

# Fluorescence Signal Amplification on DNA Microarrays via Rolling Circle Amplification (RCA)

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## Introduction

DNA microarrays are increasingly important tools for bioanalysis and clinical diagnostics [1]. However, their sensitivity is still insufficient for the analysis of small sample quantities. An elegant strategy for on-chip signal amplification for the analysis of small sample quantities is rolling circle amplification (RCA) [2,3].

To fully exploit the potential of this RCA technique, incorporation of dye-labeled nucleotides by the RCA polymerase  $\phi 29$  and the fluorescence of the amplified single-stranded RCA product have to be optimized. Thus, parameters of DNA that was enzymatically fluorescently labeled have been studied.

## Objective

Efficient fluorescent signal amplification  
for DNA microarrays

## Theory

We investigated the environment-dependent *fluorescence quantum yield*  $\phi_f$  and the *emission anisotropy*  $r$ .  $\phi_f$  is the measure for the likelihood of an absorbed photon to be reemitted, while  $r$  is the measure for the polarization dependence of the fluorophore emission.

## Experimental

Preliminary experiments with Cy3-labeled model systems showed ready integration of Cy3-dUTP and no change in  $\phi_f$  between ssDNA or dsDNA [4].

We tested further fluorophores for the same properties: Three Cy3 analogues - *Dy547* (single negative charge), *Dy548* (two negative charges), *Dy549* (three negative charges), a rhodamine analogue - *Dy555* (zwitterionic), and a boron-dipyrromethene dye - *Bodipy FL* (neutral).

## Results and Discussion

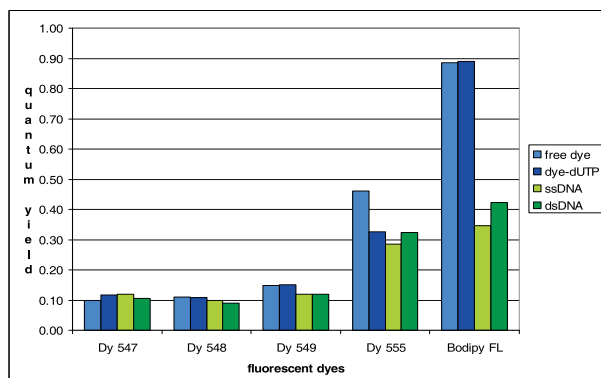


FIGURE 1. Quantum yield  $\phi_f$  in dependence of the immediate environment of the dye.  $\phi_f$  of the five fluorophores was determined for the carboxylic acids (free dye), linked to the uracil of a dUTP via a C6-linker (dye-dUTP), in single stranded DNA (ssDNA) and double stranded DNA (dsDNA).

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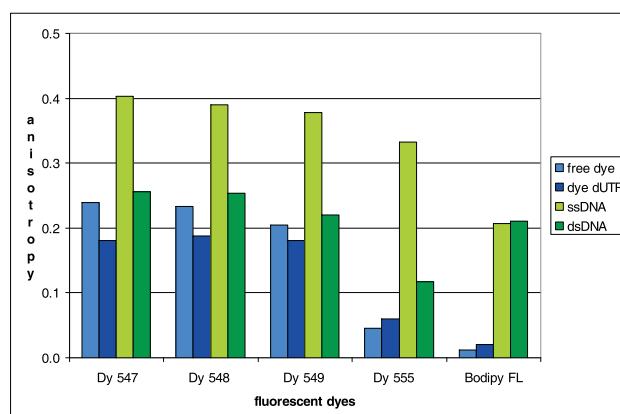


FIGURE 2. Anisotropy  $r$  in dependence of the immediate environment of the dye.  $r$  of the five fluorophores was determined for the carboxylic acids (free dye), linked to the uracil of a dUTP via a C6-linker (dye-dUTP), in single stranded DNA (ssDNA) and double stranded DNA (dsDNA).

**Incorporation:**  $\phi 29$  polymerase incorporated all dyes independent of their charge and is thus suitable for direct labeling.

**Quantum yield:** All Cy3 analogues showed little to none dependence on the immediate dye environment. Bodipy FL exhibited a clear reduction of  $\phi_f$  when linked to DNA which is likely due to quenching from neighboring guanine bases by photoinduced electron transfer.

**Anisotropy:** Except for Bodipy FL all dyes showed a higher anisotropy for ssDNA than dsDNA. The reason for the increased anisotropy for ssDNA compared to dsDNA is currently studied using time-resolved fluometry.

## Outlook

So far linear model systems were used to identify the fluorescence properties of labeled strands. RCA in solution and on a chip will be performed. It is probable that the surface of the DNA microarray will influence the polymerization and the fluorescent signal output.

The ultimate goal with this strategy is to determine the overall signal amplification for different dyes and chip surface chemistries.

## Literature

[1] Shi, Springer Ser Fluoresc (U. Resch-Genger), 2008, vol 6, 265-282. [2] Baner, Nucl. Acids Res. 1998, 34, 5073. [3] Nallur, Nucl. Acids Res. 2001, 29, 118. [4] Mayer-Enthardt, Ann. NY Acad. Sci. 2008, 1130, 287-92.

## Acknowledgements

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