## Supporting information for:

# DNA-Catalyzed Transfer of a Reporter Group

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## 1) Materials and general procedures

**Materials**: PNA monomers were purchased from Applied Biosystems. PyBOP, HCTU, resins and protected amino acids were purchased from Novabiochem. DNA was purchased from BioTeZ Berlin-Buch GmbH, Germany in HPLC quality. FAM-OH, TAMRA-OH and dry DMF ($H_2O < 0.01\%$) were purchased from Fluka. Water was purified with a Milli-Q Ultra Pure Water Purification System.

**Synthesis**: All column chromatography was performed with SDS 60 ACC silica gel using a Büchi Sepacore flash chromatographer. TLC was performed with E. Merck Silica Gel 60 F254 plates. $^{1}$H- and $^{13}$C-NMR spectra were recorded with Bruker DPX 300 spectrometer. The signals of the residual protonated solvent (DMSO-$d_6$) were used as reference signal. High resolution mass spectra (HRMS) were measured with a Hewlett-Packard GCMS 5995-A (ESI+) spectrometer.

**Solid-phase synthesis**: Manual solid phase synthesis was performed by using 5 mL polyethylene syringe reactors that are equipped with a fritted disc. Automated linear solid-phase Fmoc-synthesis was performed by using an Intavis ResPep parallel synthesizer equipped with micro scale columns for PNA synthesis.

**Preloading of resins**: The resins used in solid phase synthesis were loaded with the protected amino acids (ca. 0.12 mmol/g) according to standard protocols (see: *NovaBiochem Catalog* **2004**/**2005)**.

**Solid-phase synthesis according to Boc-strategy**: *Boc cleavage*: After treatment with TFA/*m*-cresol (95:5, 5 min, 1 mL) the resin was washed (10x 1 mL $CH_2Cl_2$, 5x 1 mL DMF). *Coupling*: 4 eq. building block (final concentration ca. 0.02 M), 4 eq. PyBOP and 8 eq. NMM were dissolved in DMF and added to the resin. After 1 h, the resin was washed (5x 1 mL DMF, 5x 1 mL $CH_2Cl_2$, 5x 1 mL DMF). *Capping*: pyridine/$Ac_2O$ (10:1, 1 mL) was added to the resin. After 5 min the resin was washed (5x 1 mL DMF, 10x 1 mL $CH_2Cl_2$).

**Automated solid-phase synthesis according to the Fmoc-strategy**: *Fmoc cleavage*: DMF/piperidine (4:1, 200 µL) was added to the resin. After 2 min, the procedure was repeated once. The resin was washed (7x 200 µL DMF). *Coupling*: A preactivation vessel was charged with a 0.6 M HCTU solution in NMP (12 µL), a 4 M NMM solution in DMF (4 µL), and a 0.2 M PNA monomer solution in NMP (40 µL). After 8 min, 50 µL of preactivation solution were transferred to the resin. After 30 min, the resin was washed (2x 200 µL DMF). *Capping*: $Ac_2O$/2,6-lutidine/DMF (5:6:89, 200 µL) was added. After 3 min the resin was washed (2x 200 µL DMF).

**Purification of probes**: For purification the combined TFA washing solutions were concentrated *in vacuo* before addition of diethylether. The precipitated crude product was dissolved in water and purified by semi preparative HPLC, performed on an Agilent 1100 series instrument (column: Varian Polaris C18 A 5µ 250x100, pore size 220 Å) using A (98.9% $H_2O$, 1% acetonitrile, 0.1% TFA) and B (98.9% acetonitrile, 1% $H_2O$, 0.1% TFA) in a linear gradient (3% A – 60% A in 30 min) with a flow rate of 6 mL/min. After the eluent was removed *in vacuo*, the product was dissolved in degassed $H_2O$.

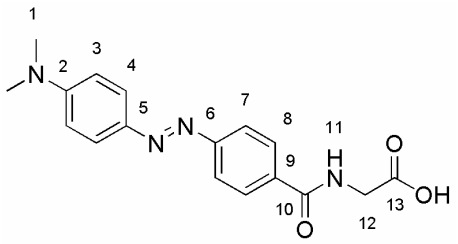
**Determination of yields**: An aliquot was diluted with Buffer (10 mM $NaH_2PO_4$, 10 mM NaCl, pH 7.0) to 1 mL and the optical density measured at 260 nm by using a quartz cuvette with a 1 cm path length. The sample concentration was calculated by using oligo calculation at www.gensetoligos.com. The absorption of FAM, TAMRA and Dabcyl at 260 nm was approximated as the one of a T-monomer.

**Characterization of probes**: Analytical HPLC was run on a Merck–Hitachi Elite LaChrom chromatograph (column: Varian Polaris C18 A 5µ 250x46, pore size 220 Å) using A (98.9% $H_2O$, 1% acetonitrile, 0.1% TFA) and B (98.9% acetonitrile, 1% $H_2O$, 0.1 % TFA) in a linear gradient (3% A – 60% A in 30 min at 55 °C) with a flow rate of 1 mL/min. Detection of the signals was achieved with a photodiode array detector at wavelength $\lambda$= 280 nm. MALDI-TOF mass spectra were recorded with a Voyager-DE Pro Biospectrometry Workstation of PerSeptive Biosystems (matrix: sinapinic acid).

## 2) Remarks to probe design

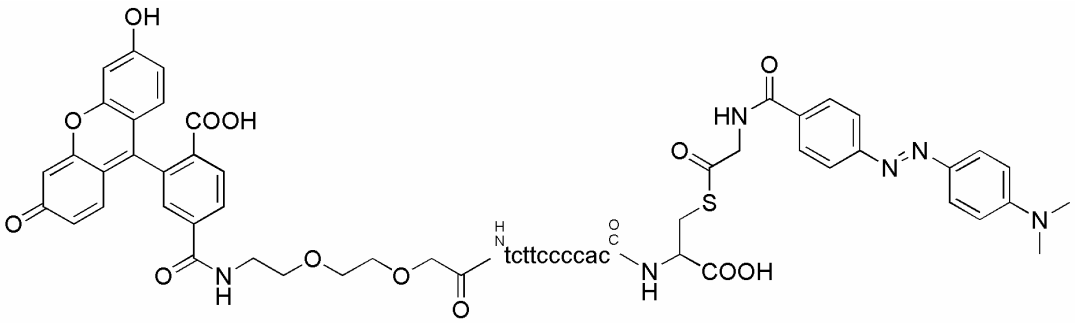
The transfer reaction was designed based on insight which was gained in DNA-directed ligation reactions. In these investigations template sequences **RasT/G** were used. It was shown that the match/mismatch selectivity of native chemical PNA ligations was increased by more than one order of magnitude when performed opposite to an unpaired templat base.[1] We speculate that perfect alignment of highly reactive groups is detrimental to sequence specificity. Rather a high flexibility may help in allowing dissociation of mismatched probe-template complexes before the reaction occurs. Previous ligation experiments revealed that the rate of the background reaction in absence of template can be reduced by using iso-cysteine as nucleophilic moiety instead of cysteine.[2] Thus, the transfer reaction was designed to feature one unpaired nucleobase between the aligned reactive probes and iso-cysteine as nucleophile in probe **2**.

### 3) Synthesis of 4-[4-dimethylamino)phenylazo]benzoylglycine (Dabcyl-Gly-OH)



To a solution of Dabcyl-*N*-hydroxysuccinimdester[3] (366 mg, 1.00 mmol) and *N*,*N*-diisopropyl-ethylamine (1.5 mL, 8.10 mmol) in DMF (25 mL) at 0 °C was added glycine (225 mg, 3.0 mmol). After stirring at room temperature for 24 h, the mixture was filtered and evaporated. The resulting red solid was washed with $CH_2Cl_2$ (5x 10 mL) and purified by column chromatography on silica gel eluting with a gradient of THF(+ 0.5 % formic acid)/cyclohexane (70 % → 20 % cyclohexane) to give the product in 43% yield (140 mg, 0.43 mmol). TLC: $R_f$ = 0.32 (THF/cyclohexane 3:2 + 0.25 % formic acid). HRMS: *m/z* = 326.1377 ($C_{17}H_{18}N_4O_3$ [$M^+$], calculated 326.1379). $^1$H-NMR (300 MHz, DMSO-$d_6$): δ [ppm] = 3.08 (s, 6 H; 1-$CH_3$), 3.95 (d, 2 H; 12-$CH_2$), 6.84 (d, 2 H; 3-CH, $^3J_{H,H}$ = 9.2 Hz), 7.83 (d, 2 H; 4-CH, $^3J_{H,H}$ = 9.2 Hz), 7.84 (d, 2 H; 7-CH, $^3J_{H,H}$ = 8.7 Hz), 8.01 (d, 2 H; 8-CH, $^3J_{H,H}$ = 8.7 Hz). $^{13}$C-NMR (75 MHz, DMSO-$d_6$): δ [ppm] = 39.8 (1-$CH_3$), 41.2 (12-$CH_2$), 111.5, 121.5, 125.1 and 128.4 (3-, 4-, 7-, 8-CH), 133.9, 142.6, 152.8 and 154.1 (2-, 5-, 6-, 9-$C_q$), 165.9 and 171.3 (10-, 13-$C_q$).

### 4) Synthesis of PNA probe 1 (FAM-AEEA-tcttccccac-Cys(S-Gly-Dabcyl)$^{COOH}$)

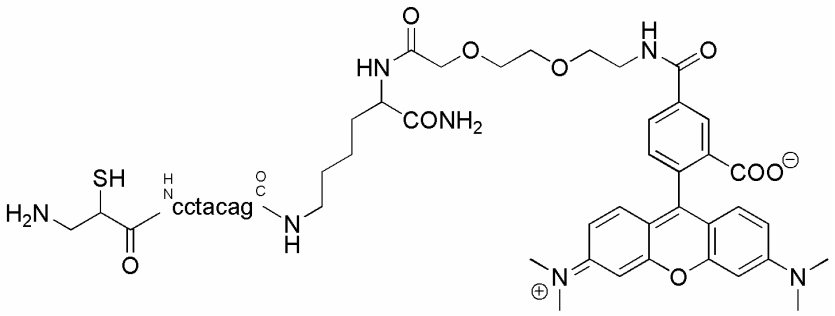



The PNA sequence Fmoc-tc$^{Bhoc}$ttc$^{Bhoc}$c$^{Bhoc}$c$^{Bhoc}$c$^{Bhoc}$a$^{Bhoc}$c$^{Bhoc}$ was assembled via automated linear solid-phase Fmoc-synthesis in 2 µmol scale on an Fmoc-Cys(Mmt)-TGA resin (20.4 mg, 0.098 mmol/g). The subsequent synthesis was continued with half of the resin (initial loading: 1 µmol). The resin was treated with DMF/piperidine (4:1, 2x 5 min, 0.5 mL) and washed (5x DMF, 5x $CH_2Cl_2$, 5x DMF). The addition of 10 eq. Fmoc-AEEA-OH[4] (final concentration ca. 0.05 M in DMF), 10 eq. PyBOP, and 25 eq. NMM (2x 1 h) was followed by washing (5x DMF, 5x $CH_2Cl_2$, 5x DMF). Subsequently, the resin was first treated with pyridine/$Ac_2O$ (10:1, 5 min, 1 mL), washed (5x DMF, 5x $CH_2Cl_2$, 5x DMF), and treated with DMF/piperidine (4:1, 2x 5 min, 0.5 mL). After washing the resin

(5x DMF, 5x $CH_2Cl_2$, 5x DMF) 10 eq. FAM-OH (final concentration ca. 0.05 M in DMF), 10 eq. PyBOP, and 20 eq. NMM (2x 1 h) were added and finally washed (5x DMF, 5x $CH_2Cl_2$, 5x DMF). The addition of pyridine/$Ac_2O$ (10:1, 5 min, 1 mL) was followed by treatment with DMF/piperidine (4:1, 5 min, 1 mL) and washing (5x DMF, 10x $CH_2Cl_2$). For Mmt-deprotection the resin was treated with $CH_2Cl_2$/TIS/TFA (93:5:2, 5x 10 min, 1 mL), washed (10x $CH_2Cl_2$, 5x DMF), and reacted with 5 eq. Dabcyl-Gly-OH (final concentration ca. 0.02 M in DMF), 4.5 eq. PyBOP and 12.5 eq. NMM (4x 1 h). After washing (5x DMF, 10x $CH_2Cl_2$) the product was cleaved from the resin by adding TFA/$H_2O$/*m*-cresol (18:1:1, 1.5 h, 0.6 mL) and further extracted with TFA (4x 0.2 mL). $OD_{260}$ = 8.16 (81.0 nmol, 8.1%). HPLC: $t_R$ = 19.2. MALDI-TOF-MS: *m/z* = 3515.8 ($[M+H]^+$, calculated 3515.4). $C_{151}H_{178}N_{56}O_{44}S$ (MW = 3513.44).

A crucial point in the synthesis of probe **1** is the formation of the thioester on the cysteine side chain of the assembled PNA-peptide conjugate. Due to sterical hindrance this reaction proceeds with ca. 60 % yield. As further workup and purification steps were carried out under acidic conditions thioester hydrolysis was not observed. It was noticed that the two hydrophobic labels facilitate HPLC purification of probe **1**.

## 5) Synthesis of PNA probe 2 (*i*Cys-cctacag-Lys($N^{\alpha}$H-AEEA-TAMRA)$^{CONH_2}$)




The Fmoc-group of the Fmoc-Lys(Boc)-MBHA resin (15.1 mg; 0.132 mmol/g) was removed by treatment with DMF/piperidin (4:1, 2x 5 min, 1 mL). After washing (5x DMF, 5x $CH_2Cl_2$, 5x DMF), the resin was reacted with 10 eq. Fmoc-AEEA-OH[4] (final concentration ca. 0.05 M in DMF), 10 eq. PyBOP, and 25 eq. NMM (2x 1 h) and washed (5x DMF, 5x $CH_2Cl_2$, 5x DMF). Thereafter, it was treated with pyridine/$Ac_2O$ (10:1, 5 min, 1 mL) followed by washing (5x DMF, 5x $CH_2Cl_2$, 5x DMF). After addition of DMF/piperidine (4:1, 2x 5 min, 1 mL) and washing (5x DMF, 5x $CH_2Cl_2$, 5x DMF), 4 eq. TAMRA-OH (final concentration ca. 0.02 M in DMF), 4 eq. PyBOP, and 10 eq. NMM (1 h) were added. Washing (5x DMF, 5x $CH_2Cl_2$, 5x DMF) was followed by treatment with pyridine/$Ac_2O$ (10:1, 5 min, 1 mL). After washing (5x DMF, 10x $CH_2Cl_2$) the PNA sequence and Boc-iCys(Trt)-OH[5] were subsequently built according to the Boc-strategy. After treatment with TFA/$H_2O$/*m*-cresol (18:1:1 +

5 mg cysteine methyl ester, 10 min) the resin was washed (10x $CH_2Cl_2$). For cleavage of the product the resin was treated with TFA/TFMSA/*m*-cresol (16:3:1, 1.5 h, 0.6 mL) and subsequently extracted with TFA (2x 0.2 mL). $OD_{260}$ = 30.4 (407 nmol, 20%). HPLC: $t_R$ = 16.4. MALDI-TOF-MS: *m/z* = 2669.3 ($[M+H]^+$, calculated 2668.7). $C_{114}H_{143}N_{47}O_{29}S$ (MW = 2667.72).

## 6) HPLC analysis of the transfer reaction

The buffer (10 mM $NaH_2PO_4$, 200 mM NaCl, 0.2 mM TCEP) was degassed and the pH adjusted to 7.0 by using a degassed 2 M NaOH solution. Subsequent manipulations were carried out while avoiding unnecessary exposure to oxygen. Aqueous 2 µM probe solutions were prepared using the buffer. Probe **2** solution (60 µL) was placed in an Eppendorf tube and the appropriate amount of DNA was added. At 25 °C probe **1** solution (60 µL) was added. The final concentration of probes **1** and **2** was 1 µM. The probes were allowed to react for 60 min while vortexing. For HPLC analysis the reaction was quenched by adding TFA (2 µL). Aliquots of 100 µL were analyzed by analytical HPLC using eluent A (98.9% $H_2O$, 1% acetonitrile, 0.1% TFA) and eluent B (98.9% acetonitrile, 1% $H_2O$, 0.1% TFA) in a linear gradient (10% A – 40% A in 30 min at 55 °C) with a flow rate of 1 mL/min. Detection of the signals was achieved with a photodiode array detector at wavelength λ= 280 nm.

The chromatogram shows the two reactant peaks (**1** and **2**) at begin of the reaction (Figure S1, bottom). The HPLC trace of the reaction in absence of DNA shows neglectible product formation (middle). In the presence of 1 eq. of DNA **RasT** a decrease of reactant peaks (**1** and **2**) and the appearance of two new product peaks (**3** and **4**) is observed (top).

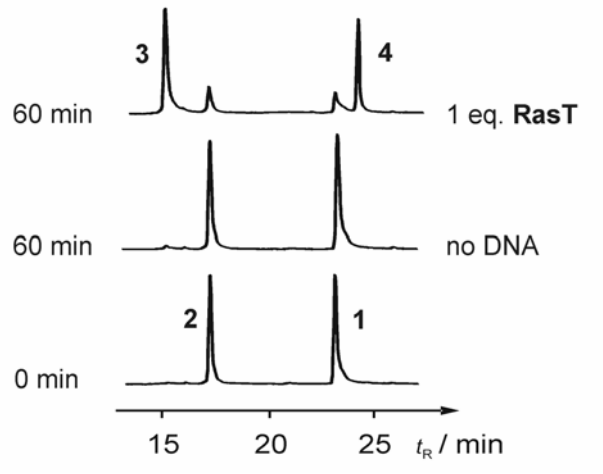



***Figure S1***. HPLC analysis of the reaction of probe **1** and **2** in the presence and absence of **RasT**. (1 µM probes, 10 mM $NaH_2PO_4$, 200 mM NaCl, 0.2 mM TCEP, pH 7.0, 25 °C).

## 7) Fluorescence-based read-out of the transfer reaction

Fluorescence spectra and time courses were recorded using a Varian Cary Eclipse spectrometer. Measurements were carried out in fluorescence quartz cuvettes (4x10 mm) in a buffered solution (10 mM $NaH_2PO_4$, 200 mM NaCl, 0.2-1.0 mM TCEP and 0.1 mg/mL roche blocking reagent). Roche blocking reagent was added to prevent adsorption of probe **2** on the glass surface. The buffer was degassed and the pH adjusted to 7.0 by using a degassed 2 M NaOH solution. Subsequent manipulations were carried out while avoiding unnecessary exposure to oxygen. The buffer (final volume 1 mL) was placed in a cuvette and the appropriate amount of probe **2** and if required DNA **RasT** or **RasG** were added. After setting the solution to the required temperature probe **1** was added and the cuvette placed in the spectrometer.

**Measurements in scan mode**: The spectrometer settings were selected as followed:

| | |
|---|---|
| FAM fluorescence: | λ-ex = 480 nm (slit 5 nm), λ-em = 495 – 570 nm (slit 10 nm) |
| TAMRA fluorescence: | λ-ex = 550 nm (slit 5 nm), λ-em = 565 – 640 nm (slit 10 nm) |

Figure S2 shows the fluorescence spectra of FAM and TAMRA at the beginning of the reaction of probe **1** and **2** (black line), after 12 min (1 eq. DNA, red) and after 60 min (0.1 eq. DNA, yellow). In the presence of match DNA **RasT** (lines) significant changes in the fluorescence spectra of FAM (increase) and TAMRA (decrease) are observed, whereas mismatch DNA **RasG** (dashed lines) provides only small changes in the fluorescence spectra.

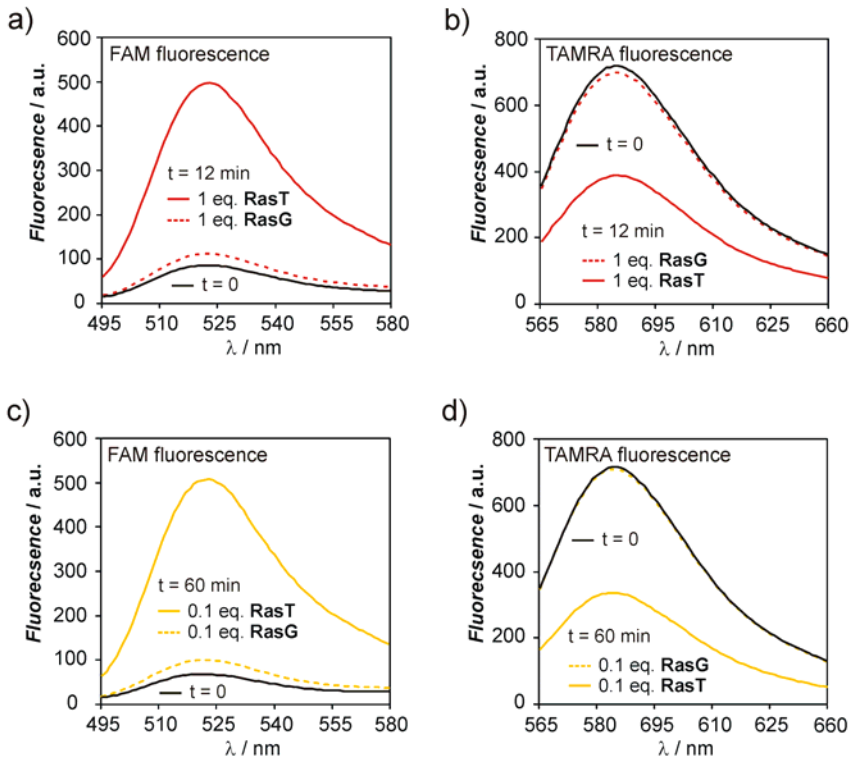



***Figure S2***. Fluorescence spectra of FAM (a, c) and TAMRA (b, d) in reactions between probe **1** and **2** in the presence of matched **RasT** and single mismatched **RasG**. Black line shows the spectrum at the beginning of the reaction. (a, b) Spectra after 12 min in the presence of 1 eq. DNA (red). (c, d) Spectra after 60 min in the presence of 0.1 eq. DNA (yellow). (200 nM probes, 10 mM $NaH_2PO_4$, 200 mM NaCl, 0.2 mM TCEP, 0.1 mg/mL roche blocking reagent, pH 7.0, 37 °C).

**Measurements in kinetic mode**: The spectrometer settings were selected as followed:

FAM fluorescence: λ-ex = 465 nm (slit 10 nm), λ-em = 525 nm (slit 10 nm)

TAMRA fluorescence: λ-ex = 558 nm (slit 10 nm), λ-em = 593 nm (slit 10 nm)

FAM and TAMRA fluorescence were monitored simultaneously. The time courses of relative $F_{FAM}/F_{TAMRA}$ ratio were determined as followed (setting signal intensity to 1 at t = 0):

relative $F_{FAM}/F_{TAMRA}$ ratio = $[F_{FAM}(t)/F_{TAMRA}(t)]/[F_{FAM}(t_0)F_{TAMRA}(t_0)]$

Based on the time courses of the fluorescence intensities the initial rates and yields of DNA-catalyzed transfer and background reactions were determined. Initial rates are determined based on the linear phase of the time courses. Yields of the background reactions, total transfer (background + DNA-catalyzed) and the DNA-catalyzed transfer reaction were calculated as followed:

yield(transfer background) = yield based on $F_{TAMRA}$(no DNA)

yield(hydrolysis background) = [yield based on $F_{FAM}$(no DNA)] – yield(transfer background)

yield(total transfer) = [yield based on $F_{FAM}$(DNA)] – yield(hydrolysis background)

yield(DNA-catalyzed transfer) = [yield based on $F_{FAM}$(DNA)] – [yield based on $F_{FAM}$(no DNA)]

***Table S1***. Initial rates of DNA-catalyzed transfer reactions and background reactions. Values are average of a duplicate of runs. (200 nM probes, 10 mM $NaH_2PO_4$, 200 mM NaCl, 0.2 mM TCEP, 0.1 mg/mL roche blocking reagent, pH 7.0, 37 °C).

| DNA concentration | 200 nM (1eq.) | | 20 nM (0.1 eq.) | | no DNA | |
|---|---|---|---|---|---|---|
| | **RasT** | **RasG** | **RasT** | **RasG** | transfer | (total) |
| initial rate / $nM \cdot s^{-1}$ | $4.16 \cdot 10^{-1}$ | $1.32 \cdot 10^{-2}$ | $7.38 \cdot 10^{-2}$ | $1.70 \cdot 10^{-3}$ | $2.42 \cdot 10^{-4}$ | $(1.29 \cdot 10^{-3})$ |

***Table S2***. Yields of transfer reactions after 12 and 24 h. Values are average of a triplicate of runs. Errors represent the standard deviation. (100 nM probes, 10 mM $NaH_2PO_4$, 200 mM NaCl, 1 mM TCEP, 0.1 mg/mL roche blocking reagent, pH 7.0, 32 °C).

| **RasT** concentration | 1 nM | 0.1 nM | 0.01 nM | no DNA | |
|---|---|---|---|---|---|
| | (0.01 eq.) | (0.001 eq.) | (0.0001 eq.) | transfer | (hydrolysis) |
| yield after 12 h / % | 56.9±0.7 | 18.1±0.9 | 5.2±0.3 | 2.5±0.2 | (13.1±0.2) |
| yield after 24 h / % | 68.8±1.2 | 24.9±0.6 | 7.4±0.4 | 3.4±0.2 | (24.4±0.3) |

## 8) Abbreviations

| | |
|---|---|
| AEEA | [2-(2-aminoethoxy)ethoxy]acetic acid |
| DMF | *N*,*N*-dimethylformamide |
| FAM | 6-carboxyfluorescein |
| Fmoc | 9-fluorenylmethoxycarbonyl |
| HCTU | 2-(6-Chloro-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate |
| λ-em | emission wavelength |
| λ-ex | excitation wavelength |
| Mmt | 4-monomethoxytrityl |
| NMM | *N*-methylmorpholine |
| NMP | *N*-methyl-2-pyrrolidon |
| PyBOP | benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate |
| TAMRA | 5-carboxytetramethylrhodamine |
| TCEP | tris(2-carboxyethyl)phosphine |
| TFA | trifluoroacetic acid |
| THF | tetrahydrofurane |
| TIS | triisopropylsilane |

## 9) Literature


[1] Ficht, S.; Dose, C.; Seitz, O. *ChemBioChem* **2005**, *6*, 2098-2103.

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