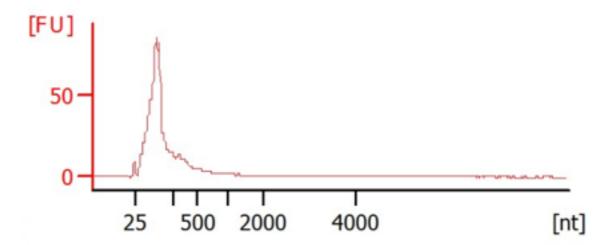
RNA isolation from lysed T. Solium eggs

RNA was isolated using RNeasy isolation kit from QIAGEN following manufacturer's instructions. Briefly, RNA was separated from other components by phenol-chloroform extraction and loaded onto a column. Column-bound RNA was subsequently washed and eluted in water. Before elution we performed on column DNAse Digest for 20 minutes with half of the samples. RNA concentration was measured using NanoDrop and BioAnalyzer. We could not detect significant differences in RNA quality or gDNA contamination when comparing on column digests with samples that were cleaned up without DNAse digest. DNAse digested samples yielded significantly lower RNA concentrations.

RNA quality control using Bioanalyzer

BioAnalyzer analysis of RNA samples was performed following the manufacturer's instructions. Most of the signals were detected below a length of 200 nucleotides, which indicates a strong degradation of RNA in our samples. Adiconis and colleagues (Nature 2013, Adiconis, Comparative analysis of RNA sequencing methods for degraded or low-input samples) previously reported successful sequencing and bioinformatics analysis using pancreas tissue samples with similar low quality. We decided to do next-generation-sequencing –based transcriptome analysis from one lysed tapeworm sample to test the feasability of this approach.



Next-Generation-Sequencing of RNA samples:

We decided to test RNA sequencing of two individual RNA samples from the same tapeworm egg. We chose the samples with the highest amount of RNA above a length of 200 nucleotides, because all molecules below that size would be lost in the sequencing preparation process. The sequencing core facility of the Max-Planck-Institute for Molecular Genetics in Berlin prepared two libraries using their in-house protocol. Subsequently, the libraries were sequenced using a P50 chip on a HiSeq2500 (Illumina). The two samples are considered technical replicates in the subsequent analysis. RNA sample 1 and 2 yielded 48 million fragments (96 miollion total reads) and 44 million fragments (88 million total reads). Fastq files were exported and used for bioinformatics analysis.

Bioinformatics:

First we aligned our sequences to the Taenia solium genome.70.98% of the reads were aligned unique mappings, 13.57% multiple mappings and only 15.42 unmapped. To identify potential targets for our toehold switches, we wanted to eliminate all T. solium specific sequences that can also be found in different tapeworm species like T. saginata or T. asiatica. For this, T. solium specific reads were barcoded and mapped against the genomes of T. Saginata and T. asiatica, all reads that were not aligned to the different species represent potential targets for our sensor platform.