

The TATAA Biocenter, Sweden

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The TATAA Biocenter is a biotechnology research center located in Göteborg, Sweden. The center focuses on molecular diagnostic methods and interacts with research groups and biotech companies around the world. One of our major partners is Bio-Rad Laboratories Ltd.

Our business idea is to develop and refine technologies in the molecular diagnostic area and transfer the results and experiences to industry and to end users. The latter we do by arranging hands-on training courses. Recently we took the initiative to start a European network on ultra-sensitive molecular diagnostic methods and on behalf of more than 50 members presented an expression of interest to the European community sixth framework program (<http://www.tataa.com/EC-networks.htm>). We also assist research teams and R&D focused companies in implementing molecular diagnostic methods in their work, and we do commissioned research in the area. In a UNESCO supported program we introduce real-time PCR based diagnostic methods to developing countries in order to fight the spreading of infectious diseases such as AIDS. As leading technology consultants we also assist global management consultant firms in technology assessments and evaluations in due diligence processes.

This article highlights some of the diagnostic methods we have developed using real-time PCR detection on the Bio-Rad iCycler iQ™ System.

The Light-up probes

The people behind the TATAA Biocenter started to develop methods for homogeneous probing in 1991. The goal was to design a simple probe that upon sequence specific binding to target nucleic acid would become luminescent. These so called Light-up probes were initially made of an oligonucleotide to which an asymmetric cyanine dye was tethered. With time the oligonucleotide was replaced by the

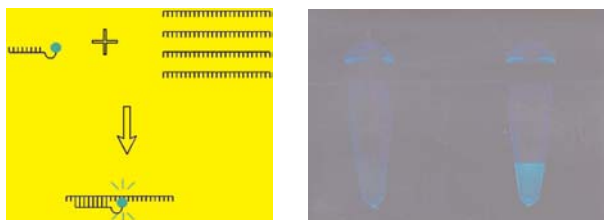


Figure 1 Left: Principle of homogeneous probing with Light-up probes. Right: Samples containing Light-up probe and non complementary (left) or complementary (right) DNA, illuminated by UV-light.

DNA mimic, peptide nucleic acid (PNA), which improved the fluorescence enhancement upon hybridisation and increased the stability of probe-target DNA complexes (Figure 1). The probe is excellent for real-time PCR applications, where it is designed to hybridise at the primer annealing temperature and to dissociate during primer extension. Hence, it does not interfere with the polymerase reaction (Figure 2).

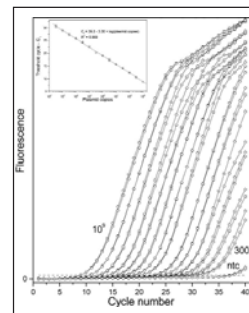
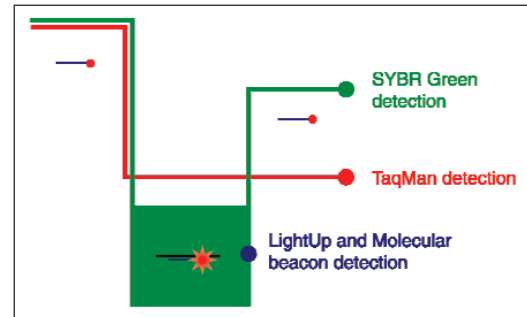


Figure 2 Top: PCR cycles with different probing systems. Neither the LightUp probe nor the Molecular Beacon binds to template during elongation. Bottom: Amplification curves using Light-up probe detection of *Gusa* gene. Serial dilution in steps of 3.2 (sqrt of 10).

Relative gene expression

In complex biological samples the PCR is usually significantly inhibited and reaction efficiencies vary among samples and also among assays. These variations can be accounted for by systematically diluting the test sample and, from the dependences of C_T on the dilution factor, calculate the efficiencies of the reactions (Figure 3). Having determined sample and assay specific efficiencies by this so called in situ calibration the expression ratio is calculated by the Q-PCR golden equation:

$$\frac{N_{0_geneA}}{N_{0_geneB}} = K_{RS} * \frac{(1 + E_{geneB})^{C_{T_geneB}}}{(1 + E_{geneA})^{C_{T_geneA}}}$$

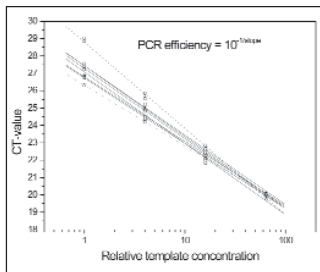


Figure 3: Determination of IgLλ expression in clinical samples by in situ calibration.

Test for non Hodgkin lymphoma

The immunoglobulin light chain exists in two versions called kappa (κ) and lambda (λ). In healthy individuals 60 % of B cells express κ and 40 % express λ. Lymphomas, like all malignant tumors, are clonal and arise from one transformed cell. Hence, in lymphoma tissue the IgLκ : IgLλ expression ratio should be altered. Figure 4 shows classification of lymphoma test samples based on measuring expression of the IgLκ and IgLλ genes.

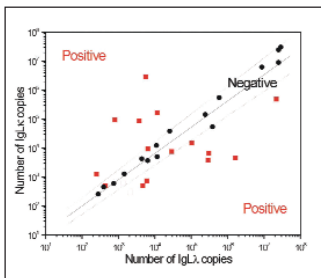


Figure 4: Plot of IgLκ and IgLλ real-time PCR CT values for 31 clinical samples. Negative samples are found within the 95 % confidence interval indicated by the dashed lines.

New dyes for real-time PCR

Current asymmetric cyanine dye reporters used in real-time PCR bind between bases in both single and double stranded DNA. Binding to primers and template interferes with the polymerisation reaction and also gives rise to some background fluorescence. To eliminate these limitations we have designed asymmetric cyanine dyes that bind in the minor groove of DNA, which makes them highly specific for double stranded nucleic acids (Figure 5).

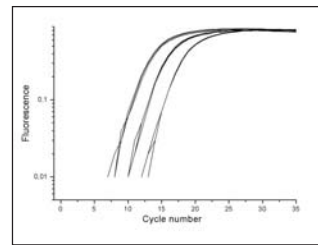


Figure 5: Real-time PCR using BEBO (detection logarithmic scale).

Single cell quantitative real time PCR

The very high sensitivity of real-time PCR makes it possible to analyse the mRNA content in individual cells. By combining real-time PCR with patch-clamp recording we study how expression of ion channels, hormones and enzymes in individual pancreatic Islet cells correlate to cell function at the single-cell level (Figure 6).

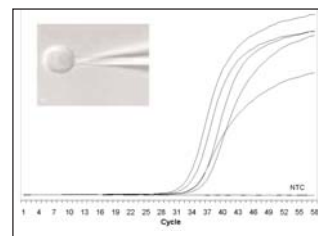


Figure 6: Glucagon cDNA amplified from a fraction of six single cells prepared from the Islets of Langerhans.

Real time immuno-PCR

We are extending the applications for real-time PCR to proteins and other biomolecules. This is done by trapping the biomolecule between two antibodies, of which one is surface immobilised and the other is tagged with a nucleic acid. The nucleic acid can then be amplified in a standard real-time PCR instrument such as the Bio-Rad iCycler™ system (Figure 7).

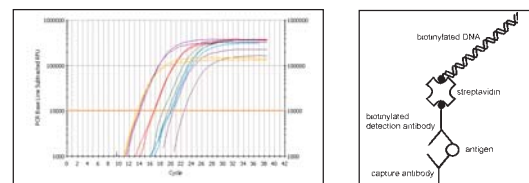


Figure 7: Real-time immuno PCR of PSA.

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