

3'-Tag RNA-sequencing

Temitayo Adebunji Olagunju^{1,2}, Chisom Ezekannagha¹, Andreas Gisel^{1,3}✉

¹International Institute of Tropical Agriculture, Ibadan, Nigeria

²University of Ibadan, Ibadan, Nigeria

³CNR, Institute for Biomedical Technologies, Bari, Italy

Competing interests: TAO none; CE none; AG none

3'Tag-Seq is an approach to produce gene expression profiling data for small budgets. The main difference to traditional RNA-seq is that 3'Tag-seq produces one read per transcript and is not resequencing the whole mRNA. This procedure allows the user to request up to 10 times fewer reads for the same transcriptome analysis and therefore is confronted with close and NOT closing to 10 times cheaper sequencing costs. 3'Tag-seq provides high-quality expression data but lacks additional information on alternative splicing events provided by the regular RNA-seq (Ma *et al.*, 2019). Since most of the transcriptome analysis is focused on expression data and differential expression analysis, 3'Tag-seq is, for these cases, the approach of choice. In our work, we outlined the procedure on how to analyse such 3'Tag-seq data, evidence the problem of false-positive counts due to expressed regions with poly T regions which could mimic in the mature RNA polyA tails. Further, we highlighted that 3'Tag-seq can give information on the position of 3'UTR regions. In a test case of the expression analysis of cassava (*Manihot esculenta*) under our experimental conditions, we find read hits in only 10996 of the 26351 annotated 3'UTR (Prochnik *et al.*, 2012); note that

additional 15031 genes do not have a 3'UTR annotation. However, most of these genes contain read hits in the downstream regions up to 1500nt after the gene end. This technology not only gives precise expression information but also data for alternative gene annotation in the region of the 3'UTR.

Acknowledgements

This work has been supported by BMGF (grant INV-008053 “Metabolic Engineering of Carbon Pathways to Enhance Yield of Root and Tuber Crops”) and Genomix4Life, Salerno, Italy

References

1. Ma F, Fuqua BK, Hasin Y, Yukhtman C, Vulpe CD, Lusic AJ and Pellegrini M (2019) A comparison between whole transcript and 3' RNA sequencing methods using Kapa and Lexogen library preparation methods. *BMC Genomics* **20**:9. <http://dx.doi.org/10.1186/s12864-018-5393-3>
2. Prochnik S, Marri PR, Desany B, Rabinowicz PD *et al.* (2012) The Cassava Genome: Current Progress, Future Directions. *Trop Plant Biol.* **5**(1): 88–94. <http://dx.doi.org/10.1007/s12042-011-9088-z>