Preparation of 27 random-primed cDNA libraries for Illumina sequencing

1 Material supplied

Thirty-five RNA samples from *Saccharomyces cerevisiae* as indicated in Table 1, delivered on dry ice.

Table 1: Samples delivered

No.	Name	Conc. (µg/µl)	Amount (µg)	Conc. (ng/µl)	Amount (µg)	Ratio 28S/18S
		customer-specified		own measurement		
1	WT 1	921	23	790	19,8	1,4
2	WT 2	927	23	856	21,4	1,6
3	WT 3	1000	25	877	21,9	1,4
4	WT 4	577	14	479	12,0	1,3
5	165 1	717	18	579	14,5	1,3
6	165 2	618	15	502	12,5	1,3
7	165 3	707	18	598	14,9	1,4
8	167 1	1132	28	974	24,3	1,4
9	167 2	1106	28	849	21,2	1,3
10	167 3	1105	28	930	23,2	1,3
11	167 4	621	16	460	11,5	1,4
12	WT 30 1	938	23	927	23,2	1,4
13	WT 30 2	909	23	972	24,3	1,5
14	WT 30 3	987	25	907	22,7	1,5
15	WT 30 4	807	20	684	17,1	1,4
16	165 30 1	904	23	770	19,3	1,4
17	165 30 2	850	21	719	18,0	1,6
18	165 30 3	869	22	639	16,0	1,5
19	165 30 4	879	22	661	16,5	1,3
20	167 30 1	1054	26	948	23,7	1,3
21	167 30 2	1181	30	989	24,7	1,3
22	167 30 3	1029	26	946	23,6	1,3
23	167 30 4	915	23	776	19,4	1,3
24	WT 60 1	1002	25	827	20,7	1,4
25	WT 60 2	953	24	766	19,2	1,3
26	WT 60 3	930	23	908	22,7	1,3
27	WT 60 4	1065	27	932	23,3	1,4
28	165 60 1	847	21	936	23,4	1,3
29	165 60 2	766	19	802	20,0	1,4
30	165 60 3	861	22	841	21,0	1,4
31	165 60 4	987	25	925	23,1	1,5
32	167 60 1	1375	34	761	19,0	1,3
33	167 60 2	1222	31	987	24,7	1,3
34	167 60 3	1092	27	910	22,8	1,1
35	167 60 4	1472	37	1.348	33,7	1,3

The RNA samples were examined by capillary electrophoresis (Fig. 1).

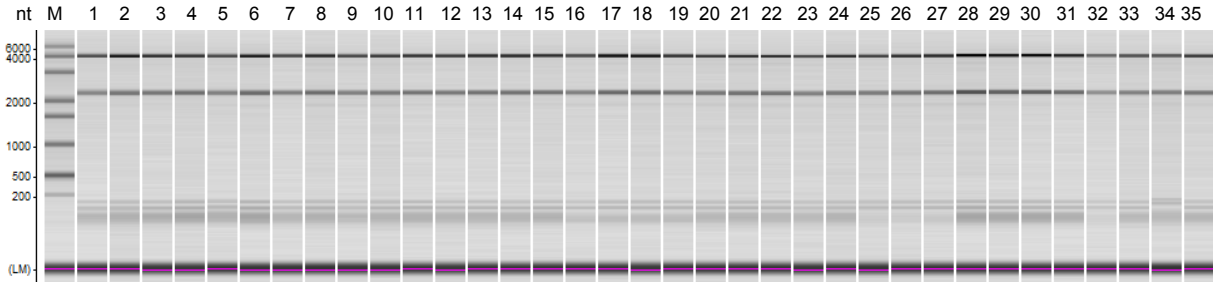

Figure 1: Analysis of the total RNA samples on a Shimadzu MultiNA microchip electrophoresis system. M = RNA marker.

2 cDNA synthesis

cDNA libraries were prepared from replicates 1 – 3.
Poly(A)+ RNA was isolated from the total RNA samples. First-strand cDNA synthesis was primed with a N6 randomized primer. After fragmentation, the Illumina TruSeq sequencing adapters were ligated in a strand specific manner to the 5' and 3' ends of the cDNA fragments. In this way, a strand specific PCR amplification of the cDNA was achieved using a proof reading enzyme (see Fig. 1). The number of cycles and the TruSeq barcode sequences, which are part of the 5' and 3' TruSeq sequencing adapters, are included in Table 2.

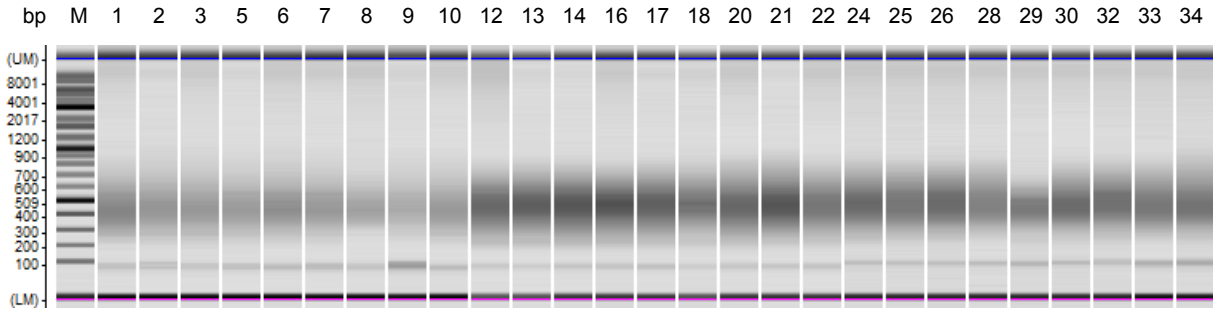

Figure 1: Analysis of the PCR-amplified cDNA samples on a Shimadzu MultiNA microchip electrophoresis system. M = 100 bp ladder.

Table 2: Properties of the cDNA samples

No.	Sample	i5 Barcode	i7 Barcode	PCR cycles
1	WT 1	AGTCTTCT	CTGAAGCT	15
2	WT 2	CATTCGCT	CTGAAGCT	15
3	WT 3	TCTACTCT	CTGAAGCT	15
5	165 1	ATCCTGTG	CTGAAGCT	15
6	165 2	TACAGGTC	CTGAAGCT	15
7	165 3	AGTCCAAC	CTGAAGCT	15
8	167 1	GGCAGCTA	CTGAAGCT	15
9	167 2	GCAGCATA	CTGAAGCT	15
10	167 3	AAGGTTCA	CTGAAGCT	15
12	WT 30 1	ACTTAGCA	TAATGCGC	15
13	WT 30 2	GTGTCTTA	TAATGCGC	15
14	WT 30 3	TGTTCTAG	TAATGCGC	15
16	165 30 1	GGAACTTA	TAATGCGC	15
17	165 30 2	GGACGTTT	TAATGCGC	15
18	165 30 3	AGGTCGTT	TAATGCGC	15

20	167 30 1	ACCTTCTT	TAATGCGC	15
21	167 30 2	TAGCTCTT	TAATGCGC	15
22	167 30 3	CGAGACTT	TAATGCGC	15
24	WT 60 1	TACTCCTT	GAATTCGT	15
25	WT 60 2	AGGCTTAG	GAATTCGT	15
26	WT 60 3	ATTAGACG	GAATTCGT	15
28	165 60 1	CGGAGAGA	GAATTCGT	15
29	165 60 2	CTAGTCGA	GAATTCGT	15
30	165 60 3	CTTAATAG	GAATTCGT	15
32	167 60 1	ATAGCCTT	GAATTCGT	15
33	167 60 2	TAAGGCTC	GAATTCGT	15
34	167 60 3	TCGCATAA	GAATTCGT	15

3 Pool generation and size fractionation

For Illumina HiSeq sequencing, the samples were pooled in approximately equimolar amounts. The cDNA pool in the size range of 350 – 600 bp was eluted from a preparative agarose gel. An aliquot of the size fractionated pool was analyzed by capillary electrophoresis (Fig. 3).

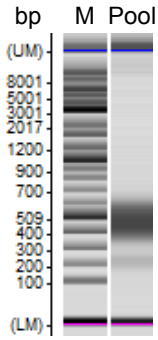

Figure 3: Analysis of the size fractionated cDNA pool on a Shimadzu MultiNA microchip electrophoresis system. M = 100 bp ladder.

Please note, the distinct bands in the size fractionated cDNA pool below 300 bp are not 'real' bands. They are rather a chip effect, which we frequently observe with gel fractionated DNA samples.

4 Description of cDNA samples

The cDNA has a size range of 350 – 600 bp. The primers used for PCR amplification were designed for TruSeq sequencing according to the instructions of Illumina.

The following adapter sequences flank the DNA insert:

TruSeq_Sense_primer i5 Barcode
5'-AATGATACGGCGACCACCGAGATCTACAC-NNNNNNNN-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-3'

TruSeq_Antisense_primer i7 Barcode
5'-CAAGCAGAAGACGGCATACGAGAT-NNNNNNNN-GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3'

The combined length of the flanking sequences is 136 bases.

5 Illumina sequencing

The cDNA pool was paired-end sequenced on an Illumina HighSeq system using 2x150 bp read length.