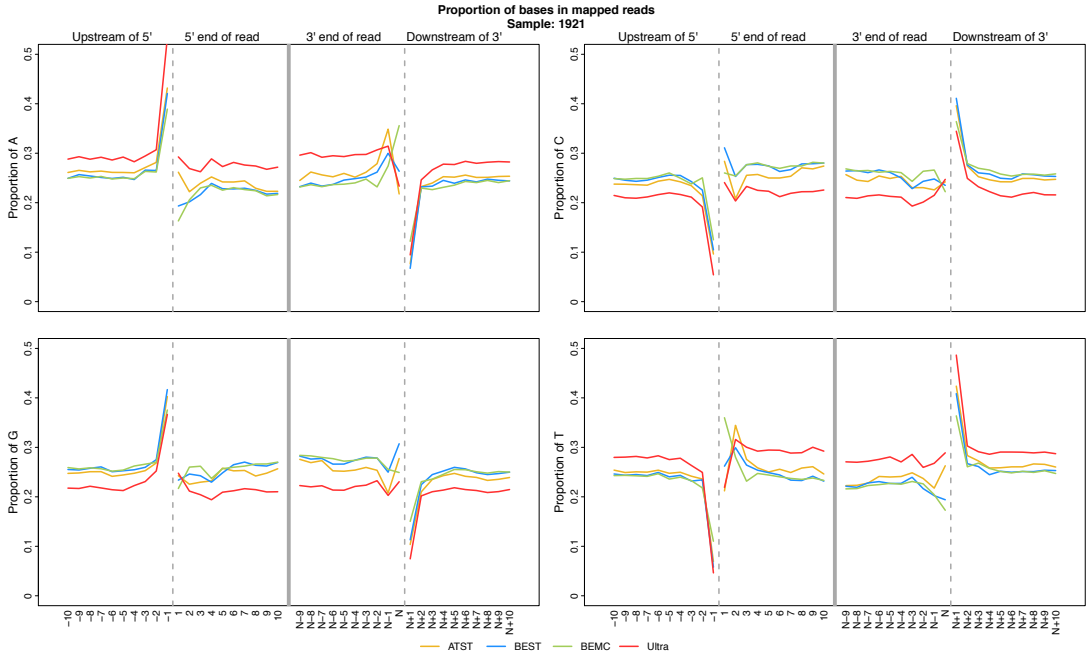

Figure S16. Sequence nucleotide composition analysis for sample 6_CN1921. Proportions of each base for the first and last 10 bases of each read as well as the flanking 10 bases in the reference genome.

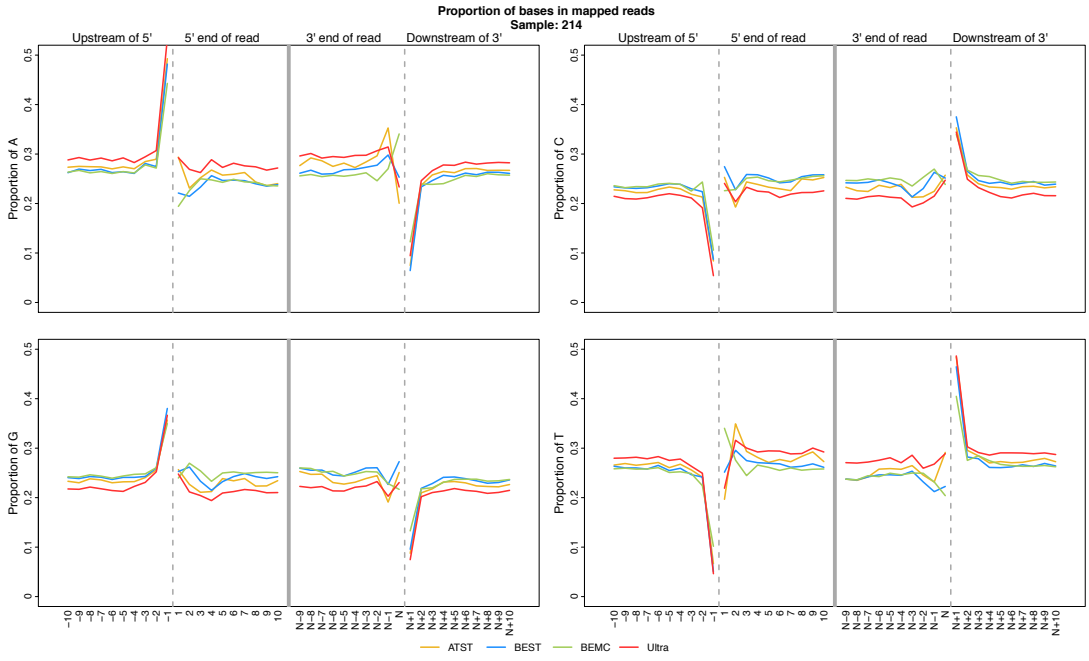

Figure S17. Sequence nucleotide composition analysis for sample 7_CN214. Proportions of each base for the first and last 10 bases of each read as well as the flanking 10 bases in the reference genome.

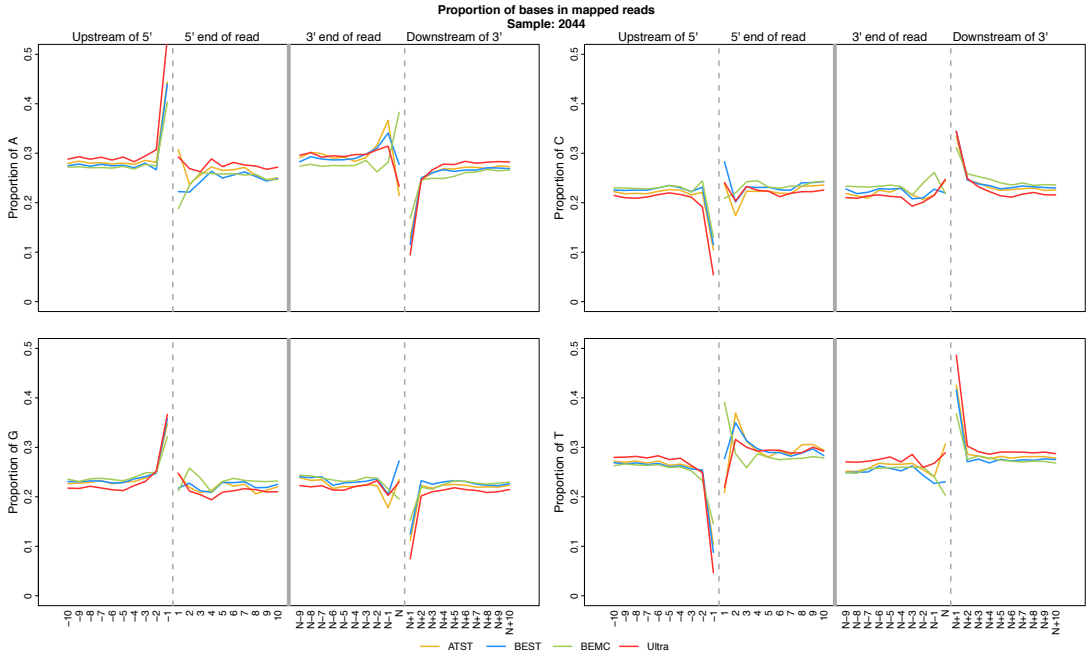

Figure S18. Sequence nucleotide composition analysis for sample 8_CN2044. Proportions of each base for the first and last 10 bases of each read as well as the flanking 10 bases in the reference genome.

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