dynamic BIOSENSORS

Application Note

Quantifying DNA exonuclease activity using a switchSENSE® hybridization assay

Keywords: exonucleases, enzymes, DNA degradation, T5

Background

DNA exonucleases are enzymes that degrade DNA by removing nucleotides one at a time. While exonucleases can be very useful tools in molecular biology for the controlled removal of DNA pieces, their presence as contaminants amongst other enzymes (e.g. in PCR solutions) can be very problematic as it results in the loss of DNA of interest. It is necessary to accurately determine the activity of exonuclease solutions before use, and, likewise, it is crucial to detect unwanted nuclease activity with high sensitivity for the manufacturing of 'exonuclease-free' products.

Here we present a simple and quick assay to quantify the activity of the T5 exonuclease with a standard switchSENSE chip on a DRX analyzer in an automated workflow.

Methods and Results

A switchSENSE Multi-Purpose Chip (MPC-48-2-Y1-S) with immobilized 48 mer ssDNA and a DRX^{yellow} analyzer were used in this study.

The 5'→3' exonuclease activity of T5 enzyme (NEB) was investigated by measuring the enzymatic degradation of a single stranded oligodeoxynucleotide substrate (=sacrificial DNA) in solution. As sacrificial DNA, a sequence complementary to the DNA immobilized on the switchSENSE chip was chosen (cNL-48B). Different concentrations of T5 exonuclease (from 0.1 U/µl to 0.001 U/µl) were prepared by dilution

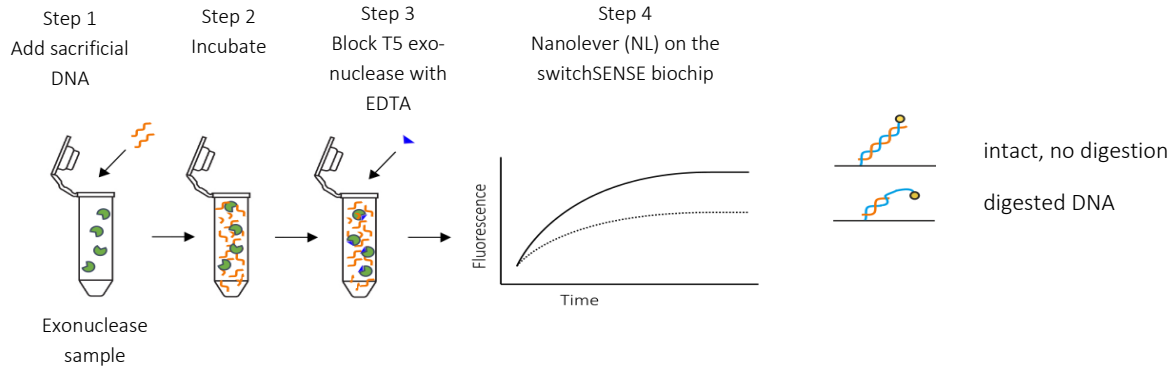

Figure 1 | Assay workflow to quantify the activity of ultra-low concentrations of T5 exonuclease with a standard switchSENSE chip on a DRX analyzer in an automated workflow.

in 1x NEBuffer 4 (NEB). To probe the activity of the enzyme solutions, sacrificial DNA (500 nM) was added and the mixture was allowed to react at an optimal temperature of 37°C, cf. steps 1 and 2 in Figure 1. After 30 min, the enzymatic reaction was stopped by adding 20 mM EDTA, which scavenges divalent ions and thus quenches the enzymatic activity (step 3).

In the 4th and final step, the reaction mixture containing digests of the sacrificial DNA as well as un-degraded DNA was then injected into the flow channel of the multi-purpose chip.

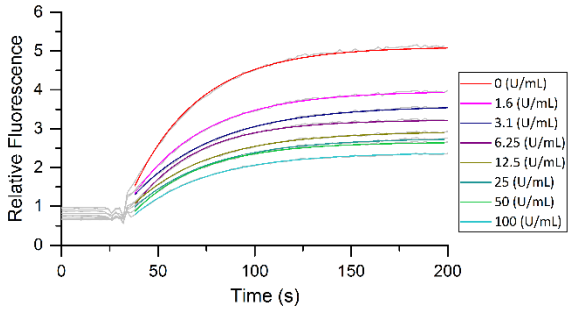

Figure 2 | Fluorescence signal during hybridization of sacrificial (initially 48mer) ssDNA to a switchSENSE multi-purpose 48mer DNA chip. The sacrificial ssDNA was previously degraded with T5 exonuclease at different concentrations. Colored lines are exponential fits and grey lines are the measured signals. Data are referenced with buffer injections.

The switchSENSE sensor was used as a highly sensitive detection system for DNA hybridization in real-time. An increase in the fluorescence signal is observed when solute DNA hybridizes to dye-labelled complementary DNA probes on the gold sensor surface. This is because the dye is less quenched by the gold as it moves away from the surface when the rigid duplex forms. A slight negative potential of -0.1 V that is applied to the gold electrode facilitates this process.

At zero T5 concentration, the sensor signal increases by a factor of 5, which reflects the hybridization of intact 48mer DNA to the sensor.

When T5 is present, lower signal end levels are obtained, which indicates that the sacrificial DNA has been enzymatically degraded. Remarkably, a hybridization signal is observed even at the highest T5 concentrations. This means that the exonuclease fails to completely 'chew up' the sacrificial DNA into individual nucleotides, but pieces of significant lengths remain undigested.

An alternative explanation for the reduced signal amplitude seems conceivable at first, too, but it can be ruled out by inspecting the real-time hybridization kinetics: one could assume that the enzyme can digest the sacrificial DNA completely down to individual nucleotides, but just did not have enough time to react on all the sacrificial DNA strands before the reaction was stopped. In this case, a decreased signal amplitude (end level) would be observed as well. However, the unchanged hybridization kinetics (coefficient of variation < 10%) reveal that the concentration of DNA hybridizing to the probe DNA on the sensor remains constant, which contradicts this notion. If the enzyme would not have had time to digest all the DNA, at least it would have reduced the concentration of sacrificial DNA to some extent. Consequently, slower hybridization kinetics should be observable, which obviously is not the case within this concentration regime of the enzyme and sacrificial DNA. Instead the enzyme produced a type of 'short' DNA, which is long enough to form a stable duplex with the DNA on the sensor at 35 °C, but is shorter than the substrate 48mer DNA, as can be seen from the decreased sensor response (end level).

Hence, by considering the signal end levels together with the real-time hybridization kinetics, we can conclude that all the sacrificial DNA strands in solution had been processed by the exonuclease during the 30-min reaction time.

To identify the concentration of T5 enzyme that is required to achieve maximal digestion for the DNA solution used here, a dose response curve was calculated from the measured fluorescence. The percentage of the sacrificial DNA degraded is plotted as a function of exonuclease concentration in Figure 3. '100% DNA degraded' denotes that all the sacrificial strands in the reaction mixture had been processed by the enzyme.

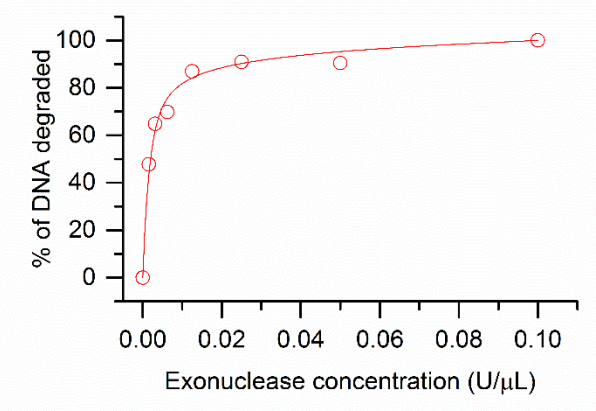

Figure 3 | The degree of DNA degradation (as obtained from the amplitude of the exponential fits in Figure 2) plotted as a function of T5 exonuclease concentration.

Conclusions

This study demonstrates that the presence of T5 exonuclease down to 0.001 U/µL could be detected and quantified using a switchSENSE hybridization assay.

Importantly, the combined analysis of signal end levels and real-time kinetics enables the user to follow the time course of the enzymatic reaction and to eventually identify the length of the digested DNA products.

The switchSENSE measurement was performed in less than 5 minutes and the liquid handling steps required to incubate the enzyme with sacrificial DNA beforehand could be performed automatically by the DRX's auto-sampler system. The study further expands the possibilities to perform experiments at different temperature and reaction time, which would improve the detection limit. It is also straightforward to adapt the assay to monitor exonucleases with an opposite directionality.

Authors I Prayanka Rajendran, Andreas Langer & Ulrich Rant
info@dynamic-biosensors.com
Dynamic Biosensors GmbH I December 2016