Abstract

Alongside ribosomal-RNAs (rRNAs) and messenger-RNAs (mRNAs), transfer-RNAs (tRNAs) are one of the large RNA groups that play an important role in protein biosynthesis. In their secondary structure, tRNAs represent a cloverleaf stucture with four different loops: a D-loop, T-loop, variable loop and an anticodon loop. The average length of tRNAs is between 74 and 95 nucleotides (nt). However, in some species (e.g. mites) the length and structure can vary widely. Recently, the mitochondrial genome of *Paraleius leontonychus*, a mite of the order Oribatida was sequenced. Annotation revealed only 20 of the expected 22 tRNAs in this species, with strong modifications in length and structure. These found tRNAs still have to be experimentally validated.

In this thesis an attempt was made to establish the YAMAT-seq a method for the high-throughput sequencing of tRNAs and to develop a standard operating procedure (SOP) for the use of this protocol on RNA extracted from mouse tissue and *Pichia pastoris* cells, where sufficient RNA amounts can be obtained. Instead of the Illumina TruSeq Small RNA sample preparation kit used in the original method, a NEBNext Small RNA Library Prep Kit was used and a new Y-3´-adapter was designed. In two different attempts various protocol modifications were tested. From these different protocol modifications, two samples showed the expected band in the range of 190 nt and 210 nt on the agarose gel.

The successful samples show which steps are important in the protocol. While the adapter amount described in the YAMAT-seq protocol (40 pmol 3´-AD and 10 pmol of each 5´-AD) seems to result in an adapter surplus, both successful samples worked with a 1:10 adapter dilution (4 pmol 3´-AD and 1 pmol of each 5´-AD). Further important points are the use of at least 10 units T4 RNA ligase and either increasing the tRNA concentration, through an additional step of tRNA enrichment by separation on a 6% PAGE gel and passive gel elution, or the reduction of the NEB protocol volume (70 µl instead of 100 µl).

Before using the YAMAT-seq protocol on mites, the successful samples of this attempt should be sequenced and results evaluated. Further protocol improvements could also be considered.