Abstract

The prediction of transfer ribonucleic acids (tRNAs) with a standard clover-leaf structure is straightforward and well-established. In most eukaryotes, tRNAs consist of a D-arm, a T-arm, an acceptor stem and an anticodon stem. However, mitochondrial tRNAs of certain species lack the D-arm or the T-arm (or both) and prediction from sequence is difficult. Extreme examples are mitochondrial tRNAs in mites in particular (1–3).

In the context of a project of Schäffer *et al.*, the mitochondrial genome of *Paraleius leontonychus*, a phoretic mite using the bark beetle as its host organisms for transport, was sequenced. To predict the structure of mitochondrial tRNAs in *P. leontonychus*, bioinformatic annotation was used. However, only 20 instead of the typical 22 mitochondrial tRNAs were annotated. Some mitochondrial tRNAs lacked one or both arms and showed mismatches in the stem loops (4).

In silico annotation is not 100% reliable. Therefore, predicted sequences have to be verified in laboratory. Sequence validation is a method to proof annotation results. In this Bachelor thesis, a standard operating procedure (SOP) for sequence validation was developed following the approach of Wende *et al.* (1). Parts of the experimental procedure were tested with the mite species *Chamobates* and *Scheloribates* and with the yeast *Pichia pastoris* CBS 7435.

The experimental procedure involved the extraction of total RNA from *Chamobates*, *Scheloribates* and *P. pastoris*. The total RNA concentrations of the extraction products were measured and the optimal number of mites needed for extraction was calculated. The extraction from 100 µL *P. pastoris* cell suspension resulted in 11.80 µg of total RNA at a concentration of 118.0 ng/µL. 35 individuals of *Scheloribates* revealed 2.38 µg of total RNA at a concentration of 23.8 ng/µL, whereas 53 individuals of *Chamobates* resulted in 4.71 µg of total RNA at a concentration of 47.1 ng/µL. The total RNA concentrations of *Chamobates* and *P. pastoris* were sufficient to perform a cDNA synthesis. However, the RNA concentration of *Scheloribates* was not sufficient, leading to the conclusion that a number of 35 mites is too small to perform a cDNA synthesis. A minimum of 53 individuals of *P. leontonychus* will be necessary for a sequence validation. 2D- and 3D-structures of mitochondrial tRNAs in *P. pastoris* and in *P. leontonychus* were generated using bioinformatic software tools. A concept for primer design was developed using the approach of Wende *et al.* (1). Based on this concept, 44 primers for *P. leontonychus* and 4 primers for *P. pastoris* were designed.