Mercury CCD diffractometer, $Mo_{K\alpha}$ radiation, $T = 23.0\,^{\circ}C$, monoclinic, space group $P2_1/n$ (No. 14), $a = 12.783(1)$, $b = 21.697(2)$, $c = 15.441(2)$ Å, $\beta = 105.957(4)^{\circ}$, $V = 4117.7(7)$ Å^3, $Z = 4$, $\rho_{calcd} = 1.082\,g\,cm^{-3}$, 28321 reflections collected, 4934 unique intensities reflections observed $[I > 4.00\sigma(I)]$, $2\theta_{max} = 55.0^{\circ}$, structure solution with direct methods (SIR92) and refinement on F with 483 parameters, R (R_w) = 0.153 (0.396), S (GOF) = 2.23. CCDC-183912 (**6**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or deposit@ccdc.cam.ac.uk).

[15] For pure π dimerization, see: a) M. R. Gleiter, B. Kanellakopulos, C. Krieger, F. A. Neugebauer, *Liebigs Ann.* **1997**, 473–483, and references therein; b) P. A. Capiomont, B. Chion, J. Lajzérowicz, *Acta Crystallogr. Sect. B* **1971**, *27*, 322–326.

Expanding the Reaction Scope of DNA-Templated Synthesis**

Zev J. Gartner, Matthew W. Kanan, and David R. Liu*

The translation of amplifiable information into chemical structure is a key component of nature's approach to generating functional molecules. The ribosome accomplishes this feat by catalyzing the translation of RNA sequences into proteins. Developing general methods to translate amplifiable information carriers into synthetic molecules may enable chemists to evolve non-natural molecules in a manner analogous to the cycles of translation, selection, amplification, and diversification currently used by nature to evolve proteins. As an initial step towards this goal, we recently examined the generality of DNA-templated synthetic chemistry.[1, 2] We demonstrated the ability of DNA-templated synthesis to direct a modest collection of chemical reactions without requiring the precise alignment of reactive groups into DNA-like conformations. Indeed, the distance independence and sequence fidelity of DNA-templated synthesis allowed the simultaneous, one-pot translation of a model library of more than 1000 templates into the corresponding thioether products, one of which was enriched by in vitro selection for binding to the protein streptavidin and amplified by the polymerase chain reaction (PCR).

The range of reactions known to be supported by DNA-templated synthesis,[2] however, remains a tiny fraction of those used either by synthetic chemists or by nature to generate molecules with desired properties. Many reactions central to the construction of natural or synthetic molecules have yet to be developed in a DNA-templated format despite their known compatibility with water.[3] We describe here the development of several useful DNA-templated reactions, including the first reported DNA-templated organometallic couplings and carbon–carbon bond forming reactions other than pyrimidine photodimerization.[4, 5] Collectively, these reactions represent an important additional step towards the in vitro evolution of non-natural synthetic molecules by enabling the DNA-templated construction of a much more diverse set of structures than has been previously achieved.

We first investigated the ability of DNA-templated synthesis to direct reactions that require a non-DNA-linked activator, catalyst, or other reagent in addition to the principal reactants. To test the ability of DNA-templated synthesis to mediate such reactions without requiring structural mimicry of the DNA backbone, we performed DNA-templated reductive aminations between amine-linked template **1** and benzaldehyde- or glyoxal-linked reagents (**2** and **3**) with millimolar concentrations of $NaBH_3CN$ at room temperature in aqueous solutions. Products formed efficiently when the template and reagent sequences were complementary. In contrast, control reactions in which the sequence of the reagent did not complement that of the template, or in which $NaBH_3CN$ was omitted, yielded no significant product (Table 1 and Figure 1). While DNA-templated reductive aminations to generate products closely mimicking the

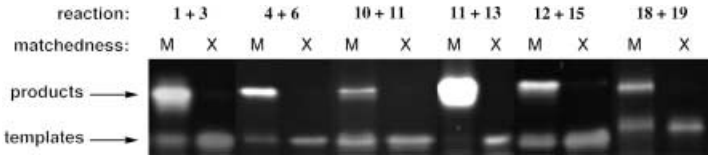

Figure 1. Analysis by denaturing polyacrylamide gel electrophoresis of representative DNA-templated reactions listed in Tables 1 and 2. The structures of reagents and templates correspond to the numbering in Tables 1 and 2. Lanes 1, 3, 5, 7, 9, and 11: reaction of matched (complementary) reagents and templates under the conditions listed in Tables 1 and 2 (the reaction of **4** and **6** was mediated by DMT-MM). Lanes 2, 4, 6, 8, 10, and 12: reaction of mismatched (noncomplementary) reagents and templates under conditions identical to those used in lanes 1, 3, 5, 7, 9, and 11, respectively.

structure of double-stranded DNA have been previously reported,[6, 7] the above results demonstrate that reductive amination to generate structures unrelated to the phosphoribose backbone can take place efficiently and sequence specifically. We also performed DNA-templated amide bond formations[8, 9] between amine-linked templates **4** and **5** and carboxylate-linked reagents **6**–**9** mediated by 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and *N*-hydroxysulfosuccinimide (sulfo-NHS) to generate amide products in good yields at pH 6.0 and 25 °C (Table 2). Product formation was sequence specific, dependent on the presence of EDC, and surprisingly insensitive to the steric encumbrance of the amine or carboxylate group. Efficient DNA-templated amide formation was also mediated by the water-stable activator 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium

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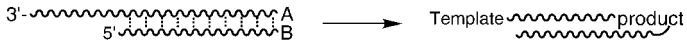
Table 1. Product yields of the various reactions.[a]



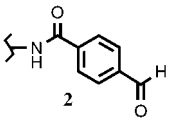
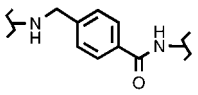
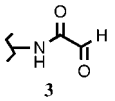
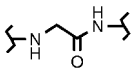
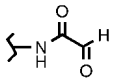
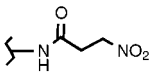
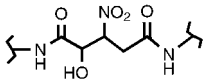
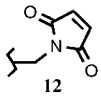
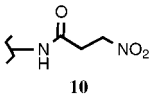
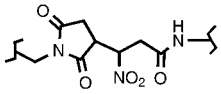
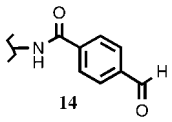
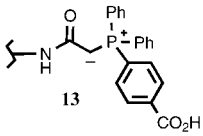
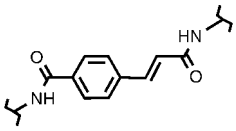
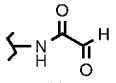
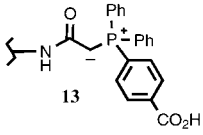
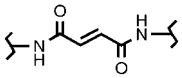
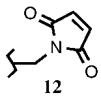
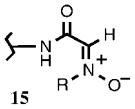
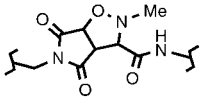
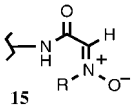
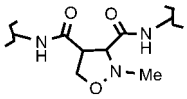
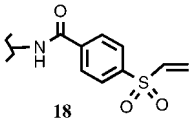
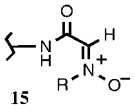
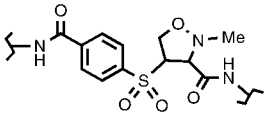
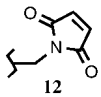
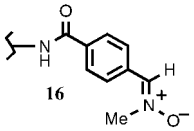
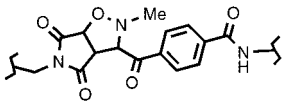
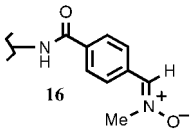
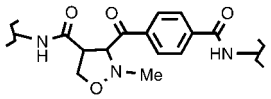
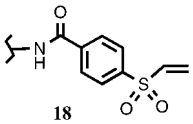
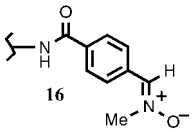
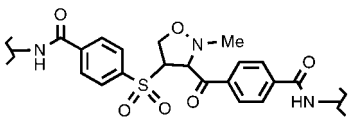
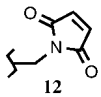
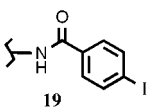
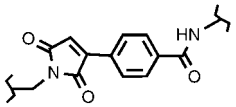
A	B	Conditions	Product	Yield [%]
 **1**	 **2**	a		81
 **1**	 **3**	a		70
 **11**	 **10**	b		45
 **12**	 **10**	b		42
 **14**	 **13**	c		93
 **11**	 **13**	c		>97
 **12**	 **15**	d		53 (R = Me) 42 (R = Bn)
 **17**	 **15**	d		54
 **18**	 **15**	d		47
 **12**	 **16**	d		41
 **17**	 **16**	d		15
 **18**	 **16**	d		44
 **12**	 **19**	e		54
17	**19**	f		26

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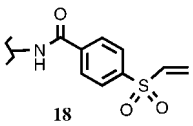
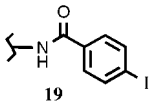
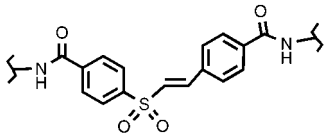
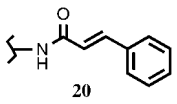
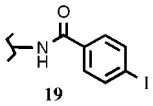
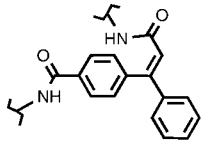
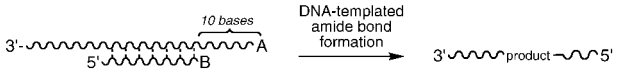
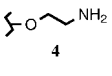
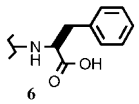
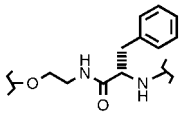
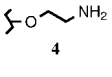
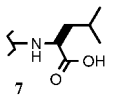
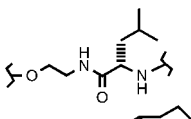
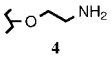
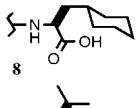
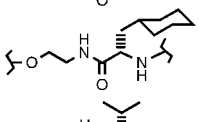
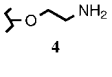
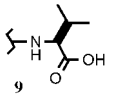
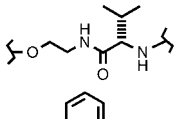
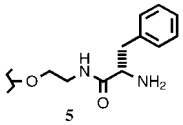
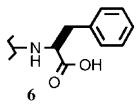
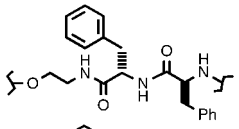
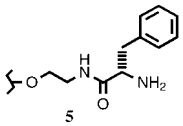
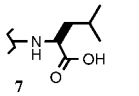
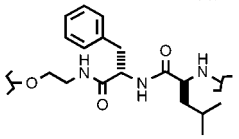
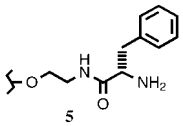
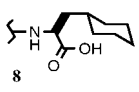
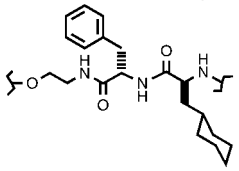
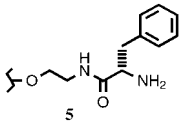
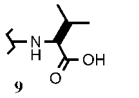
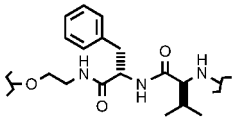
Table 1. (Continued)

A	B	Conditions	Product	Yield [%]
 **18**	 **19**	f		51
 **20**	 **19**	f		31

[a] Product yields of all reactions with matched template and reagent sequences under the specified conditions were greater than 20-fold higher than that of control reactions with scrambled reagent sequences. Reactions were conducted at 25 °C with one equivalent each of template and reagent at 60 nM final concentration unless otherwise specified. Conditions: a) 3 mM $NaBH_3CN$, 0.1 M 2-[*N*-morpholino]ethanesulfonic acid (MES) buffer pH 6.0, 0.5 M NaCl, 1.5 h; b) 0.1 M [2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino-1-propanesulfonic acid (TAPS) buffer pH 8.5, 300 mM NaCl, 12 h; c) 0.1 M pH 8.0 TAPS buffer, 1 M NaCl, 55 °C, 1.5 h; d) 50 mM 3-[*N*-morpholino]propanesulfonic acid (MOPS) buffer pH 7.5, 2.8 M NaCl, 22 h; e) 120 nM **19**, 1.4 mM Na_2PdCl_4, 0.5 M NaOAc buffer pH 5.0, 18 h; f) premix Na_2PdCl_4 with two equivalents of $P(p\text{-}SO_3C_6H_4)_3$ in water 15 min, then add to reactants in 0.5 M NaOAc buffer pH 5.0, 75 mM NaCl, 2 h (final [Pd] = 0.3 mM, [**19**] = 120 nM). The olefin geometry of products from **13** and the regiochemistries of cycloaddition products from **15** and **16** are presumed, but not verified.

Table 2. DNA-templated amide bond formation mediated by EDC and sulfo-NHS or by DMT-MM for a variety of substituted carboxylic acids and amines.[a]



A	B	Product	Yield [%]
 **4**	 **6**		79, 59
 **4**	 **7**		73, 54
 **4**	 **8**		81, 62
 **4**	 **9**		79, 46
 **5**	 **6**		58, 66
 **5**	 **7**		47, 64
 **5**	 **8**		56, 71
 **5**	 **9**		58, 53

[a] In each row, the yields of DMT-MM-mediated reactions between reagents and templates complementary in sequence are followed by yields of EDC and sulfo-NHS-mediated reactions. Conditions: 60 nM template, 120 nM reagent, 50 mM DMT-MM in 0.1 M MOPS buffer pH 7.0, 1 M NaCl, 16 h, 25 °C; or 60 nM template, 120 nM reagent, 20 mM EDC, 15 mM sulfo-NHS, 0.1 M MES buffer pH 6.0, 1 M NaCl, 16 h, 25 °C. In all cases, control reactions with mismatched reagent sequences yielded little or no detectable product.

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chloride (DMT-MM)[10] instead of EDC and sulfo-NHS (Table 2 and Figure 1). The efficiency and generality of DNA-templated amide bond formation under these conditions, together with the large number of commercially available chiral amines and carboxylic acids, make this reaction an attractive candidate in future DNA-templated syntheses of structurally diverse small-molecule libraries.

As a result of the importance of carbon–carbon bond forming reactions in both chemical and biological synthesis, we explored several such reactions in a DNA-templated format. Both the reaction of nitroalkane-linked reagent **10** with aldehyde-linked template **11** (nitro-aldol or Henry reaction) and the conjugate addition of **10** to maleimide-linked template **12** (nitro-Michael addition) proceed efficiently and with high sequence specificity at pH 7.5–8.5 and 25 °C (Table 1). In addition, the sequence-specific DNA-templated Wittig reaction between stabilized phosphorus ylide reagent **13** and aldehyde-linked templates **14** or **11** provided the corresponding olefin products in excellent yields at pH 6.0–8.0 and 25 °C (Figure 2). Finally, the DNA-templated 1,3-dipolar cycloaddition between nitrone-linked reagents **15** and **16** and olefin-linked templates **12**, **17**, or **18** also afforded products sequence specifically at pH 7.5 and 25 °C (Table 1). While these cycloadditions in general proceeded efficiently with maleimides, vinyl sulfones, and acrylamides, unactivated alkenes provided products in much lower yield (< 10 %).

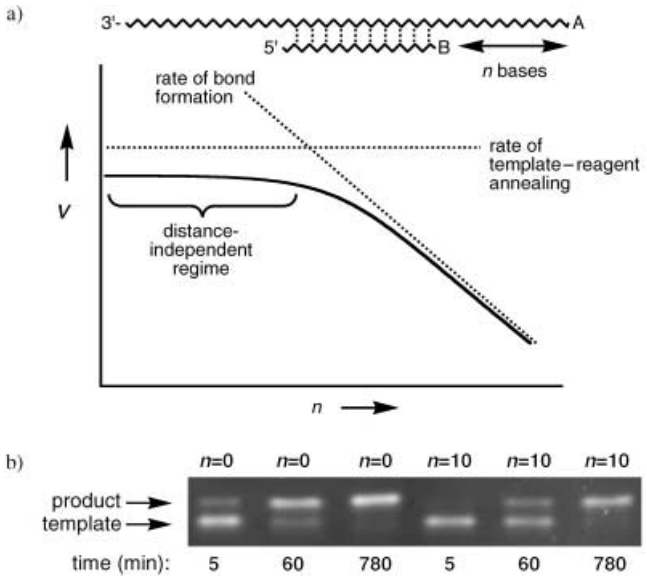

Figure 2. a) Conceptual model for distance-independent DNA-templated synthesis. As the distance between the reactive groups of an annealed reagent and template (n) is increased, the rate of bond formation (V) is presumed to decrease. For those values of n in which the rate of bond formation is significantly higher than the rate of template–reagent annealing, the rate of product formation (solid line) remains constant. In this regime, the DNA-templated reaction shows distance independence. b) Denaturing polyacrylamide gel electrophoresis of a DNA-templated Wittig olefination between complementary **11** and **13** with either zero bases (lanes 1–3) or ten bases (lanes 4–6) separating annealed reactants. Although the apparent second order rate constants for the $n = 0$ and $n = 10$ reactions differ by threefold ($k_{app}(n = 0) = 9.9 \times 10^3\,\text{M}^{-1}\text{s}^{-1}$ while $k_{app}(n = 10) = 3.5 \times 10^3\,\text{M}^{-1}\text{s}^{-1}$), product yields after 13 h at both distances are nearly quantitative. Control reactions containing sequence mismatches yielded no detectable product (not shown).

Organometallic couplings constitute a powerful class of complexity-building synthetic reactions. We performed DNA-templated Heck couplings in the presence of water-soluble Pd precatalysts to test the ability of DNA templates to direct organometallic reactions in aqueous solution. In the presence of 170 μM Na_2PdCl_4, aryl iodide reagent **19** and a variety of olefin-linked templates including maleimide **12**, acrylamide **17**, vinyl sulfone **18**, or cinnamamide **20** provided Heck coupling products in modest yields at pH 5.0 and 25 °C (Table 1). Adding two equivalents of $P(p\text{-}SO_3C_6H_4)_3$ per equivalent of Pd prior to template and reagent addition typically increased overall yields twofold for the coupling with olefins **17**, **18**, and **20**. Control reactions containing sequence mismatches or lacking the Pd precatalyst yielded no product. To the best of our knowledge the above DNA-templated nitro-aldol addition, nitro-Michael addition, Wittig olefination, dipolar cycloaddition, and Heck coupling represent the first reported nucleic acid-templated organometallic reactions and carbon–carbon bond forming reactions other than pyrimidine photodimerization.[4, 5]

We previously discovered[1] that some DNA-templated reactions demonstrate distance independence—the ability to form product at a rate independent of the number of intervening bases between the annealed reactants. We hypothesized (Figure 2a) that distance independence arises when the rate of bond formation in the DNA-templated reaction is greater than the rate of template–reagent annealing. Although only a subset of chemical reactions fall into this category, any DNA-templated reaction that affords comparable product yields when the reagent is annealed at various distances from the reactive end of the template is of special interest because it can be encoded at a variety of template positions. To evaluate the ability of the DNA-templated reactions developed above to take place efficiently when reactants are separated by distances relevant to library encoding, we compared the yields of reductive amination, amide formation, nitro-aldol addition, nitro-Michael addition, Wittig olefination (Figure 2b), dipolar cycloaddition, and Heck coupling when zero or ten bases separated annealed reactive groups (Figure 2a, $n = 0$ versus $n = 10$). Among the reactions described above or in our previous work,[1] amide bond formation, nitro-aldol addition, Wittig olefination, Heck coupling, conjugate addition of thiols to maleimides, and S_N2 reactions between thiols and α-iodo amides demonstrate comparable product formation when reactive groups are separated by zero or ten bases. Our findings indicate that these reactions can be encoded during synthesis by nucleotides that are distal from the reactive end of the template without significantly impairing product formation.

Taken together, these results expand considerably the reaction scope of DNA-templated synthesis. A wide variety of reactions proceed efficiently and selectively only when the corresponding reactants are programmed with complementary sequences. By augmenting the repertoire of known DNA-templated reactions to now include carbon–carbon bond forming and organometallic reactions (nitro-aldol additions, nitro-Michael additions, Wittig olefinations, dipolar cycloadditions, and Heck couplings) in addition to previously reported amide bond formation,[8, 9] imine formation,[11]

  1433-7851/02/4110-1799 $ 20.00+.50/0

reductive amination,[6, 7] S_N2 reactions,[1, 12, 13] conjugate additions of thiols,[1] and phosphoester or phosphonamide formation,[14, 15] these results may enable the sequence-specific translation of libraries of DNA into libraries of structurally and functionally diverse synthetic products. Since minute quantities of templates encoding desired molecules can be amplified by PCR,[1] the yields of DNA-templated reactions are arguably less critical than the yields of traditional synthetic transformations. Nevertheless, many of the reactions developed above proceed efficiently. In addition, by demonstrating that DNA-templated synthesis in the absence of proteins can direct a large diversity of chemical reactions, our findings support previously proposed hypotheses[16–18] that nucleic-acid-templated synthesis may have translated replicable information into some of the earliest functional molecules such as polyketides, terpenes, and polypeptides prior to the evolution of protein-based enzymes. The diversity of chemistry shown here to be controllable simply by bringing reactants into proximity using DNA hybridization without any apparent structural requirements provides an experimental basis for these possibilities. The translation of amplifiable information into a wide range of structures is a key requirement of our ongoing efforts to apply nature's molecular evolution approach to the discovery of non-natural molecules with new functions.

Experimental Section

Functionalized templates and reagents were typically prepared by treating 5′-NH_2-terminated oligonucleotides (for template **1**), 5′-NH_2-$(CH_2O)_2$-terminated oligonucleotides (for all other templates) or 3′-OPO_3-$CH_2CH(CH_2OH)(CH_2)_4NH_2$-terminated oligonucleotides (for all reagents) with the appropriate NHS esters (0.1 volumes of a 20 mg mL^{-1} solution in DMF) in 0.2 M sodium phosphate buffer at pH 7.2 and 25 °C for 1 h to provide the template and reagent structures shown in Tables 1 and 2. For amino-acid-linked reagents **6**–**9**, 3′-OPO_3-$CH_2CH(CH_2OH)$-$(CH_2)_4NH_2$-terminated oligonucleotides in 0.2 M sodium phosphate buffer at pH 7.2 were treated with 0.1 volumes of a 100 mM bis[2-(succinimidyloxycarbonyloxy)-ethyl]sulfone (BSOCOES, Pierce) solution in DMF for 10 min at 25 °C, followed by 0.3 mL of a 300 mM solution of amino acid in 300 mM NaOH for 30 min at 25 °C.

Functionalized templates and reagents were purified by gel filtration using Sephadex G-25 followed by reverse-phase HPLC (0.1 M triethylammonium acetate/acetonitrile gradient) and characterized by MALDI mass spectrometry. DNA-templated reactions were conducted under the conditions described in Tables 1 and 2, and products were characterized by denaturing polyacrylamide gel electrophoresis and MALDI mass spectrometry.

The sequences of oligonucleotide templates and reagents are as follows (5′ to 3′ direction, *n* refers the number of bases between reactive groups when template and reagent are annealed as shown in Figure 2): **1**: TGGTACGAATTCGACTCGGG; **2** and **3** matched: GAGTCGAATTCGTACC; **2** and **3** mismatched: GGGCTCAGCTTCCCCA; **4** and **5**: GGTACGAATTCGACTCGGGAATACCACCTT; **6**–**9** matched ($n = 10$): TCCCGAGTCG; **6** matched ($n = 0$): AATTCGTACC; **6**–**9** mismatched: TCACCTAGCA; **11**, **12**, **14**, **17**, **18**, **20**: GGTACGAATTCGACTCGGGA; **10**, **13**, **16**, **19** matched: TCCCGAGTCGAATTCGTACC; **10**, **13**, **16**, **19** mismatched: GGGCTCAGCTTCCCCATAAT; **15** matched: AATTCGTACC; **15** mismatched: TCGTATTCCA; template for $n = 10$ versus $n = 0$ comparison: TAGCGATTACGGTACGAATTCGACTCGGGA

Reaction yields were quantitated by denaturing polyacrylamide gel electrophoresis followed by staining with ethidium bromide, UV visualization, and CCD-based densitometry of the product and template starting material bands. Calculations of the yields assumed that the templates and products stained with equal intensity per base; for those cases in which products are partially double stranded during quantitation, changes in staining intensity may result in higher apparent yields.

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