Preparation of 30 cDNA libraries for Illumina sequencing

1 Material supplied

Thirty RNA samples from *Salmonella typhimurium* (strain SL1344) as indicated in Table 1, delivered on dry ice.

Table 1: Samples delivered

Sample ID	Name	ID	Conc. (ng/µl)	Amount (µg)	Conc. (ng/µl)	Amount (ng)
			customer-specified		own measurement	
1	1_1	ID-006413	29,2	0,44	31,1	0,40
2	1_2	ID-006414	57,0	0,86	39,0	0,51
3	1_3	ID-006415	34,1	0,51	34,8	0,45
4	1_4	ID-006416	32,4	0,49	33,4	0,43
5	1_5	ID-006417	60,4	0,91	43,7	0,57
6	1_6	ID-006418	69,6	1,04	53,0	0,69
7	1_7	ID-006419	38,5	0,58	44,2	0,58
8	1_8	ID-006420	77,3	1,16	53,7	0,70
9	1_9	ID-006421	88,9	1,33	51,5	0,67
10	1_10	ID-006422	45,3	0,68	35,0	0,45
11	2_1	ID-006423	36,5	0,55	31,6	0,41
12	2_2	ID-006424	39,3	0,59	37,6	0,49
13	2_3	ID-006425	36,1	0,54	32,6	0,42
14	2_4	ID-006426	36,2	0,54	39,1	0,51
15	2_5	ID-006427	69,3	1,04	38,9	0,51
16	2_6	ID-006428	41,9	0,63	37,3	0,48
17	2_7	ID-006429	24,8	0,37	27,6	0,36
18	2_8	ID-006430	57,8	0,87	40,1	0,52
19	2_9	ID-006431	57,1	0,86	36,3	0,47
20	2_10	ID-006432	23,2	0,35	24,6	0,32
21	3_1	ID-006433	32,7	0,49	33,5	0,44
22	3_2	ID-006434	37,9	0,57	33,0	0,43
23	3_3	ID-006435	34,0	0,51	31,3	0,41
24	3_4	ID-006436	27,1	0,41	29,7	0,39
25	3_5	ID-006437	44,0	0,66	27,8	0,36
26	3_6	ID-006438	59,1	0,89	43,6	0,57
27	3_7	ID-006439	29,7	0,45	31,3	0,41
28	3_8	ID-006440	53,9	0,81	40,7	0,53
29	3_9	ID-006441	50,2	0,75	33,5	0,44
30	3_10	ID-006442	31,2	0,47	29,7	0,39

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

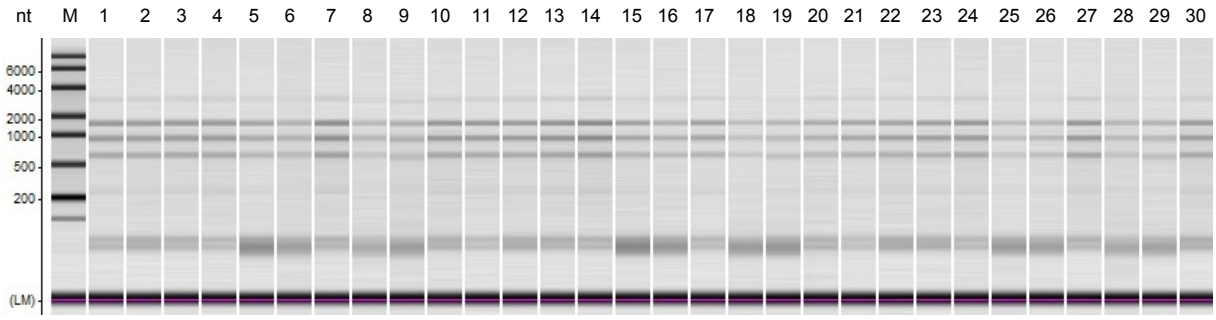

Figure 1: Analysis of the total RNA samples on a Shimadzu MultiNA microchip electrophoresis system. M = RNA marker

2 cDNA synthesis (Adapter ligation method)

The RNA samples were first fragmented using ultrasound (4 pulses of 30 s each at 4°C). Then, an oligonucleotide adapter was ligated to the 3' ends of the RNA molecules. First-strand cDNA synthesis was performed using M-MLV reverse transcriptase and the 3’ adapter as primer. The first-strand cDNA was purified and the 5' Illumina TruSeq sequencing adapter was ligated to the 3' end of the antisense cDNA. The resulting cDNA was PCR-amplified to about 10-20 ng/µl using a high fidelity DNA polymerase (cycle numbers are indicated in Table 2). The TruSeq barcode sequences, which are part of the 5' and 3' TruSeq sequencing adapters, are included in Table 2. The cDNA was purified using the Agencourt AMPure XP kit (Beckman Coulter Genomics) and was analyzed by capillary electrophoresis (Fig. 2).

Table 2: Description of cDNA samples

Seq ID	Sample	i5 Barcode	i7 Barcode	PCR cycles
1	1_1	AGGCTATA	ATTACTCG	12
2	1_2	GCCTCTAT	ATTACTCG	12
3	1_3	AGGATAGG	ATTACTCG	12
4	1_4	TCAGAGCC	ATTACTCG	12
5	1_5	CTTCGCCT	ATTACTCG	12
6	1_6	TAAGATTA	TCCGGAGA	13
7	1_7	ACGTCCTG	TCCGGAGA	13
8	1_8	GTCAGTAC	TCCGGAGA	13
9	1_9	ATAGAGAG	TCCGGAGA	13
10	1_10	AGAGGATA	TCCGGAGA	13
11	2_1	CTCCTTAC	CTGAAGCT	12
12	2_2	TATGCAGT	CTGAAGCT	12
13	2_3	TACTCCTT	CTGAAGCT	12
14	2_4	AGGCTTAG	CTGAAGCT	12
15	2_5	ATTAGACG	CTGAAGCT	12
16	2_6	CGGAGAGA	CGGCTATG	12
17	2_7	CTAGTCGA	CGGCTATG	12
18	2_8	CTTAATAG	CGGCTATG	12
19	2_9	ATAGCCTT	CGGCTATG	12
20	2_10	TAAGGCTC	CGGCTATG	12
21	3_1	TCGCATAA	TCCGCGAA	12
22	3_2	AATTTGGT	TCCGCGAA	12
23	3_3	GCTACGGT	TCCGCGAA	12
24	3_4	CATCCCGT	TCCGCGAA	12

Seq ID	Sample	i5 Barcode	i7 Barcode	PCR cycles
25	3_5	CTTATAGT	TCCGCGAA	12
26	3_6	AGGTCGTT	AGCGATAG	12
27	3_7	ACCTTCTT	AGCGATAG	12
28	3_8	TAGCTCTT	AGCGATAG	12
29	3_9	CGAGACTT	AGCGATAG	12
30	3_10	TTCGCTGT	AGCGATAG	12

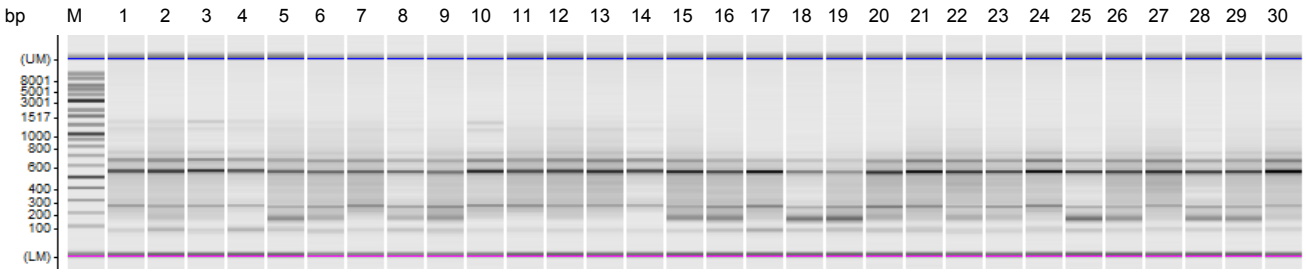

Figure 2: Analysis of PCR-amplified cDNA samples on a Shimadzu MultiNA microchip electrophoresis system. M = 100 bp ladder

3 Pool generation and size fractionation

For Illumina NextSeq sequencing, the samples were pooled in approximately equimolar amounts. The cDNA pool in the size range of 200 – 700 bp was eluted from a preparative agarose gel. An aliquot of the size fractionated pool was analyzed by capillary electrophoresis (Figure 3).

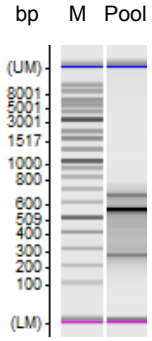

Figure 3: Analysis of the size-fractionated cDNA pool on a Shimadzu MultiNA microchip electrophoresis system. M = 100 bp ladder

4 Sample description

The primers used for PCR amplification were designed for TruSeq sequencing according to the instructions of Illumina.

The following adapter sequences flank the cDNA inserts:

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 sequenced on an Illumina NextSeq 500 system using 75 bp read length.