LEXOGEN
Enabling complete transcriptome sequencing

Amendment:
Fragmentation of SIRV502 in SIRV mixes batch No 216652830

Dear Customer,

Thank you for having purchased the Spike-In RNA Variant set (SIRVs, Lexogen Cat. No. 025.03) for isoform determination and quantification. Unfortunately, one out of the 69 SIRV transcripts, SIRV502, was found to be fragmented.

We have produced the SIRV mixes of your batch (No 216652830) as described in the User Guide (p. 20f). Due to length similarities and because of incomplete denaturation during capillary electrophoresis few transcripts migrate together, as shown in ***Fig. 1A*** for the affected PreMix 4B. The presence of SIRV transcripts in the merged peaks had to be confirmed by correlating peak areas with the expected partial contributions. With hindsight, this was not conclusive for SIRV502.

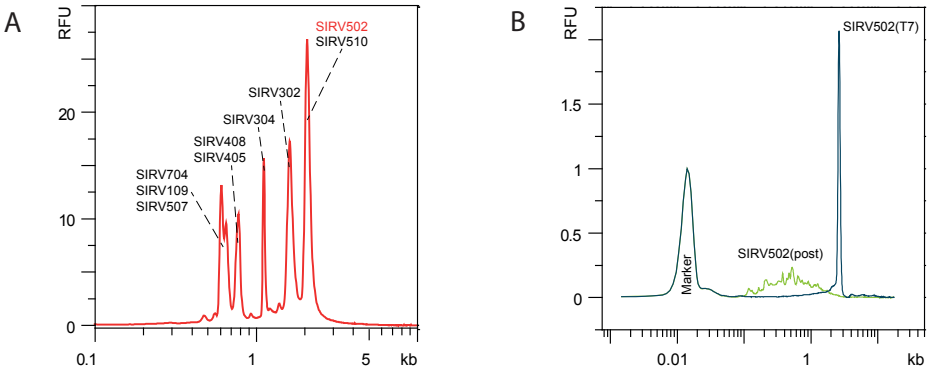

Figure 1 | Capillary electrophoresis analysis of SIRV PreMix 4B and SIRV502.
(A) Bioanalyzer trace of PreMix 4B. SIRV502 cannot be resolved as a single peak but runs jointly with SIRV510. (B) Overlaid Fragment Analyzer traces of SIRV502 RNA immediately after T7 transcription (blue) and in stock solution used for SIRV mixing (green).

The re-examination of the SIRV502 stock solution used to prepare the mixes revealed a substantial fragmentation of SIRV502 ***(Fig. 1B)***. This was further evidenced by NGS data from RNA-Seq libraries prepared by using a poly(A) selective protocol. No telling reads from SIRV502 were detected, the transcript-specific region in the 3' part of SIRV5 exon 4 shows no coverage ***(Fig. 2)***. All other 68 SIRV transcripts are represented appropriately in the NGS data.

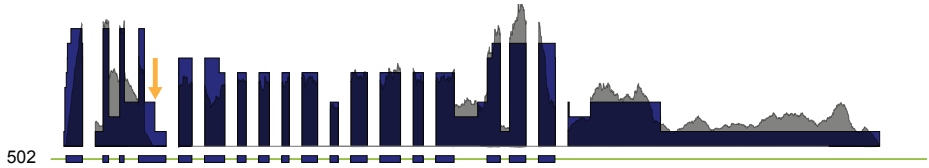

Figure 2 | Overlay of actual and expected SIRV5 read coverage.
The expected SIRV5 coverage in Mix E0 is shown in blue, the actual read coverage in grey with the exon-intron structure of SIRV502 aligned below. The yellow arrow indicates the site of the expected coverage originating exclusively from transcript SIRV502.

Implications

- If you apply a poly(A) directed protocol (oligo(dT) bead based RNA selection or oligo(dT) based oligo priming), then exclude SIRV502 from your calculations since it cannot be detected quantitatively.
- If you apply a random priming NGS library preparation protocol, you will detect SIRV502 at lower levels as stated.
- All other expected absolute and relative concentration values are not influenced by the drop out of SIRV502, because it entered all mixes (E0, E1 and E2) via SubMix 4, which corresponds to a uniform 25% in each mix.

File updates

These updated files can be downloaded from our web-site www.lexogen.com/sirvs/#sirvsdownloads:

- The Excel file "SIRV-sequence-design-overview.xls" has been updated to v1.2 to include a comment for SIRV502, explaining its status.
- The annotation files in "SIRV_Sequences_151124.zip" have been updated. SIRV502 has been removed from the correct version "SIRV_C_151124a.gtf" and the insufficient version "SIRV_I_151124a.gtf" but is kept in the over-annotated version "SIRV_O_151124a.gtf". The fasta file has been renamed "SIRV_151124a.fasta" without modification of its content.
- The Certificate of Analysis has been updated accordingly "SIRVs_216652830_Certificate-of-Analysis.pdf ".

We sincerely apologize for any inconvenience this causes. If you have any questions, please contact us at info@lexogen.com or call Lexogen Support at +43(0) 1 345 1212-41.

Best regards,

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Spike-in RNA Variant Control Mixes