

Preparation of 2 cDNA libraries for Illumina sequencing

1 Material supplied

Two RNA samples from *Chara braunii* as indicated in Table 1, delivered on dry ice.

Table 1: Samples delivered

No.	Sample	Conc. (ng/μl)	Amount (μg)	Conc. (ng/μl)	Amount (μg)	Ratio 28S/18S	Recovery after poly(A)+ isolation (%)
		Customer-specified		Own measurements			
1	DH RNA Nodules	18,9	0,7	13,2	0,4	N/A	1,9
2	DH RNA Control	194,8	272,0	197,0	2,2	1,2	0,8

2 Analysis of total RNA and poly(A)+ isolation

The total RNA samples were examined by capillary electrophoresis (Fig. 1). Poly(A)+ RNA was isolated from the total RNA samples. Recovery rates are shown in Table 1.

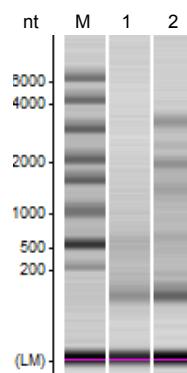


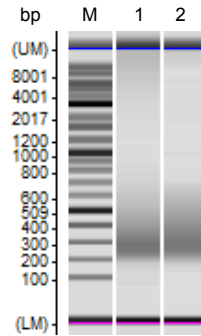
Figure 1: Analysis of the total RNA samples on a Shimadzu MultiNA microchip electrophoresis system. M = RNA marker.

3 cDNA synthesis

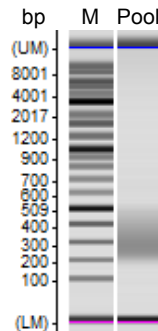
The poly(A)+ RNAs were first fragmented using ultrasound (1 pulse of 30 s at 4°C). Then, an oligonucleotide adapter was ligated to the 3' end of the RNA molecules. First-strand cDNA synthesis was performed using M-MLV reverse transcriptase and the 3' adapter as primer. The first-strand cDNA was purified 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 (Figure 2).

Table 2: Description of cDNA samples

No.	Sample	PCR cycles	i5 Barcode	i7 Barcode
1	DH RNA Nodules	19	CAAGTGGG	TCCGCGAA
2	DH RNA Control	16	AGATTG	TCCGCGAA



4 Pool generation



5 Sample description

The following adapter sequences flank the cDNA inserts:

TruSeq_Sense_primer i5 Barcode
5'-AATGATACGCGACCAACGAGATCTACAC-NNNNNNNN-ACACTCTTTCCTACACGACGCTCTTCCGATCT-3'

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

The combined length of the flanking sequences is 136 bases.

6 Illumina sequencing