small rnaseq-methods

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1 Methods

All samples are processed using sRNA-seq pipeline implemented in bcbio-nextgen project. Raw reads will be examined for quality issues using FastQC to ensure library generation and sequencing are suitable for further analysis. 3' end adapter were trimmed from reads using cutadapt [3]. Trimmed reads were aligned to miRBase 21 [1] using seqbuster [4].

miRNAs counting was done with isomiRs package discarting any sequence with only 1 count. Normalilzation and differential expression at the gene level were called with DESeq2 [2], which has been shown to be a robust, conservative differential expression calller.

2 Bibliography

References

[1] Ana Kozomara and Sam Griffiths-Jones. miRBase: annotating high confidence microRNAs using deep sequencing data. *Nucleic acids research*, 42(Database issue):D68–73, 2014.

[2] Michael I Love, Wolfgang Huber, and Simon Anders. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biology*, 15(12):550, December 2014.

[3] Marcel Martin. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal*, 17(1):pp. 10–12, February 2011.

[4] Lorena Pantano, Xavier Estivill, and Eulàlia Martí. SeqBuster, a bioinformatic tool for the processing and analysis of small RNAs datasets,

reveals ubiquitous miRNA modifications in human embryonic cells. *Nucleic Acids Research*, 38(5):e34, 2010.