ROSLIN

Transcriptome Annotation Construction Software (TACoS): leveraging long and short read attributes for a fully source aware transcriptome annotation

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BBSRC
20 Years of Pioneering
Great British Bioscience

Motivation

- Functional Annotation of Animal Genomes (FAANG) Consortium
- Public annotations (Ensembl, NCBI, etc.)
  - Mostly just model organisms
  - Require funding to annotate
  - Take time to annotate
  - Annotation decisions are not as transparent and not customizable
  - Slower to adapt to new technologies
  - Require public data
- RNAseq annotations
  - For non-model organisms
  - Highly dependent on analysis pipelines used
  - Likely to be of lower quality

FAANG
Functional Annotation of Animal Genomes

www.clipartkid.com/images/391/back-gallery-for-taco-clip-art-black-and-white-hJUUMH-clipart.jpg

Iso-Seq Motivation

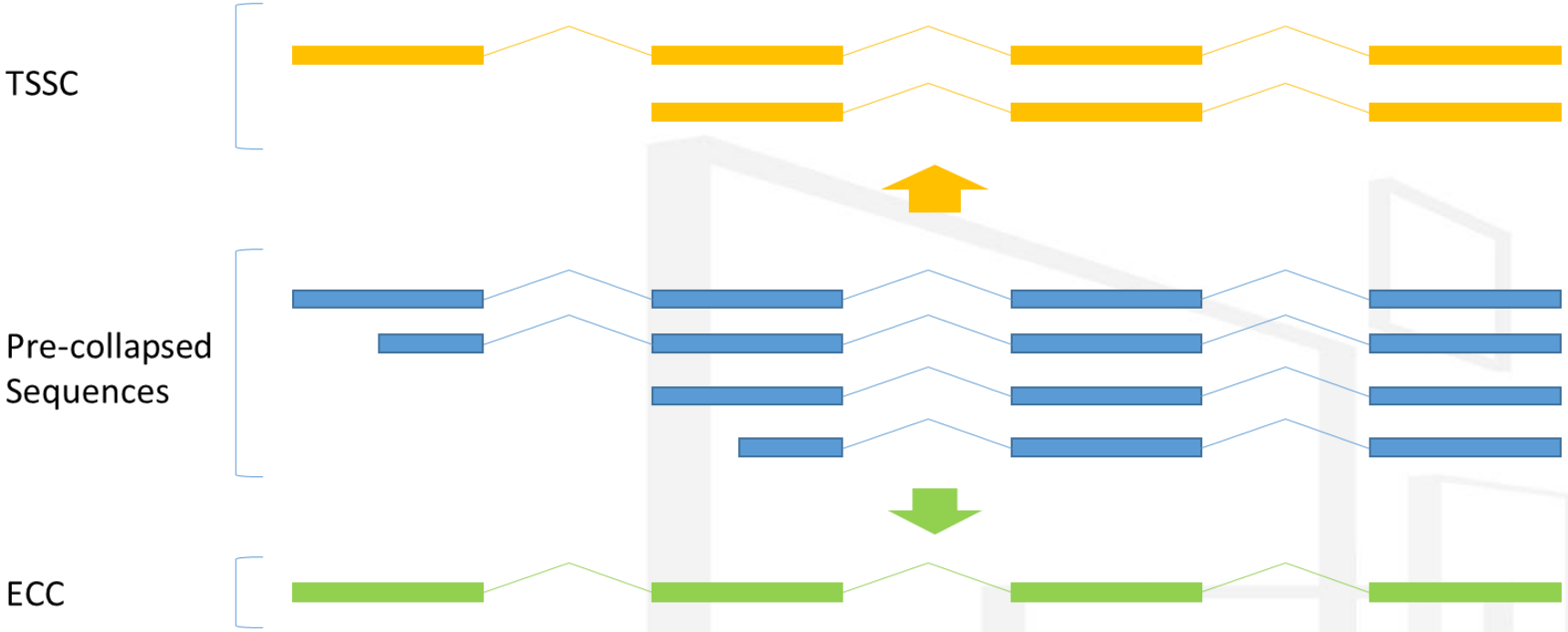
- Collapsing redundant transcripts
- Merging annotations
- Preparing for use by FAANG

FAANG

Functional Annotation of Animal Genomes

Iso-Seq Collapsing

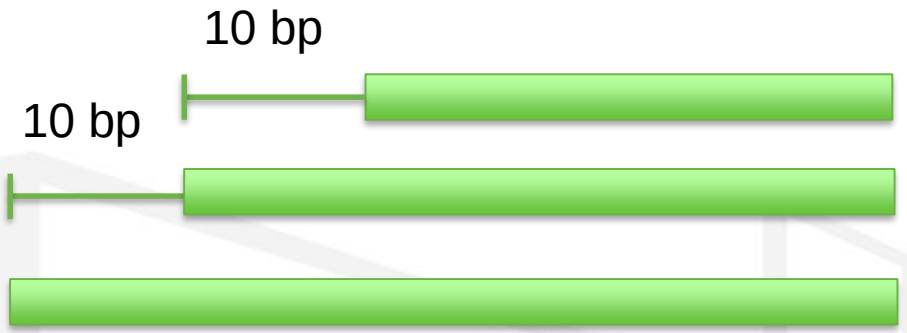
TSSC – Transcription Start Site Collapse
ECC – Exon Cascade Collapse



But what about 5’ cap selected libraries?

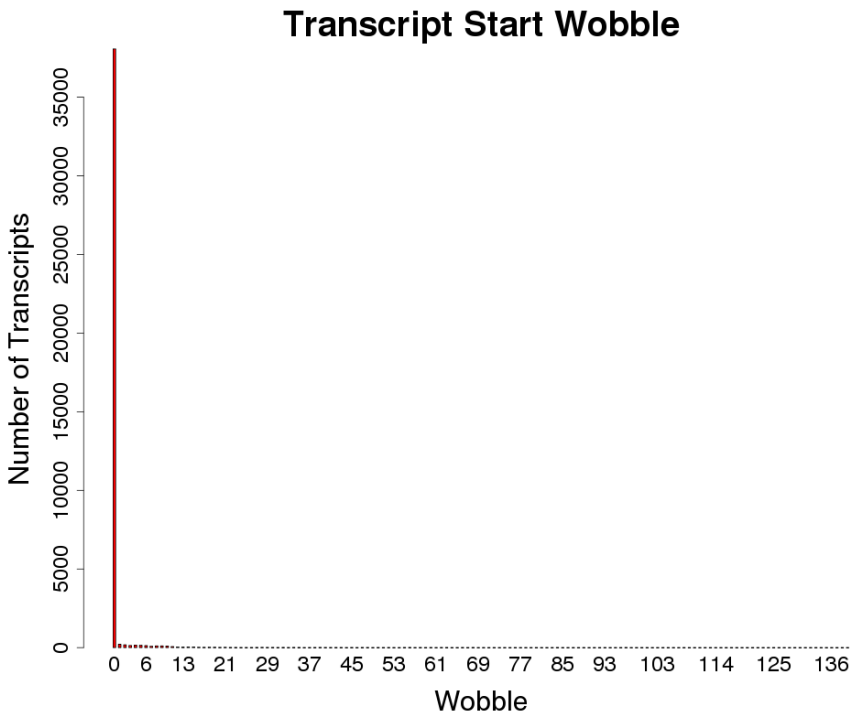
TACoS Collapsing

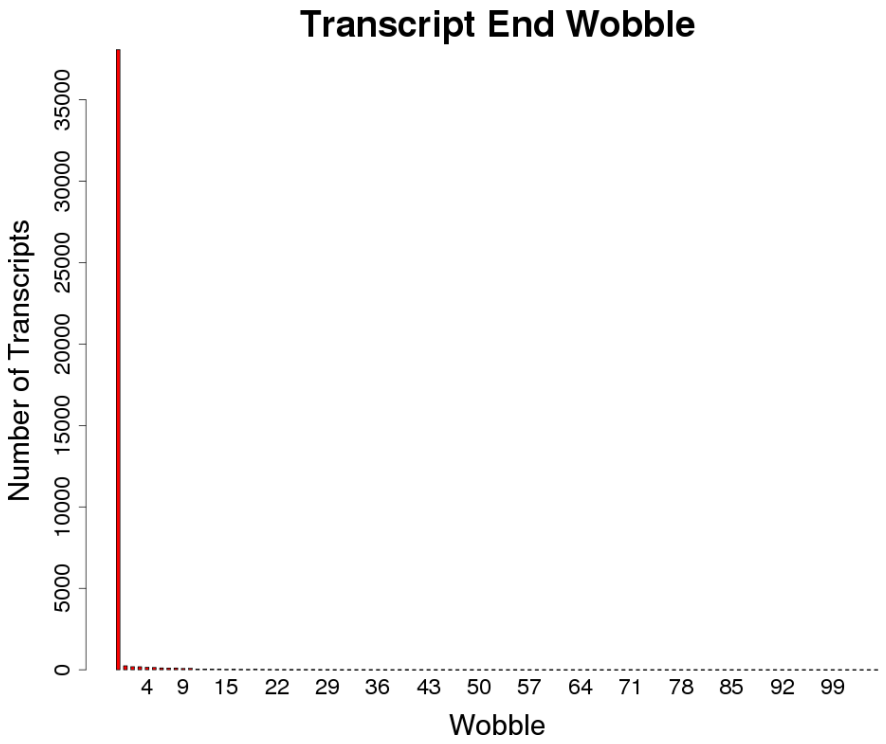
- Threshold for:
  - Transcript start
  - Transcript end
  - Exon start
  - Exon End



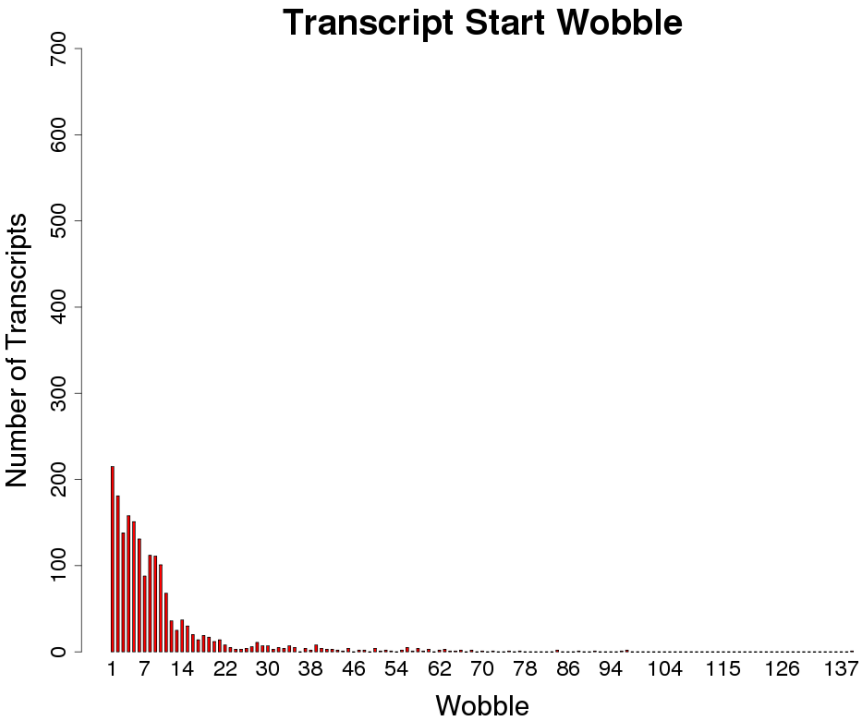
- Grouping:
  - How much wobble walking should be allowed?
- Current implementation:
  - 10 bp difference allowed
  - Unlimited wobble walking

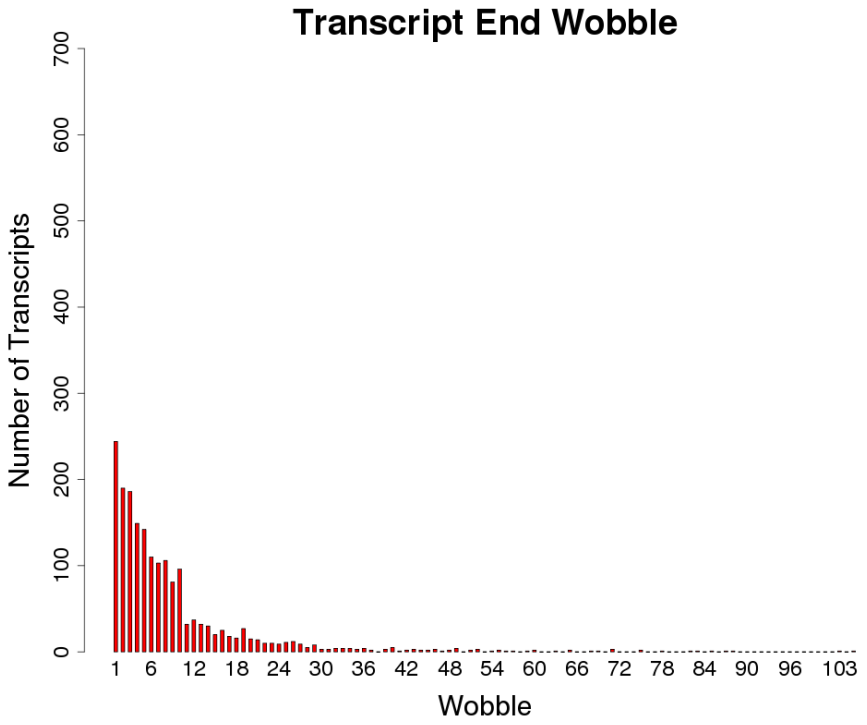
Wobble: Transcript Start/End



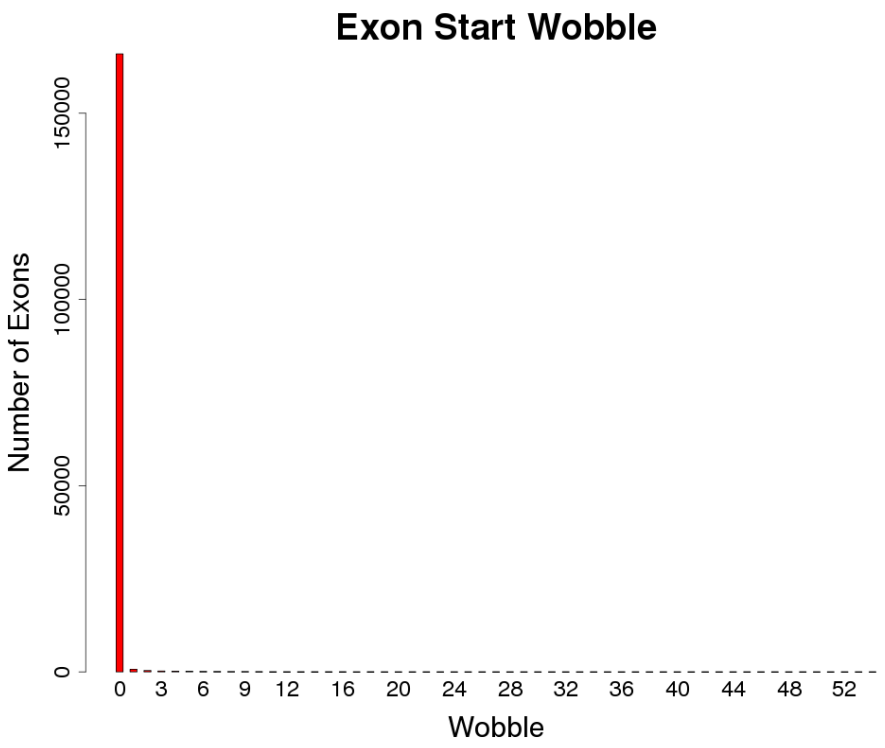


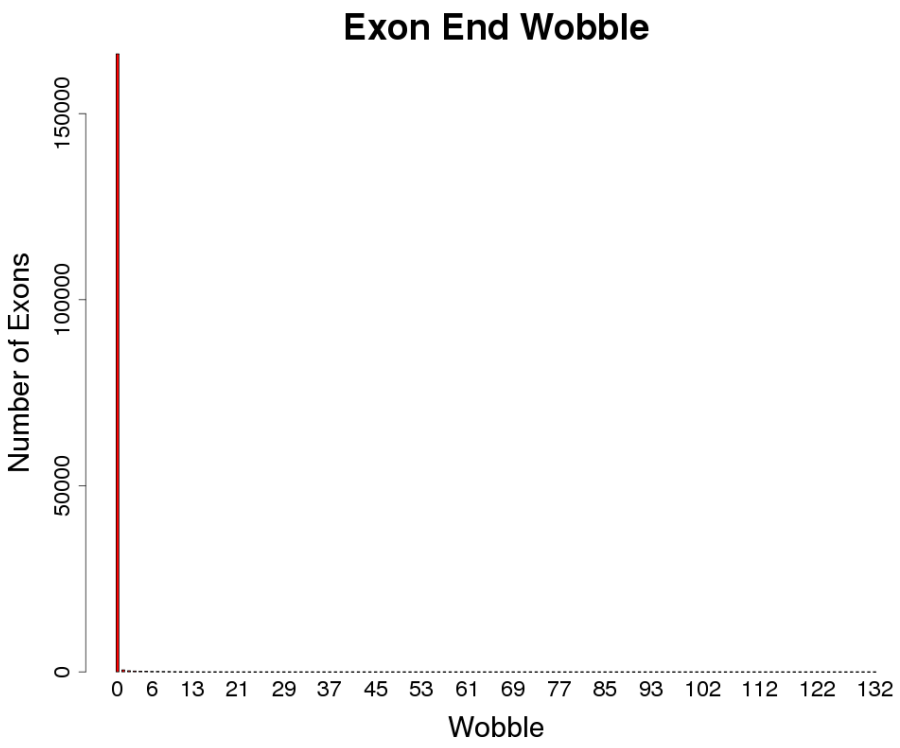
Wobble: Transcript Start/End



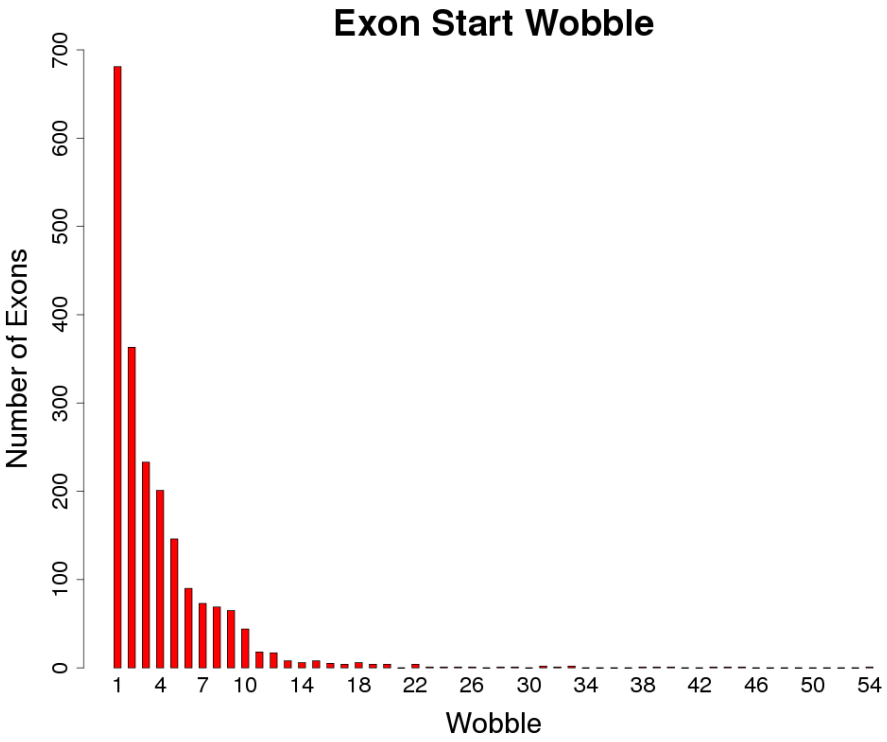


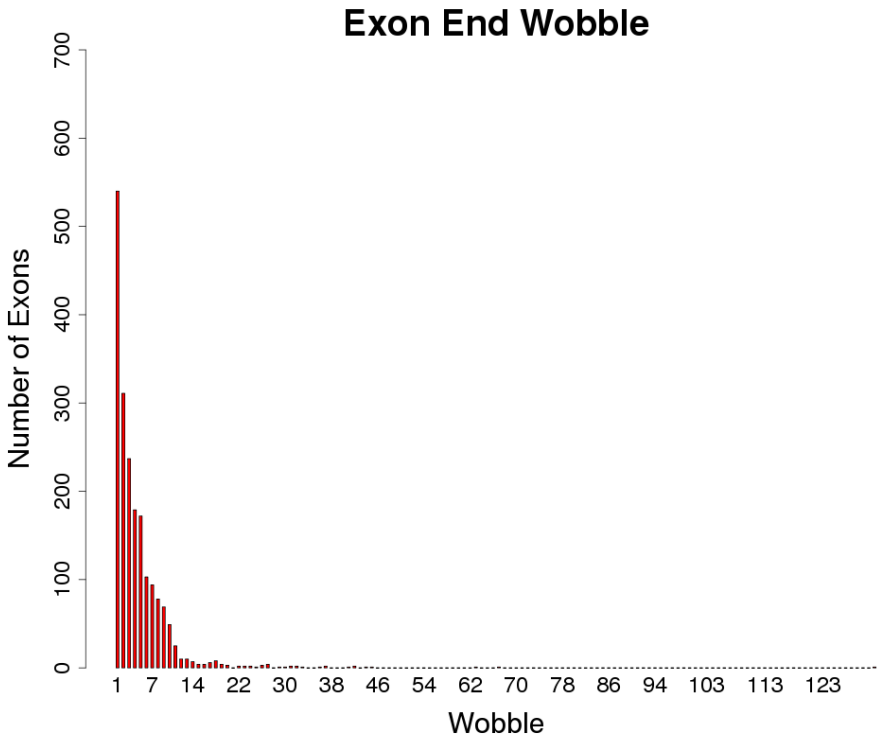
Wobble: Exon Start/End





Wobble: Exon Start/End





TACoS Collapsing

- Control over transcript collapsing
  - Define threshold for transcription start/end and exon boundaries
- Manages 5’ cap selected and non cap selected sequencing data
  - Allows for Iso-Seq Tofu Collapse style of collapsing
- Provides source information for all predicted events
  - Support for each final model
  - Support for each transcript feature (TSS/TTS, splice junctions)
- Flags uncertainties
  - Poly A truncation
  - Genomic issues (assembly gap)*
  - RT Switch*

*future feature

https://tacobueno-live-iglusjax.stackpathdns.com/assets/food/tacos/Taco_Crispy_Beef_990x725.jpg

Additional Features

- Variant Calling
  - SNP and Indels
  - Can be used for phasing
- Wide vs narrow transcription start/end sites
- Wobbly splice junctions
- Can be used from FLNC stage or post-ICE stage
  - Adjust coverage and identity settings

FLNC – Full length non-chimeric reads
ICE – Iterative Clustering for Error Correction

https://tacobueno-live-iglusjax.stackpathdns.com/assets/food/tacos/Taco_Crispy_Beef_990x725.jpg

TACoS Annotation Merging

- Manages short read data merge with Iso-Seq
  - Uses transcript start and end sites from Iso-Seq, verifies splice junctions with RNAseq
  - CAGEseq for transcription start sites*
- Merge public annotation with Iso-Seq*
  - Attach public ID's and flag differences

- Provides source information for all predicted events
- Flags uncertainties
  - Genomic issues*
  - Multi-mapping issues*
- Collapse models for RNAseq count based software*
  - Kallisto, Salmon etc.

*future feature

https://tacobueno-live-iglusjax.stackpathdns.com/assets/food/tacos/Taco_Crispy_Beef_990x725.jpg

Similar Tools

- EVM
  - Weighted inputs
  - Does not weight features
- Augustus
  - Does not yet incorporate Iso-Seq
- IDP
  - Requires Iso-Seq and RNAseq from same sample
  - Incorporates validation pre-assembly
- Anyone know of any other tools?

TACoS Summary

- Primarily based on Iso-Seq data
- Manages Iso-Seq from after error correction to transcriptome annotation
- Integrates other data
  - Sequencing Data: RNAseq, CAGEseq, CHIPseq*
  - Database Data: Public annotations*, protein databases*
- Biotype classification*

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ICE-Cycle

- Tool for splitting FLNC sequences by size for running ICE
- Define overlapping windows
- Collect split groupings
- Re-run ICE with split groupings
- Merge all ICE runs
- Not pretty…

github.com/rkizen/ice_cycle

FLNC – Full length non-chimeric reads
ICE – Iterative Clustering for Error Correction

Acknowledgement

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