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1) IN YOUR OWN WORDS briefly describe the each of the following classes of RNAs and their functions. Give at least one full sentence for each description and another full sentence for each function.

* rRNA: Most abundant RNA in humans based on percent of total RNA mass. Works with proteins to make ribosomes.
* tRNA: Most abundant RNA in humans based on the number of molecules. Works with ribosomes to turn mRNA into proteins.
* mRNA: Abundant RNA that is smaller than rRNA or tRNA, but still large compared to other RNAs. Contains the sequences used by ribosomes and tRNA to make proteins.
* hnRNA: Estimated to be 2 to 4% of the mRNA weight. It is just pre-mRNA that has yet to be processed and turned into full mRNA.
* snRNA: Abundant RNA that are way smaller than rRNA or tRNA. Probably helps transcribe split gene products.
* snoRNA: Abundant RNA that are way smaller than rRNA or tRNA. Helps regulate ribosome and spliceosome function.
* miRNA: Small RNA present in high levels. They help regulate gene expression.
* circRNA: Strand ends are connected so it appears to be a circle. Have many roles, such as being transcription factors, and are extra stable thanks to their lack of free 5’ and 3’ ends.
* lncRNAs: Represent non-coding RNAs that are longer than 200 nucleotides. Some may encode peptides or play a role in epigenetic modification, but overall they thought to be non-functional.

2) Which of the RNAs in Table 1 are most abundant based on the number of molecules? Which are most abundant based on total RNA mass?

rRNA is the most abundant based on total RNA mass while tRNA is the most abundant based on the number of molecules present.

3) Given the relative abundances by mass, what percentage of your RNAseq reads would come from rRNA if you extracted total RNA and created sequencing libraries using a technique that did not purify or bias the relative composition of the sample?

rRNA makes up around 80 to 90% of the total RNA mass. It would be reasonable to expect that, if we do not try to compensate for this, a similar percent of our reads would come from rRNA.

4) Which of the RNAs in Table 1 can be separated from each other based on size (using gel electrophoresis or another technique)? In other words…

* If you were to run all of the RNAs on a gel and cut out bands that correlate to different sizes of RNAs, how many different bands would you get if you assume that all RNAs in a class are exactly the size listed as their average size in the table (which is not reality) and that you can easily separate bands of RNAs that are 500 bases (0.5kb) different from each other in size?

There would be 7 visible bands.

* Would any of the bands have more than one class of RNA grouped together in it? If so, which ones would be grouped together?

Yes. The 0.1 to 0.6 band would contain tRNA, circular RNA, snRNA, snoRNA, and 7SL. The other types of RNA in the chart should be further then 0.5kb apart from one another and produce their own bands.

* What about if you could increase your resolution to 50 bases (0.05kb) of difference between the bands? How many bands would you have? Would any of the RNA classes still be grouped together?

You would see more bands. There would probably be about 9 bands. Most of the 0.1 to 0.6 band would be broken up. However, the tRNA, snRNA, and snoRNA still overlap so they may appear together as one single band.

5) Based on Table 1, what would be left if you were to get rid of all of the rRNAs and use size selection to eliminate anything smaller than 0.5kb? Specifically, what RNA classes would remain what percentage of the total starting material would you have left?

You would still have rRNA, mRNA, hnRNA, circular RNA, Xist, and lncRNA. We would still have between 83.0923 and 97.45% of the total starting RNA mass left.

6) Which of the RNAs in Table 1 will be included in an experiment that extracts only polyadenylated transcripts?

It would include lncRNA and mRNA.

7) What is junk RNA and what causes it?

Junk RNA are non-functioning strands of non-coding RNA. They are thought to be caused by pervasive transcription, which means that most of the genome is transcribed, even if it doesn’t necessarily have a clear functional purpose. RNA polymerase prefers promoter regions, but also has a small chance to set down on any accessible DNA. This could result in inappropriate, nonfunctional transcripts.

8) If you had discovered a novel (unannotated) noncoding RNA, how would you determine whether it was functional or simply junk? Specifically, if you wanted to prove that it was functional, what would you look for in terms of the following:

* Expression levels

Presence is a burden. Higher presence indicates it may have function.

Synthesizing RNA is costly. Higher synthesis rate indicates it may have function.

May have deleterious effects. If so, may indicate there is some sort of valuable function that has kept it from being eliminated by natural selection.

* Expression profiles

Less restrictions on expression is more indicative of function.

* Distribution of the RNA in the cell

Exportation to the cytoplasm may be a sign of function, as most non-functional RNA stay nuclear. However, sub-nuclear compartmentalization cannot be used as evidence to concretely support functionality.

* RNA processing

Presence of strong processing signals may indicate functionality. As may an absence of polyadenylation, which are often seen in non-functional RNA. But there are exceptions.

* Conservation

Lower mutation rates and conservation are associated with functionality. Many of the non-functional RNA at a rate consistent with genetic drift. However it is important to consider the context as there are exceptions.

* Causal roles

Perform elimination on RNA sequence to see if it effects fitness. Reduction in fitness is associated with a functional strand of RNA.