Preparation of four TSS cDNA libraries from Illumina sequencing

1 Material supplied

Six total RNA samples from *Streptomyces venezuelae* as specified in Table 1, delivered on dry ice.

Table 1: Samples delivered

No.	Sample	Conc. (ng/µl)	Volumen (µl)	Conc. (ng/µl)	Total amount (µg)	Ratio 23S/16S
		customer-specified		own measurement (see Fig.1)		
1	SvenRNA-10hr	195,6	90	169,1	13,7	1,5
2	SvenRNA-14hr	228,4	90	160,0	12,6	1,5
3	SvenRNA-18hr	189,1	90	148,3	12,0	1,1
4	SvenRNA-24hr	180,0	90	162,4	13,6	1,0

2 rRNA depletion

From the total RNA samples, ribosomal RNA molecules were depleted using the Ribo-Zero rRNA Removal Kit for bacteria (Epicentre). The electrophoresis images of the RNA samples before and after rRNA depletion are shown in Fig. 1.

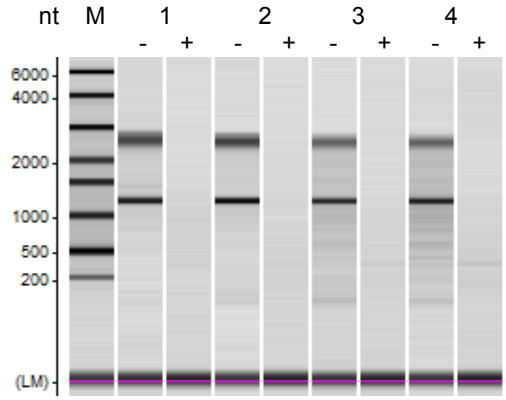

Figure 1: Analysis of RNA samples before (-) and after (+) RNA depletion on a Shimadzu MultiNA microchip electrophoresis system. M = RNA marker

3 TSS (5' tag RACE) library construction

First, to the 5' momophosphate groups (5'P) the 5' Illumina TruSeq sequencing adapters were ligated, which carry sequence tags ATTACTCG and TCCGGAGA (in a proportion of 50% of each adapter). The samples were then treated with 5' RNA polyphosphatase (5'PP; Epicentre) in order to convert 5' triphosphate (5'PPP) structures into 5' monophosphate ends. To the formed 5'P groups the 5' Illumina TruSeq sequencing adapters were ligated, which carry sequence tag CGCTCATT and GAGATTCC (50% each). Then, first-strand cDNA was synthesized using a N6 randomized primer. After fragmentation, the 3' Illumina TruSeq sequencing adapter was ligated to the 5' ends of the 1.-strand antisense cDNA fragments. The tagged 5' cDNA fragments were then specifically amplified with PCR (number of cycles indicated in Table 2) using a proof reading enzyme. The 5' cDNAs were purified using the Agencourt AMPure XP kit (Beckman Coulter Genomics) and were analyzed by capillary electrophoresis (Figure 2).

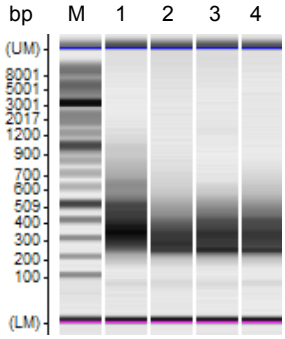

Figure 4: Analysis of the PCR-amplified 5' cDNA fragments on a Shimadzu MultiNA microchip electrophoresis system. M = 100 bp ladder

Table 2: Properties of the cDNA samples

No.	Sample	Barcode	PCR cycles
1	SvenRNA-10hr	TACAGGTC	19
2	SvenRNA-14hr	AGTCCAAC	19
3	SvenRNA-18hr	GGCAGCTA	19
4	SvenRNA-24hr	GCAGCATA	19

For Illumina NextSeq sequencing, the cDNA preparations were pooled in approximately equimolar amounts and size fractionated in the size range of 240 – 450 bp on a preparative agarose gel.

4 Description of cDNA samples

The cDNAs have a size range of 240 – 450 bp. 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_prime Barcode
5'-AATGATACGGCGACCACCGAGATCTACAC-NNNNNNNN-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-3'

5' ends of cDNA from processed RNAs (before 5'PP treatment)
(linker 5-15 nt) (linker 5-15 nt)
NNATTACTCGNN-cDNA insert and NNTCCGGAGANN-cDNA insert

5' ends of cDNA from primary transcripts (after 5'PP treatment)
(linker 5-15 nt) (linker 5-15 nt)
NNCGCTCATTNN-cDNA insert and NNGAGATTCCNN-cDNA insert

TruSeq_Antisense_prime
5'-CAAGCAGAAGACGGCATACGAGATGTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT-3'

The combined length of the flanking sequences is 128 bases.

5 Ilumina sequencing

The cDNA pool was sequenced on an Illumina NextSeq 500 system using 75 bp read length.