# IAB Transcriptome

Thanks to our IAB team in RNA laboratory sample management (RNA isolation, transcriptome library preparation, sample pooling, as well as data analysis), IAB guarantees high quality and consistency for all samples in a respective project.

## Introduction

In transcriptome sequencing (RNA-Seq) (RNA isolation, transcriptome library preparation, sample pooling, as well as data analysis) genes that are transcribed into messenger RNA (mRNA) and translated into protein in the cells. However, it can be challenging to isolate enough high-quality, non-fragmented RNA sufficiently and then there are crucial few stepstones allowing successful analysis, including the decision of the correct workflow (see library prep QC). IAB Transcriptome thus differs depending on measured QC metrics and adjusts workflow to your needs.

## IAB Expertise

The IAB uses different library preparation protocols according to the sample nature and QC metrics of the sample (e.g. origin, concentration, quality) and project requirements.

- Poly(A) mRNA isolation is the most cost-effective solution for high-quality RNA samples (inapplicable for the procaryotic cells). This approach is not species-specific and is suitable for all organisms with a Poly(A) tail on the 3' end of mRNA. mRNA is isolated via Oligo d(T) magnetic beads (**Fig. 1**). This approach does not support analysis of non-coding RNA's, miRNA's and other RNA's that do not undergo translation to the proteins.

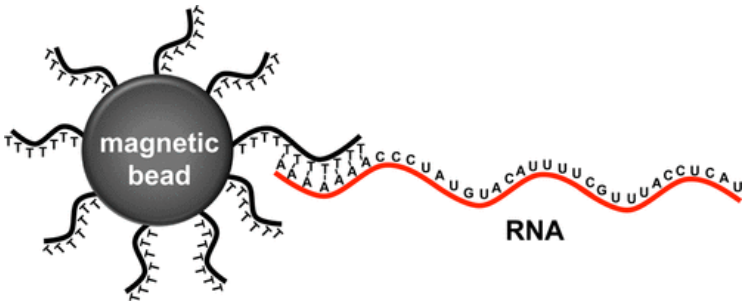


**Figure 1** Mechanism of mRNA separation via Oligo d(T) magnetic beads. After the mRNA is binded to the bead, the sample is placed on the magnetic stand and the supernatant is discarded.

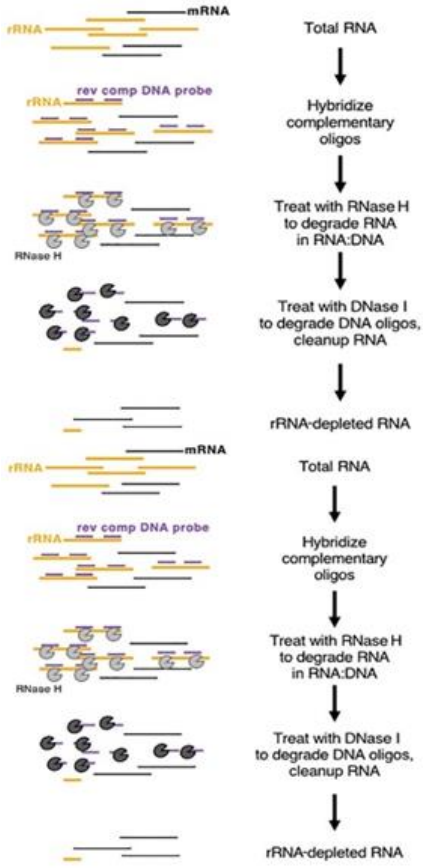


**Figure 2** Scheme of rRNA depletion. Species-specific ssDNA probes are hybridized to the RNA and cleaved by the RNase H, which degrades DNA-RNA hybrids.

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- In lower-quality/quality RNA samples, the rRNA is depleted from total RNA isolated by the species-specific rRNA probes (**Fig. 2**). IAB has experience with various rRNA depletion kits (even the custom kits) and is able to prepare libraries from a variety of RNA's origin. Or highly targeted solutions – Jumpcode, TWIST...

- For ultra-low quality and volume of RNA samples, IAB follows a protocol of post-transcriptional rRNA depletion. NGS libraries are prepared from total RNA, and the rRNA depletion is done at the final level. This approach is also recommended for FFPE samples.

# Material and methods

**Poly(A) mRNA isolation:**

Required amount of RNA: 10 – 1000 ng
Required RIN (RNA Integrity Number) score: 7 – 10
Required volume: ≥ 15 ng/µl

**rRNA depletion isolation:**

Required amount of RNA: 10 – 1000 ng
Required RIN (RNA Integrity Number) score: 2 – 7
Required volume: ≥ 15 ng/µl

**Post-transcriptional rRNA depletion solution:**

Required amount of RNA: 250 pg – 10 ng
Required RIN (RNA Integrity Number) score: 1 – 10 (including FFPE samples)
Required volume: ≥ 15 ng/µl

# Results

Next to the DRAGEN (Illumina), which offers an accurate and fast analysis of transcriptome data, we use CLC Genomics Workbench (QIAGEN) for a more fully customizable and reliable analysis of your samples. Complete QC report, a broad range of graphical representations of differentially expressed genes, GO enrichment analysis, fusions detection and much more (Fig 3, 4, 5).

Contact us for more information.

**3 Fusions**

**3.1 SLC34A2-ROS1**

| | |
|---|---|
| Fusion name | SLC34A2-ROS1 |
| 5' gene | SLC34A2 |
| 5' chromosome | 4 |
| 3' gene | ROS1 |
| 3' chromosome | 6 |
| Reported transcript 5' | NM_001177998.2 |
| Reported transcript 3' | NM_002944.2 |
| Translocation name | SLC34A2(NM_001177998.2):r.1_419_ROS1(NM_002944.2):r.5448_7368 |
| P-value | 0.00 |
| Z-score | 209.04 |
| Fusion crossing reads | 70 |
| 5' read coverage | 94 |
| 3' read coverage | 70 |

| 5' position | 3' position | Translocation Name | Count |
|---|---|---|---|
| 25,664,330 | 117,329,446 | SLC34A2(NM_001177998.2):r.1_419_ROS1(NM_002944.2):r.5448_7368 | 70 |
| 25,664,330 | 117,324,415 | SLC34A2(NM_001177998.2):r.1_419_ROS1(NM_002944.2):r.5757_7368 | 19 |
| 25,664,330 | 117,326,414 | SLC34A2(NM_001177998.2):r.1_419_ROS1(NM_002944.2):r.5566_7368 | 5 |

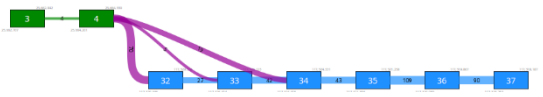


**Figure 3**

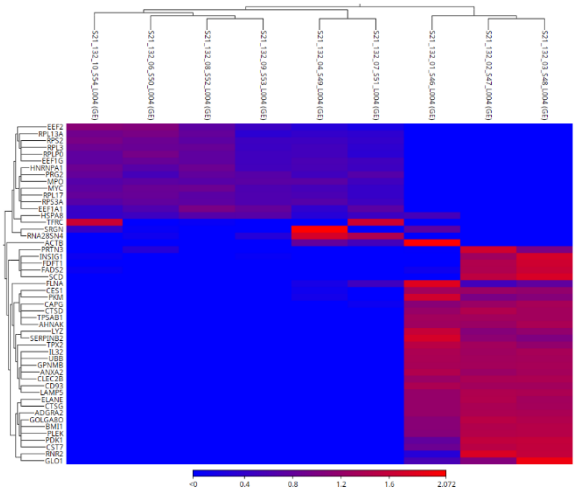

**Figure 4**

Example of detected and reported SLC34A2-ROS1 fusion with graphical representation. CLC Genomics Workbench v 22.1.

| Type | Uniquely mapped | Non-specifically mapped | Mapped | % of total mapped with type |
|---|---|---|---|---|
| Gene (total) | 16 313 328,00 | 599 891,00 | 16 913 219,00 | 98,56 |
| - Intron | 1 749 168,00 | 52 981,00 | 1 802 149,00 | 10,5 |
| - Exon (total) | 14 564 160,00 | 546 910,00 | 15 111 070,00 | 88,06 |
| - Exon | 6 067 754,00 | 186 652,00 | 6 254 406,00 | 36,45 |
| - Exon-exon | 8 496 406,00 | 360 258,00 | 8 856 664,00 | 51,61 |
| Intergenic | 233 816,00 | 12 643,00 | 246 459,00 | 1,44 |
| Total | 16 547 144,00 | 612 534,00 | 17 159 678,00 | 100 |

| Biotype | % of total |
|---|---|
| mRNA | 95,03 |
| misc_RNA | 3,23 |
| rRNA | 0,93 |
| ncRNA | 0,81 |

**Figure 5**

# Conclusions

IAB transcriptome solution represents a set of proved protocols tailored for different RNA inputs with individual focus on the quality/quantity of samples and required type of transcriptomic analysis.

# About IAB

The experienced team follows IAB Transcriptome workflow with strict quality procedures to perform the essential QC steps on your samples and generate reliable and repeatable results.

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