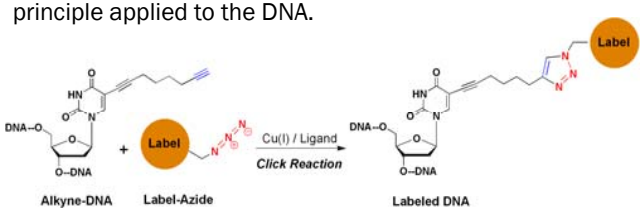
Introduction:

Labelled nucleic acids (NAs) such as oligonucleotides, DNA or RNA, are of daily use for different standard laboratory techniques like PCR assays, FISH experiment or microarrays. State-of-the-art labelling technologies suffer from poor incorporation rates of the label and of instabilities of intermediate products. The new proprietary labelling technology of baseclick GmbH, based on *Click-Chemistry*, overcomes this weakness and enables the NA labelling in an unprecedented, highly efficient and site specific way. The *Click-Reaction* minimises the amount of label to be used during the labelling reaction significantly, increasing the production efficiency while decreasing the lost of high valuable material. Below the *Click-Chemistry* principle applied to the DNA.

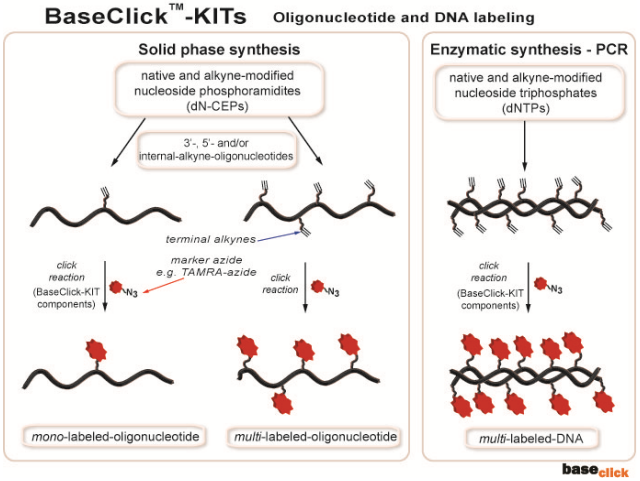


Before the *Click-Reaction* takes place it is important to quantify the NAs present in solution in order to define the exact amount of label-azide to be used. This step is quickly and efficiently afforded by analysing a small volume of 1 µL of the NA solution with the Implen NanoPhotometer™. A big advantage of the instrument is that the sample can be recollected after the measurement, avoiding any lost of valuable material. Once the *Click-Reaction* is completed a quantitatively and qualitatively analyse due to the absorption spectrum of the labelled NAs is again performed by the NanoPhotometer™. This process validates important experiments and ensures high quality products for the end-user. The small sample volume and the easy handling of the instrument are a real benefit for the daily laboratory work.

Methods:

Highly efficient labelling with BaseClick™-KITS:

For NA labelling the baseclick alkyne-phosphoramidites or alkyne-dNTPs were incorporate into the nucleic acids and afterwards labelled with label-azides (single- or multi-labelling). BaseClick™-KITs contain all the necessary reagents to label the alkyne-modified oligonucleotides and alkyne-modified PCR-products with label-azides of choice via Cu(I)-catalyzed azide-alkyne cycloaddition reaction (*Click-Reaction*, below).

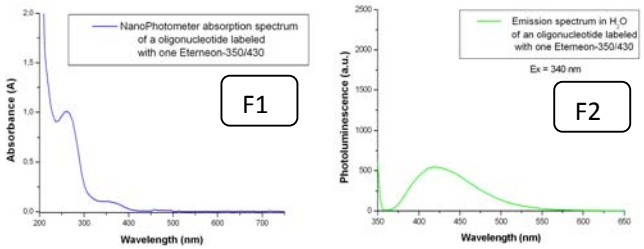


Oligonucleotides* bearing one or more alkynes moiety - single or multiple labelling - are usually modified with 2 to 5 equivalents (eq.) of the corresponding label-azide (e.g. TAMRA-dye azide). After the addition of precomplexed Cu(I)/Ligands a, complete conversion to the labelled oligo is observed within a time span between 30 min and 4 hours. After a simple precipitation step, labelled oligos can be recovered in nearly quantitative yields, monitored by the NanoPhotometer™ spectrum. A spin-column purification may be necessary for water soluble labels or when using a larger excess of label.

* Alkyne-modified oligos and alkyne-modified PCR-products can be prepared by solid phase synthesis using baseclick alkyne-phosphoramidites or enzymatic synthesis / PCR using baseclick alkyne-dNTPs. Additionally, alkyne-modified oligos can be directly purchased from the baseclick GmbH and other baseclick licensed companies as IDT, Metabion, Ella-Biotech and IBA.

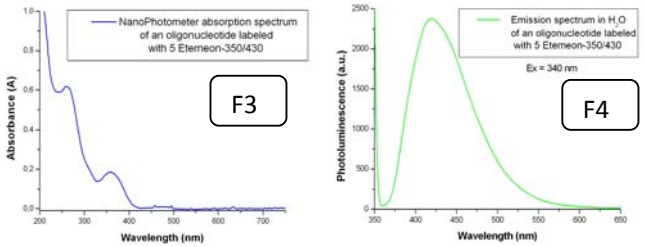
Results:

Single labelling: An oligonucleotide (22mer) bearing one internal alkyne was reacted with two equivalents of Eterneon™-(350/430)-azide for 4h at 37°C. After an ethanol precipitation and a spin-column purification, 89% of the labelled oligo were recovered (measured with the NanoPhotometer™, F1). The MALDI-mass analysis of the crude product shows the labelled conjugate as the sole product of this reaction.

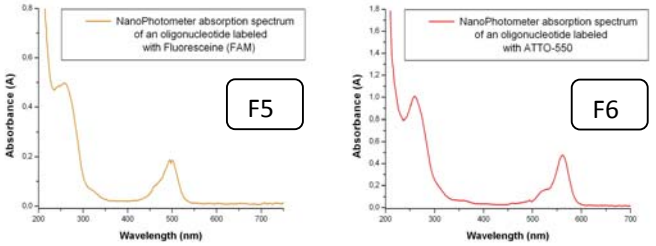


Multiple labeling: The *Click-Reaction* enables furthermore the multiple postsynthetic labelling of alkyne modified NAs. Complete high-density functionalization of several alkyne moieties within the NAs can be achieved without the formation of by-products.

An oligonucleotide (22mer) bearing five internal alkynes was reacted with five equivalents of Eterneon™-(350/430)-azide for 4h at 37°C. After an ethanol precipitation and a spin-column purification, 85% of the labelled oligo were recovered (measured with the NanoPhotometer™, F3). The MALDI-mass analysis of the crude product shows the 5 times labelled conjugate as the sole product of this reaction.



The presented NanoPhotometer™ spectra show clearly the typical NA absorption (260 nm) along with the dye absorption proportional to the amount of dye attached to the NAs (see F1 vs F3). Mass analysis (data not shown) and emission spectra (F2 vs F4) confirm the NP data. Examples of NanoPhotometer™-measurements for oligos labelled with different types of dyes like fluoresceine (FAM, F5) and ATTO-550 (F6) are reported as well..



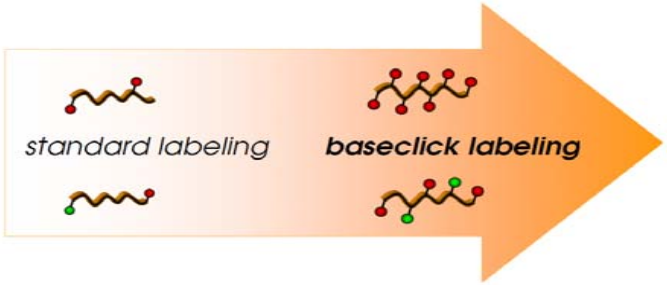
References:

P. M. E. Gramlich, C. T. Wirges, A. Manetto, T. Carell. Postsynthetic DNA Modification through the Copper-Catalyzed Azide-Alkyne Cycloaddition Reaction. *Angew. Chem. Int. Ed.* **2008**, *47*, 8350-8358.

P. M. E. Gramlich, S. Warncke, J. Gierlich, T. Carell. Click-Click-Click: Single to Triple Modification of DNA. *Angew. Chem. Int. Ed.* **2008** 3442-3444.

Summary:

Labelled nucleic acids are versatile used in different fields of research and diagnostics purposes and therefore essential for a wide spectrum of biochemical techniques. Here we report about a highly efficient single- and multi-labelling of nucleic acids via *Click-Chemistry*. Using this baseclick GmbH patented method, nucleic acids can be easily labelled with various molecules, amongst them dyes, quenchers, affinity tags, but also peptides, lipophilic groups, nanoparticles, enzymes, antibodies, solid phases and many more.



A quick determination of the nucleic acid concentration by spectrophotometric measurements plays an important rule before, during and after the labelling process. The Implen NanoPhotometer™ is the ideal tool for a fast, accurate and easy to handle analysis of labelled nucleic acid products. The small sample volume of less than 1 µl and the recovery of the analysed samples in order to avoid waste of expensive material are important advantages of the Implen NanoPhotometer™ and submit the best process outcome.

Reference-customers:

The NanoPhotometer™Pearl

SMALL. FAST. ACCURATE

0.3µl sample volume
2 ng/µl – 18,750 ng/µl detection range for dsDNA
3.5 sec. per measurement
Cuvette capability included
Accurate results/ no calibration over lifetime

Beside the application folder BIOTECHNOLOGY there are further NanoPhotometer application folders available:

- *Zoology*
- *Botany*
- *Microbiology*
- *Genetics*
- *Anthropology*
- *Ecology*
- *Cell Biology*
- *Virology*
- *Pathology*
- *Immunology*
- *Veterinary Medicine*

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Small Volume Spectrophotometric applications in

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DNA-labelling via Click Chemistry