Optimizing Cost-Effective Gene Expression Phenotyping Approaches in Cattle Using 3' mRNA Sequencing

Ruwaa I. Mohamed[1], Taylor B. Ault-Seay[2], Sonia Moisa[2], Jonathan Beever[2], Agustin Rius[2], Troy Rowan[1,2,*]

[1]Genome Science and Technology Program, Bredesen Center, University of Tennessee, Knoxville, TN, USA.

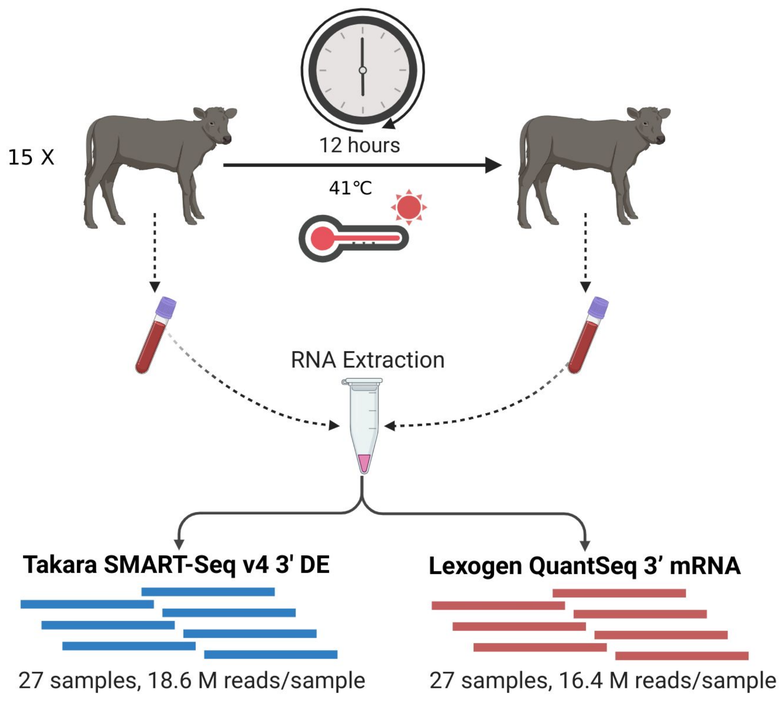
[2]Animal Science Department, University of Tennessee Institute of Agriculture (UTIA), Knoxville, TN, USA.

Background

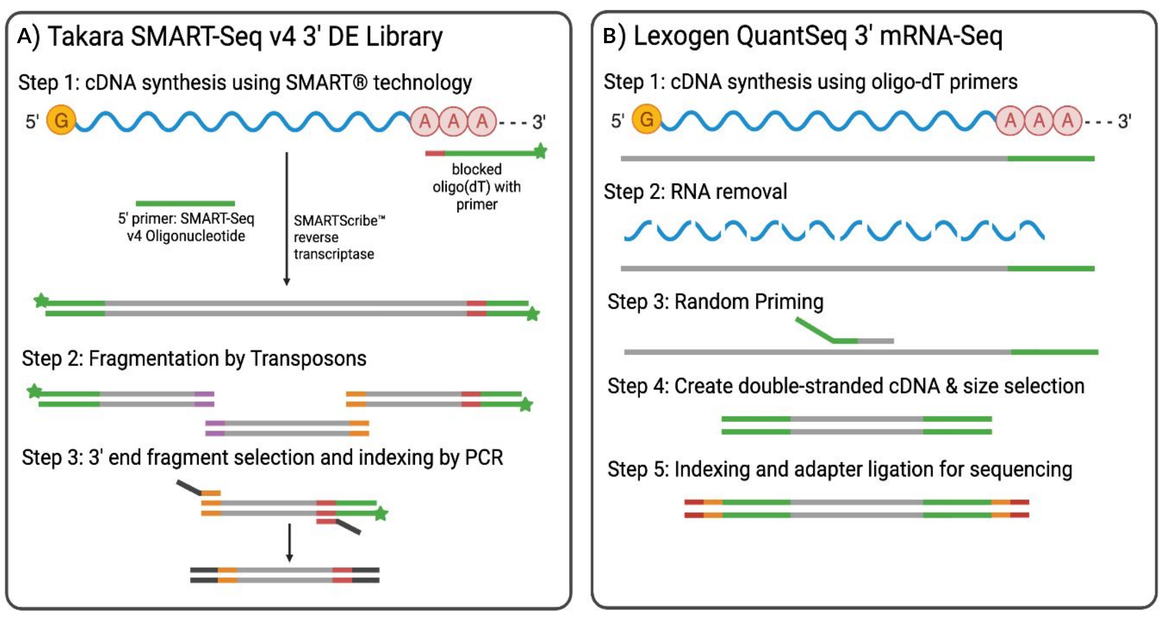
Direct measurements of phenotypes like meat quality, methane emission, and disease susceptibility are difficult and expensive to measure at scale but are critically important to livestock production. The rapidly declining cost of next-generation sequencing presents opportunities for population-level molecular phenotyping. While the cost of whole transcriptome sequencing has declined recently, its required sequencing depth makes it an expensive choice for wide-scale molecular phenotyping. Our work aims to use and optimize 3′ RNA-Seq approaches for collecting cost-effective proxy molecular phenotypes for cattle from easy-to-collect tissue samples (*i.e.*, whole blood). When paired with high-throughput miniaturized library preparation, 3' RNA-Seq is a powerful, ultra-affordable approach to quantifying population-level gene expression.

Conclusion: In our testing data, the Takara SMART-Seq v4 3' DE kit with 5M reads/sample provided the maximum amount of information at the lowest cost. The all-in collection, reagent, library preparation, and sequencing cost is <$25/sample.

Study Design



1) Which sequencing library?



2) How many reads per sample (sequencing depth)?

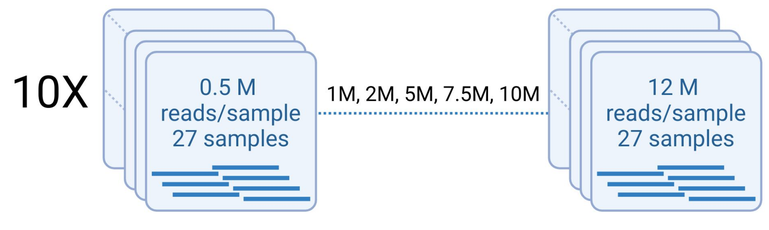


Figure 3: Number of expressed genes (A), informative genes (B), and DEGs (C) starts to plateau at 5M reads per sample.

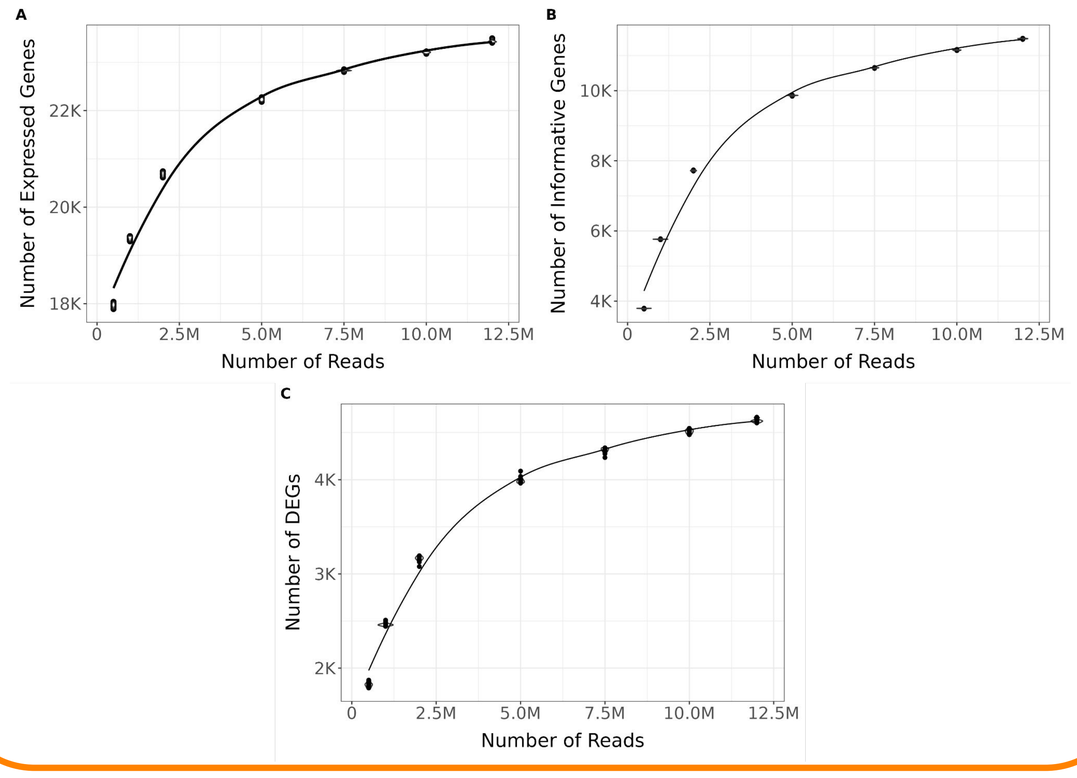
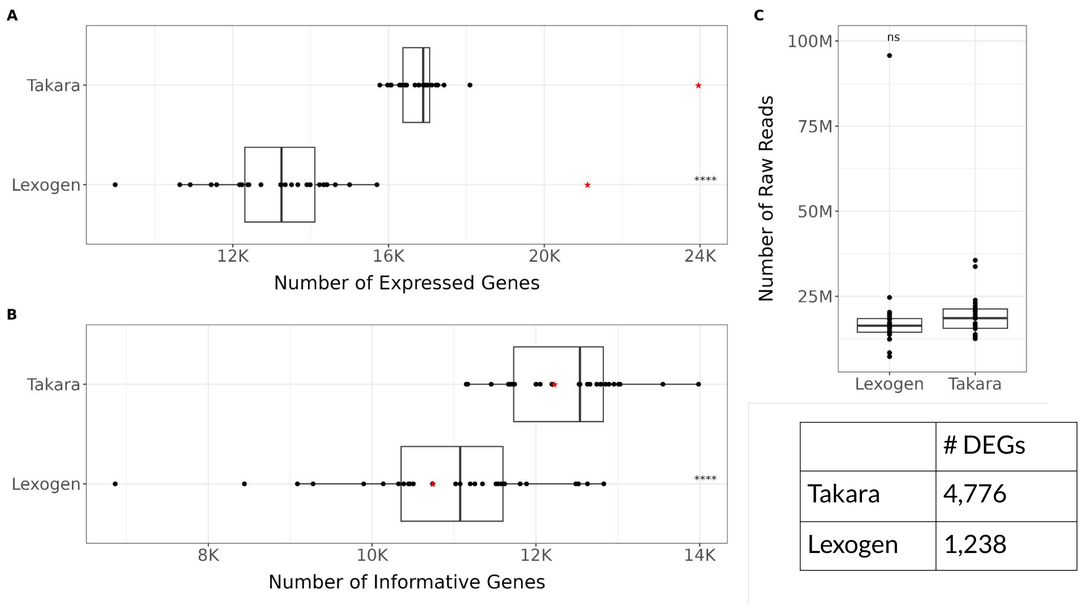


Figure 1: Takara libraries captured significantly more (A) expressed genes and (B) informative genes compared to Lexogen libraries. (C) The number of raw reads was not significantly different between both libraries.



	# DEGs
Takara	4,776
Lexogen	1,238

Figure 2: Takara libraries detected more variants compared to Lexogen libraries

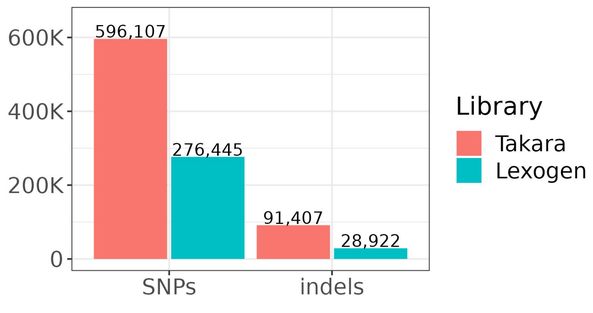
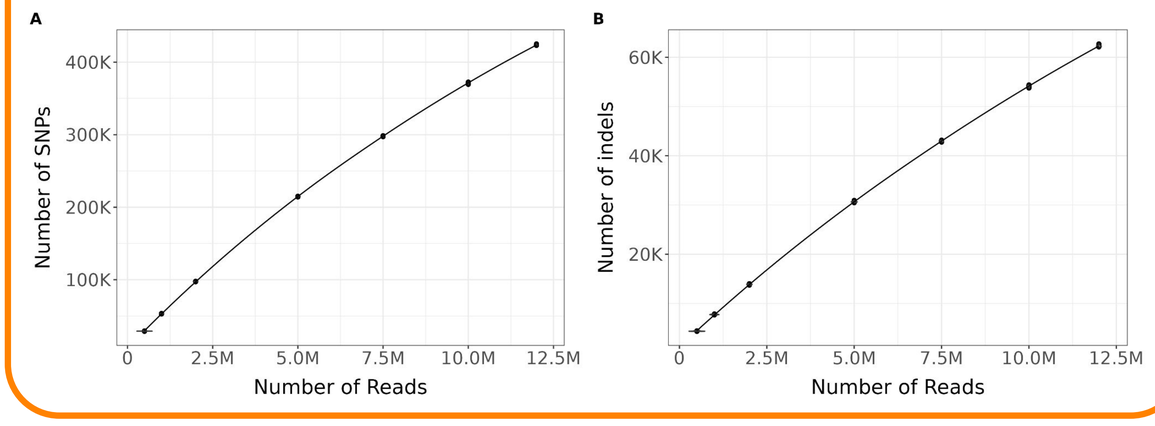


Figure 4: Number of high-quality variants increases almost linearly with the number of reads per sample.



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