sRNA isolation, cloning, and sequencing (480)

Filtering (rRNA, tRNA, chloroplast DNA, exclusion of sRNAs < 16 nt), generation of a non-redundant dataset of sRNAs (152)

BLASTN search against Physcomitrella genomic trace files and EST database

sRNA sequences present in genomic trace files (66)
sRNA sequences present in ESTs (22)

21 sRNAs present in ESTs identical to genomic trace files (67)

Clustering of identified sequences

Trimming of singlets and contig sequences

Prediction of hairpin-like structure using RNAshapes

25 miRNAs with 33 precursors from direct cloning and 29 miRNAs with 31 precursors from microHARVESTER prediction

MiRNAs (59) (30 from cloning approach, 29 predicted by microHARVESTER)

Small RNA gel blot analysis (20 and 9 miRNAs identified through cloning and microHARVESTER, respectively)

Verified miRNAs (18)

Prediction

MiRNA prediction with the microHARVESTER program using all plant miRNAs present in Rfam (version 8.1)

MiRNA prediction from Physcomitrella genomic trace files and ESTs (123)

Clustering of identified sequences (43)

Trimming of singlets and contig sequences

sRNAs unable to form hairpin like structure (42 and 14 from cloning and microHARVESTER, respectively)

Target prediction using RNAhybrid (59 targets for 30 miRNAs)

BLASTX against UniProt/TrEMBL database (gene annotation, biological function)