

Supplementary to

Highly efficient library preparation for Ion Torrent sequencing using Y-adapters

Table S1. Oligonucleotide sequences used in this study.

Name ¹	Sequence (5' -> 3') ²
ITy-TOP-ID042	C*C*A*T*CTCATCCCTGCGTGTCTCCGACTCAGAGCACGAAT*C*G*A*T
ITy-BOT-long-ID042	T*C*G*A*TTCGTGCTCTGAGICGTCTATCACCGACTGCCCATAGA*G*A*G*G
ITy-BOT-short-ID042	T*C*G*A*TTCGTGCTCTGATCTATCACCGACTGCCCATAGA*G*A*G*G
ITy-TOP-ID043	C*C*A*T*CTCATCCCTGCGTGTCTCCGACTCAGCTTGACACCG*C*G*A*T
ITy-BOT-long-ID043	T*C*G*C*GGTGTCAAGCTGAGICGTCTATCACCGACTGCCCATAGA*G*A*G*G
ITy-BOT-short-ID043	T*C*G*C*GGTGTCAAGCTGATCTATCACCGACTGCCCATAGA*G*A*G*G
ITy-TOP-ID044	C*C*A*T*CTCATCCCTGCGTGTCTCCGACTCAGTTGGAGGCCAG*C*G*A*T
ITy-BOT-long-ID044	T*C*G*C*TGGCCTCCAAGTCTATCACCGACTGCCCATAGA*G*A*G*G
ITy-BOT-short-ID044	T*C*G*C*TGGCCTCCAAGTCTATCACCGACTGCCCATAGA*G*A*G*G
ITy-BOT-long-2In-ID044	T*C*G*C*IGGCCTCCAAGTCTATCACCGACTGCCCATAGA*G*A*G*G
ITy-BOT-long-3In-ID044	T*C*G*C*IGGCCTCCAAGTCTATCACCGACTGCCCATAGA*G*A*G*G
ITy-BOT-opt-1In-ID042	T*C*G*A*TTIGTGCTAGTCTATCACCGACTGCCCATAGA*G*A*G*G
ITy-BOT-opt-2In-ID042	T*C*G*A*TTIGTICTAGTCTATCACCGACTGCCCATAGA*G*A*G*G
ITy-BOT-opt-1In-ID009	T*C*G*T*TICGCTCAAGTCTATCACCGACTGCCCATAGA*G*A*G*G
ITy-BOT-opt-2In-ID009	T*C*G*T*TICGCICAAGTCTATCACCGACTGCCCATAGA*G*A*G*G
ITy-TOP-ID042-0PT	CCATCTCATCCCTGCGTGTCTCCGACTCAGAGCACGAATCGAT
ITy-BOT-ID042-0PT	TCGATTTCGTGCTAGTCTATCACCGACTGCCCATAGAGAGG
ITy-TOP-ID042-1PT	C*C*ATCTCATCCCTGCGTGTCTCCGACTCAGAGCACGAATCGA*T
ITy-BOT-ID042-1PT	T*CGATTTCGTGCTAGTCTATCACCGACTGCCCATAGAGAG*G
ITy-TOP-ID042-2PT	C*C*ATCTCATCCCTGCGTGTCTCCGACTCAGAGCACGAATCG*A*T
ITy-BOT-ID042-2PT	T*C*G*ATTTCGTGCTAGTCTATCACCGACTGCCCATAGAGA*G*G
ITy-TOP-ID042-3PT	C*C*A*TCTCATCCCTGCGTGTCTCCGACTCAGAGCACGAATC*G*A*T
ITy-BOT-ID042-3PT	T*C*G*ATTTCGTGCTAGTCTATCACCGACTGCCCATAGAG*A*G*G
ITy-TOP-ID042-2PT-BC	CCATCTCATCCCTGCGTGTCTCCGACTCA*G*AGCACGAATCGAT
ITy-BOT-ID042-2PT-BC	TCGATTTCGTGCT*C*GTCTATCACCGACTGCCCATAGAGAGG
ITy-TOP-ID042-4PT-BC	C*C*ATCTCATCCCTGCGTGTCTCCGACTCA*G*AGCACGAATCGA*T
ITy-BOT-ID042-4PT-BC	T*CGATTTCGTGCT*C*GTCTATCACCGACTGCCCATAGAGAG*G
ITy-TOP-ID009-0PT	CCATCTCATCCCTGCGTGTCTCCGACTCAGTGAGCGGAACGAT
ITy-BOT-ID009-0PT	TCGTTCCGCTCAAGTCTATCACCGACTGCCCATAGAGAGG
ITy-TOP-ID009-1PT	C*C*ATCTCATCCCTGCGTGTCTCCGACTCAGTGAGCGGAACGA*T
ITy-BOT-ID009-1PT	T*CGTTCCGCTCAAGTCTATCACCGACTGCCCATAGAGAG*G
ITy-TOP-ID009-2PT	C*C*ATCTCATCCCTGCGTGTCTCCGACTCAGTGAGCGGAACG*A*T
ITy-BOT-ID009-2PT	T*C*G*TTCCGCTCAAGTCTATCACCGACTGCCCATAGAGA*G*G
ITy-TOP-ID009-3PT	C*C*A*TCTCATCCCTGCGTGTCTCCGACTCAGTGAGCGGAAC*G*A*T
ITy-BOT-ID009-3PT	T*C*G*TTCCGCTCAAGTCTATCACCGACTGCCCATAGAG*A*G*G
ITy-TOP-ID001	C*C*A*T*CTCATCCCTGCGTGTCTCCGACTCAGCTAAGGTAA*C*G*A*T
ITy-BOT-ID001	T*C*G*T*TACCTTAGAGTCTATCACCGACTGCCCATAGA*G*A*G*G
ITy-TOP-ID006	C*C*A*T*CTCATCCCTGCGTGTCTCCGACTCAGCTGCAAGTT*C*G*A*T
ITy-BOT-ID006	T*C*G*A*ACTTGCAGAGTCTATCACCGACTGCCCATAGA*G*A*G*G
ITy-TOP-ID009	C*C*A*T*CTCATCCCTGCGTGTCTCCGACTCAGTGAGCGGAA*C*G*A*T

ITy-BOT-ID009	T*C*G*T*TCCGCTCAAGTCTATCACCGACTGCCCATAGA*G*A*G*G
ITy-TOP-ID011	C*C*A*T*CTCATCCCTGCGTGTCTCCGACTCAGTCCTCGAAT*C*G*A*T
ITy-BOT-ID011	T*C*G*A*TTCGAGGAAGTCTATCACCGACTGCCCATAGA*G*A*G*G
ITy-TOP-ID042	C*C*A*T*CTCATCCCTGCGTGTCTCCGACTCAGAGCACGAAT*C*G*A*T
ITy-BOT-ID042	T*C*G*A*TTCGTGCTAGTCTATCACCGACTGCCCATAGA*G*A*G*G
ITy-TOP-ID070	C*C*A*T*CTCATCCCTGCGTGTCTCCGACTCAGCCTACTGGT*C*G*A*T
ITy-BOT-ID070	T*C*G*A*CCAGTAGGAGTCTATCACCGACTGCCCATAGA*G*A*G*G
ITy-TOP-ID073	C*C*A*T*CTCATCCCTGCGTGTCTCCGACTCAGTCTGCCTGT*C*G*A*T
ITy-BOT-ID073	T*C*G*A*CAGGCAGAAGTCTATCACCGACTGCCCATAGA*G*A*G*G
ITy-TOP-ID074	C*C*A*T*CTCATCCCTGCGTGTCTCCGACTCAGCGATCGGTT*C*G*A*T
ITy-BOT-ID074	T*C*G*A*ACCGATCGAGTCTATCACCGACTGCCCATAGA*G*A*G*G

¹ Naming: ITy, Ion Torrent Y-adapter oligonucleotide; TOP, top oligonucleotide; BOT, bottom oligonucleotide; ID, number of the Ion Xpress index sequence; PT, adapter oligonucleotide containing given number of phosphorothioate bonds; opt, optimized adapter oligonucleotide; BC, phosphorothioate bonds located upstream of the barcode within the key sequence.

² * phosphorothioate bond; all bottom oligonucleotides are phosphorylated at their 5'-end.

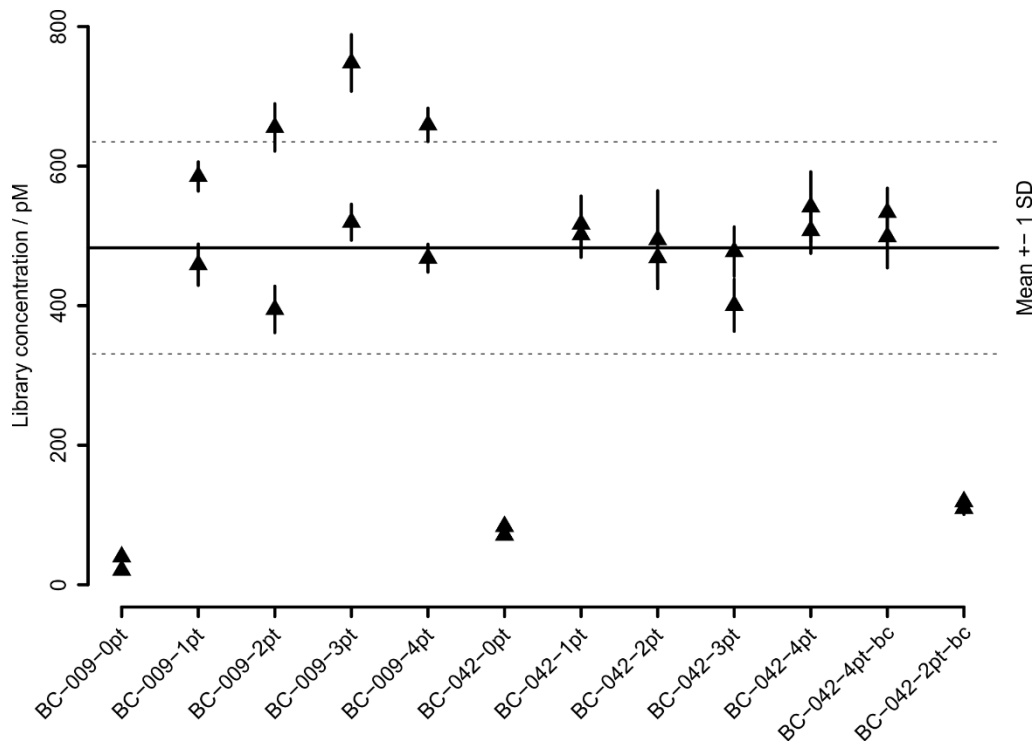
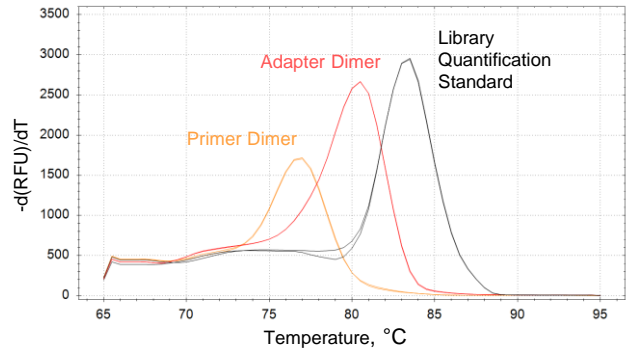
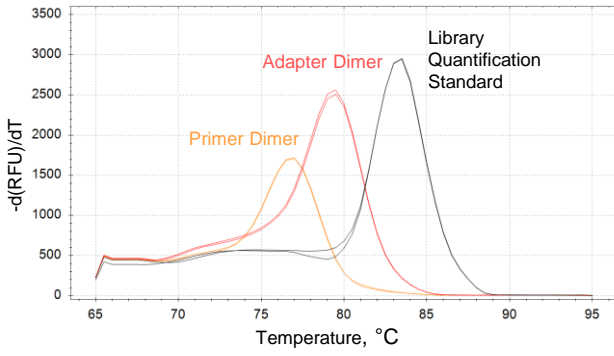


Figure S1. Library yield depending on the number and position of phosphorothioate bonds in the adapter oligonucleotides. The used adapters contained different numbers of phosphorothioate bonds at different positions within the oligonucleotide. Oligonucleotide designations: BC, barcode number of the Ion Xpress index sequence; pt, adapter oligonucleotide containing given number of phosphorothioate bonds; bc, phosphorothioate bonds located upstream of the barcode within the key sequence. For further details and the oligonucleotide sequences, see Table 1 in the main document. All libraries were prepared of the same batch of fragmented *E. coli* DNA in two independent experiments; vertical lines indicate the standard deviation calculated from three dilutions used in qPCR quantification. The horizontal line indicates the mean concentration of all libraries shown in the plot, the dashed lines indicate the mean \pm 1 standard deviation.

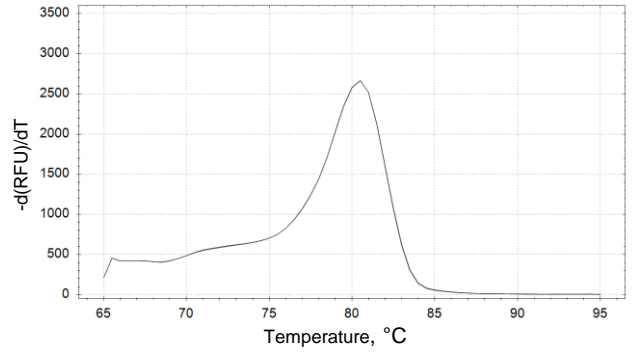
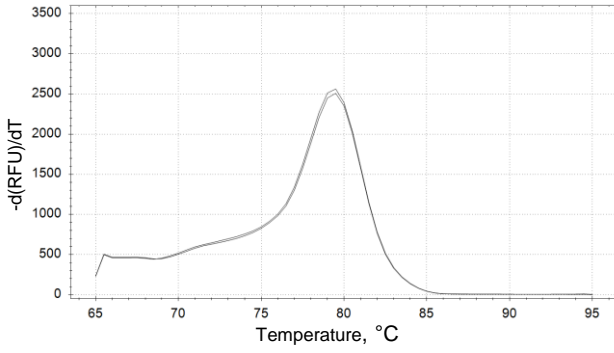
Standard-Adapter

Y-Adapter

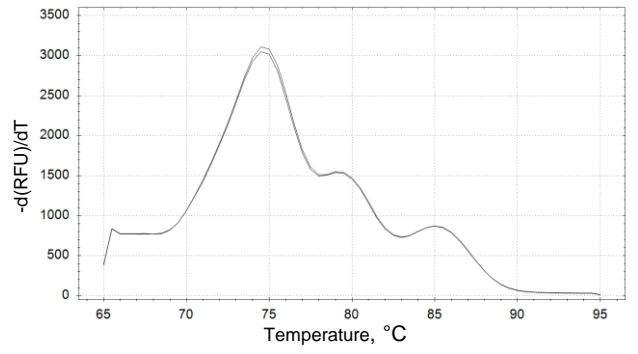
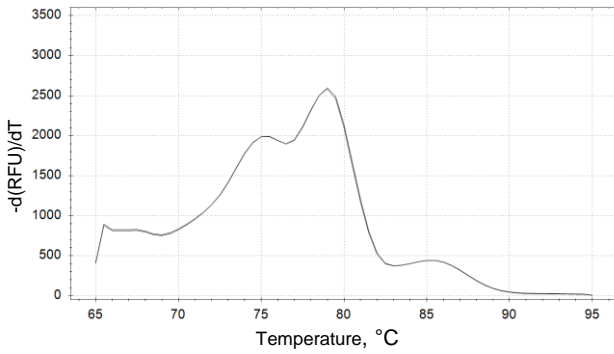
Reference



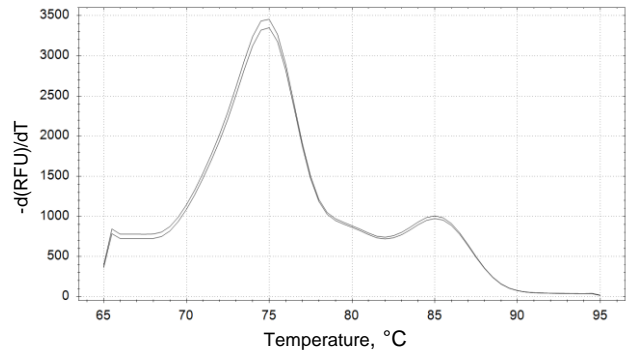
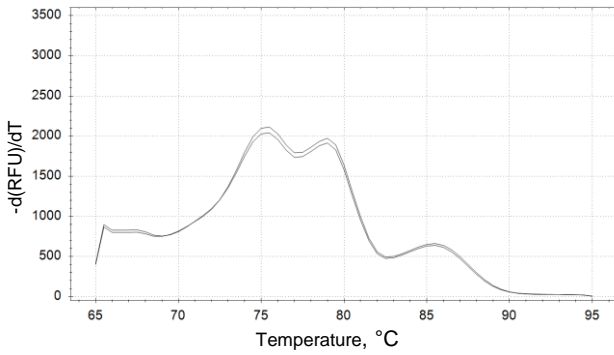
0 ng DNA



2 ng DNA



10 ng DNA



50 ng DNA

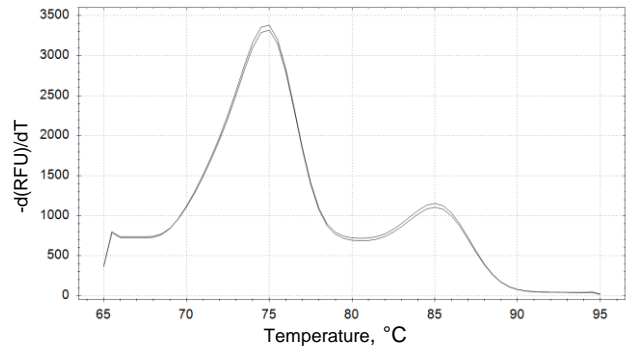
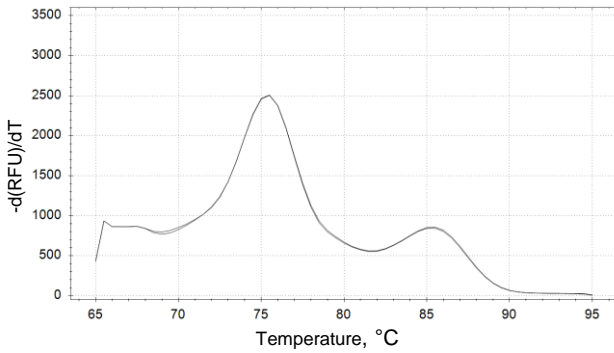


Figure S2. Melting curve analysis of libraries prepared using standard- and Y-adapters. Melting curves were recorded after qPCR quantification for 0 ng, 2 ng, 10 ng, and 50 ng DNA input. In comparison with the reference curves, the presence of adapter dimers is clearly visible. Reference was assembled of single peaks for non-template control (primer dimer; yellow), library without input (adapter dimer; red) and library quantification standard (153 bp; black).