Highlights of the Workshop Discussions

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1. Detection and Quantification of snoRNAs

Summary

- A spectrum of snoRNA detection methods was reviewed.
- Methods review indicated that different snoRNA detection approaches have different strengths and weaknesses and as such methods should be aligned with specific research aims (e.g., novel vs. known snoRNAs, quantitative vs. qualitative analysis, subcellular localization, or precursor/derivative detection).
- Benchmarking of reverse transcriptases (RTs) revealed that enzyme selection can significantly impact snoRNA quantification and biotype ratios.
- Attention needs to be given to emerging snoRNA-derived transcripts and isoforms.
- Spike-in controls are vital for managing technical variability, but there remains a need for consensus on their design and application.

Suggestions

- Implement standardized protocols, clearly reporting all methodological details: RT selection, spike-in control design, primer/probe sequences, and quantification strategies.
- Fully define bioinformatics pipelines, indicating any parameter or tool modifications from defaults.
- Make entire analysis pipelines openly available using tools such as NextFlow or Snakemake.
- Develop and share validated spike-in controls and promote benchmarking datasets for method comparisons.

2. snoRNA Annotation, Nomenclature, and Database Integration

Summary

- Annotation challenges persist, including inconsistent naming across species, transcript length variability (the existence of disease-causing mutations in processed spacers was highlighted), and difficulty distinguishing paralogs.
- Agreement was reached to retain existing naming conventions while enhancing clarity through lookup tables for cross-species and cross-database use.
- Confidence in annotations should be provided using evidence codes based on experimental validation, conservation, and genomic features.

Suggestions

- Continue with current naming conventions, supplemented with comprehensive context and lookup tables for cross-database/species comparisons.
- Integrate ongoing annotation work with major databases and assign confidence scores for each annotation.
- Highlight transcript boundaries (5', 3') in case of precursor entries
- Encourage the development and use of shared resources for annotation quality and validation.

3. Functional Analysis and Validation

Summary

- Genetic manipulation (knockdown, knockout, overexpression, rescue) of snoRNAs faces complications from redundancy, host gene effects, and clonal variability.
- No single method is universally adequate; a multifaceted approach (genetic, biochemical, structural, computational) is essential.
- Non-ribosomal functions of snoRNAs should not be overlooked.
- Approaches such as cryo-EM, in vitro reconstitution, and biochemical assays should be considered, when relevant.

Suggestions

- Apply a combination of genetic, biochemical, structural, and computational methods for functional validation.
- Carefully consider potential indirect effects of genomic manipulations, particularly host gene expression for intron-encoded snoRNAs.

- Utilize inducible systems where feasible, and validate not only snoRNA levels but also integrity (5' and 3' ends) and rRNA modifications (when relevant).
- Provide thorough documentation of protocols, observations, and limitations in publications.

4. rRNA Basepairing and Modification Mapping and Quantification

Summary

- There is currently no universally adopted system for rRNA basepairing and modification coordinates.
- A combined approach is advised: report relative to a well-characterized fixed reference sequence (with ±10 nucleotide flanking context) and, when possible, supplement with secondary structure-based annotations.
- Method sensitivity can vary greatly. Clear method benchmarks and the systematic use of spike-ins are essential for reliable quantification.

Suggestions

- Use both sequence-based and structure-based annotation for rRNA basepairing and modifications, always reporting flanking sequence context.
- Publish method-specific benchmarks (detection limits, input requirements, bias profiles) and use spike-ins for method calibration.
- Mask variable nucleotide positions and support mapping across reference sequences.

5. Towards Community Recommendations

Summary

- The group endorses a tiered guideline approach: foundational requirements for reporting, plus optional modules for advanced quality control.
- Guidelines should remain flexible to accommodate technological advances and harmonize with established standards (e.g., ENCODE).

Suggestions

- Co-develop tiered, flexible guidelines encompassing both basic and advanced requirements.
- Make guidelines open-access, community-reviewed, and seek endorsement from major scientific societies.
- Ensure mechanisms for ongoing community input and guideline updates.

Next Steps

Drafting Recommendations

The organizing committee will draft a consortium paper summarizing best practices, the pros and cons of each approach, reporting standards, and critical pitfalls.

Publication and Adoption

Final recommendations will be published in an open-access journal with strong visibility and shared with major societies (RNA Society, Translacore, EMBO, and others).

Ongoing Collaboration

Continuous engagement with the community will support updates, feedback collection, and exploration of future workshops.