SQANTI evidence

Long-read transcriptomics producing evidence-driven annotations

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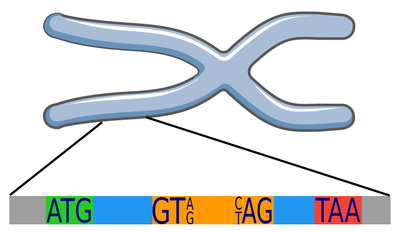
Introduction

The rise of long-read sequencing has unveiled the true complexity of transcriptomes, exposing the limitations of current genome annotation methods in capturing isoform diversity and alternative splicing.

To address this challenge, we developed **SQANTI-evidence**, a novel pipeline that integrates long-read transcriptomes curated with the SQANTI suite into the **AUGUSTUS** framework to guide structural gene prediction.

By combining curated long-read evidence with high-confidence training sets, the method delivers accurate and biologically meaningful annotations, as demonstrated in *Paniagua et al.* (2025).

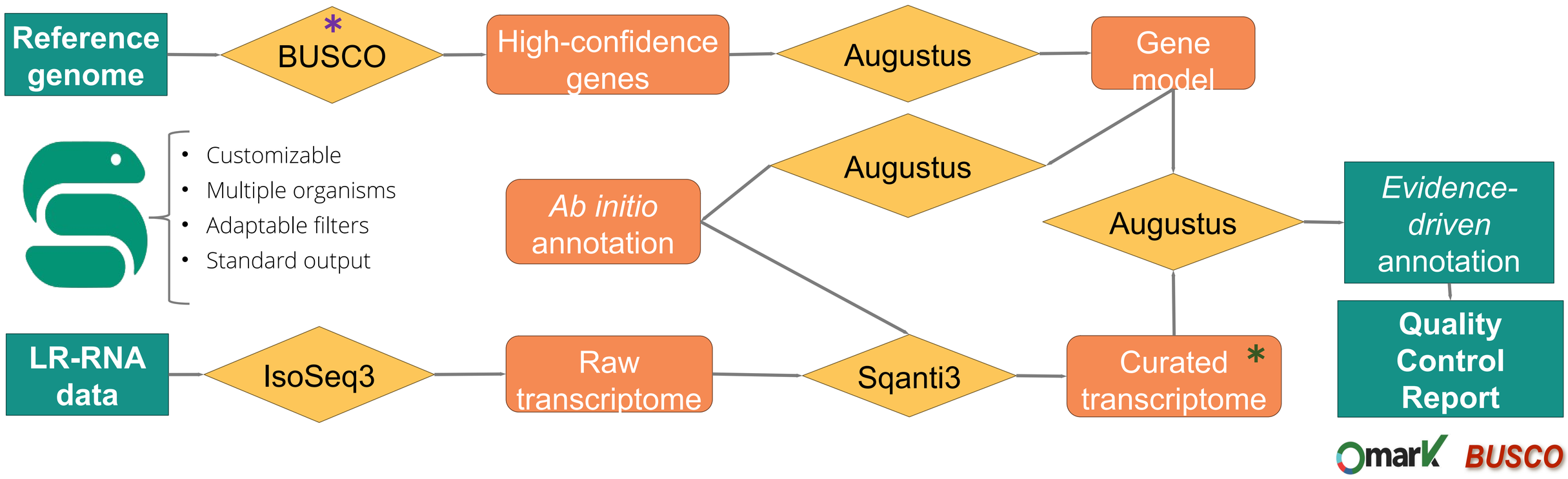
Ab-initio



Evidence-based

Evidence-driven

Methods



- Customizable
- Multiple organisms
- Adaptable filters
- Standard output

Results & Conclusion

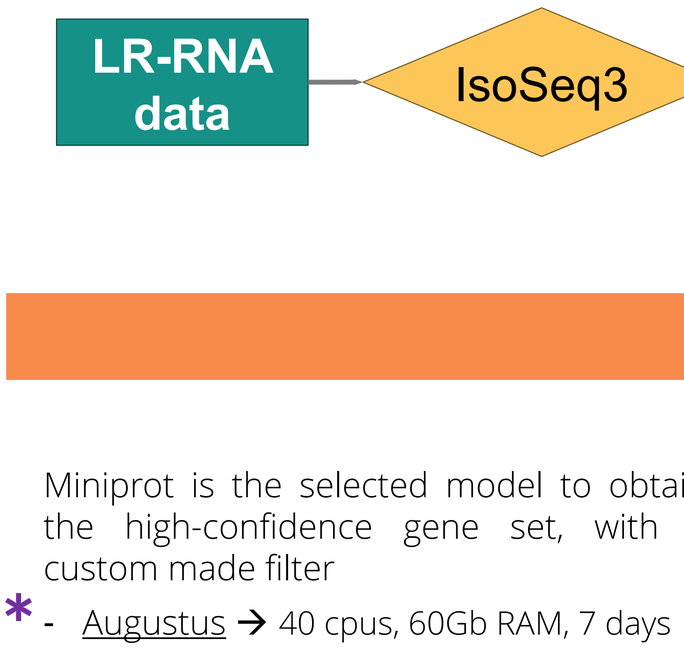
Miniprot is the selected model to obtain the high-confidence gene set, with a custom made filter

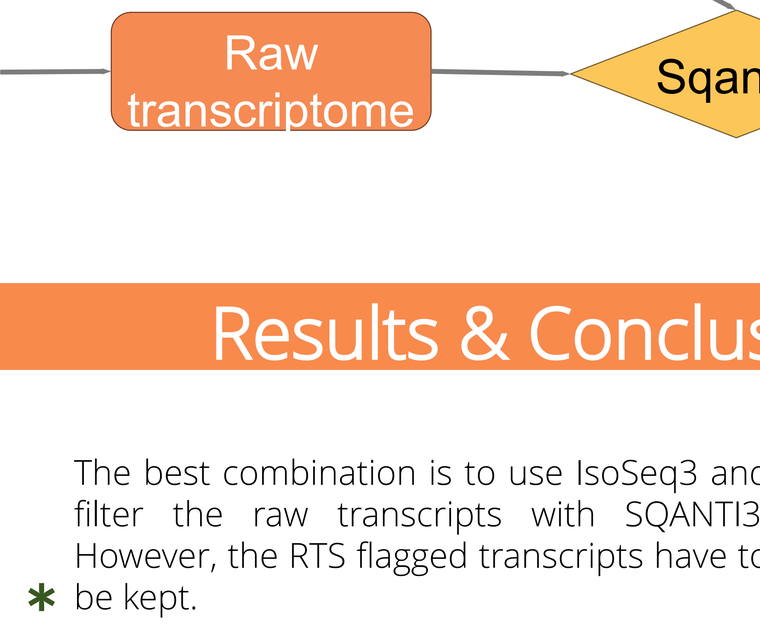
* - Augustus → 40 cpus, 60Gb RAM, 7 days

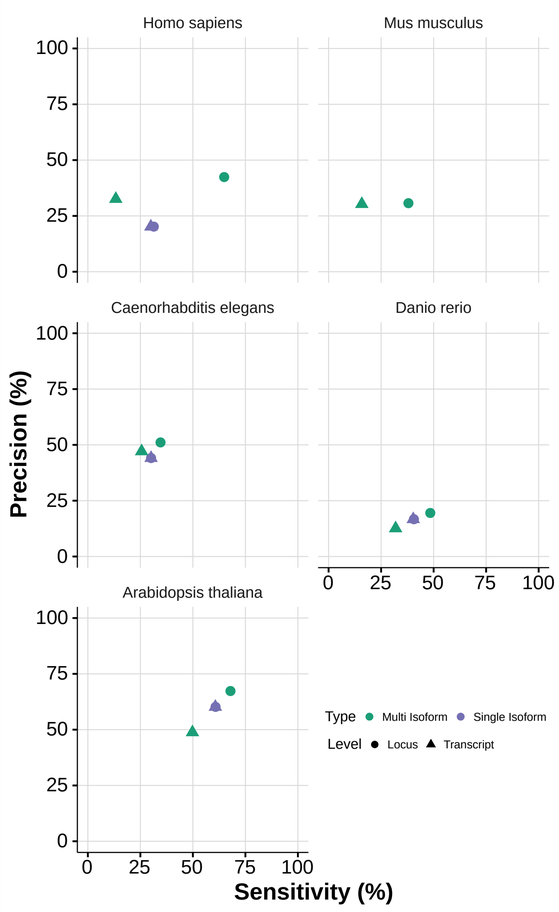
- Miniprot → 40 cpus, 20Gb RAM, 40 mins

The best combination is to use IsoSeq3 and filter the raw transcripts with SQANTI3. However, the RTS flagged transcripts have to be kept.

* IsoQuant does not produce better results. The combination of both is a slight upgrade







The main challenge is to **compare reliably** between newly assembled transcripts and reference annotation.

With the recent explosion in the number of isoforms per gene, achieving an accurate representation using a single predictive model or data from only one tissue has become virtually impossible.

- **SQANTI-evidence** provides a rapid, efficient, end-to-end solution for genome annotation from long-read RNA-Seq data.
- It can generate **high-quality annotations** for any organism, even in the absence of prior reference annotations.

Extensive benchmark with more organisms and tools will follow after the final datasets have been decided upon

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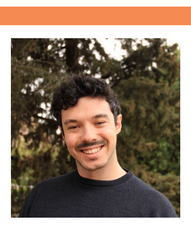

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eosc

OSCARS Open Science Clusters' Action for Research & Society

CSIC Consejo Superior de Investigaciones Científicas