LEXOGEN

Enabling complete transcriptome sequencing

SENSE mRNA-Seq Library Preparation Kit Technical Note: Reproducibility

Introduction

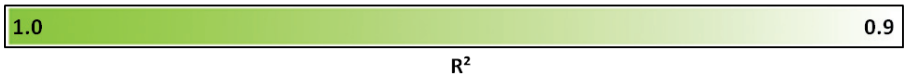
The quantification and control of technical variability is an essential part of any experiment. By minimizing technical variation introduced during steps such as RNA extraction, library construction, and sequencing, quantification more accurately reflects the true biological concentrations of transcripts and permits more repeatable and precise sample-to-sample comparisons. This technical note briefly examines the precision and repeatability of transcript quantification with the SENSE mRNA-Seq library preparation kit.

Experiment

The data analyzed here was first presented in an application note available online[1]. In brief, seven barcoded NGS libraries were made from the same pool of total RNA spiked with ERCC controls[2] and sequenced on the same lane of an Illumina HiSeq. The libraries were generated with differing amounts of input RNA, and duplicates were made starting from the RNA pool for most levels. The FPKM values for all ERCCs were calculated using cufflinks, and linear regressions were computed for each possible pair of libraries regardless of the amount of input RNA.

Table 1 Pairwise linear regressions of ERCC FPKM from libraries with varying inputs.

	2 µg rep 1	2 µg rep 2	1 µg rep 1	1 µg rep 2	500 ng rep 1	500 ng rep 2	50 ng rep 1
2 µg rep 1	-	0.994	1.026	1.003	0.961	1.007	0.987
2 µg rep 2	0.946	-	1.017	1.004	0.980	0.974	0.950
1 µg rep 1	0.959	0.948	-	0.959	0.925	0.941	0.916
1 µg rep 2	0.943	0.950	0.961	-	0.944	0.938	0.892
500 ng rep 1	0.941	0.943	0.965	0.968	-	0.956	0.974
500 ng rep 2	0.942	0.955	0.972	0.951	0.950	-	0.959
50 ng rep 1	0.948	0.963	0.961	0.928	0.956	0.967	-



Results

Both the mean R^2 (0.953 ± 0.011 standard deviation) and mean slope (0.967 ± 0.035) were high, indicating a large degree of repeatability, even with varying amounts of RNA input. No obvious relationship was observed when comparing libraries generated with the same amount of input RNA to those generated with different amounts (i.e. R^2 values were not higher between libraries with the same input RNA). This suggests that the protocol is resistant to changes in reproducibility due to variation in the amount of input material, and indicates that transcript quantification is precise even with widely varying inputs. This precise quantification even with differing amounts of input RNA allows experiments to be safely performed even when the availability of total RNA for some samples may be low.

Conclusions

The SENSE mRNA-Seq library preparation kit generates libraries with low technical variability and high reproducibility, enabling the precise quantification of transcripts. Transcript quantification with SENSE libraries is furthermore highly robust and resistant to changes in input RNA level.

[1] Nadeau, C. and Seitz A. (2012). SENSE: A fast RNA-Seq library preparation protocol with superior strand-specificity. Application note available online at www.lexogen.com and in the December 2012 issue of Nature Methods.

[2] Baker, S. *et al.* (2005). External RNA Controls Consortium: a progress report. *Nat. Methods* **2**, 731-734.

Lexogen GmbH · Campus Vienna Biocenter 5 · 1030 Vienna · Austria
Correspondence should be addressed to info@lexogen.com.