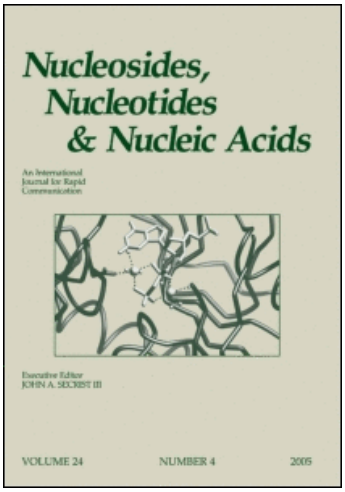

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

The Accurate Detection of One Point Mutations by Ligation of Short Oligonucleotides

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To cite this Article Zarytova, V. F. , Pyshnyi, D. V. , Krivenko, A. A. , Dymshitz, G. M. and Ivanova, E. M.(1998) 'The Accurate Detection of One Point Mutations by Ligation of Short Oligonucleotides', Nucleosides, Nucleotides and Nucleic Acids, 17: 9, 2143 — 2147
To link to this Article: DOI: 10.1080/07328319808004757
URL: http://dx.doi.org/10.1080/07328319808004757

NUCLEOSIDES & NUCLEOTIDES, 17(9-11), 2143-2147 (1998)

THE ACCURATE DETECTION OF ONE POINT MUTATIONS BY LIGATION OF SHORT OLIGONUCLEOTIDES

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ABSTRACT: A new approach which is based on the selective ligation of the cooperative tandem of three short oligonucleotides (octa-, tetra- and octamers) is proposed to reveal a point mutation in DNA. The ligation product is registered only when the correct complex of the tandem and DNA is formed. If there is one substitution base in the binding site of tetranucleotide in DNA, no product of ligation is observed.

INTRODUCTION

The discrimination of a single base substitution in DNA sequences is a serious problem of molecular biology and DNA diagnostic. Molecular hybridization of DNA (normal or mutated) with oligonucleotides is one of the most direct and reliable way to solve this problem. Long oligonucleotides, which are able to provide the adequate recognition of the target regions, can form with DNA mismatched duplexes with a T_m closed to T_m of the correct duplexes. Oligonucleotide ligation assay allows point mutation to be discriminated due to a decrease of efficacy of ligation of two oligonucleotides when their complex with DNA has mismatch by the ligation site [1]. However sensitivity of this method is insufficient for the unequivocal answer of mutation because the substrate specificity of ligase can provide any formation of ligation product even when incorrect complex is formed [2-4]. Therefore the way to enhance the selectivity of ligation is to use the oligonucleotides which cannot form mismatched complexes with DNA template under condition of ligation.

We suggested that for this purpose tandem *octamer-tetramer-octamer* suits in the best way since tetramer has very low hybridization properties and can form complementary com-

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plex only when it is flanked by additional oligonucleotides or their derivatives (effectors) [5, 6]. In this work we demonstrated that the ligation of tandem *octamer-tetramer-octamer* allows any mismatched complex *DNA template·tetramer* to be discriminated with high accuracy.

RESULTS AND DISCUSSION

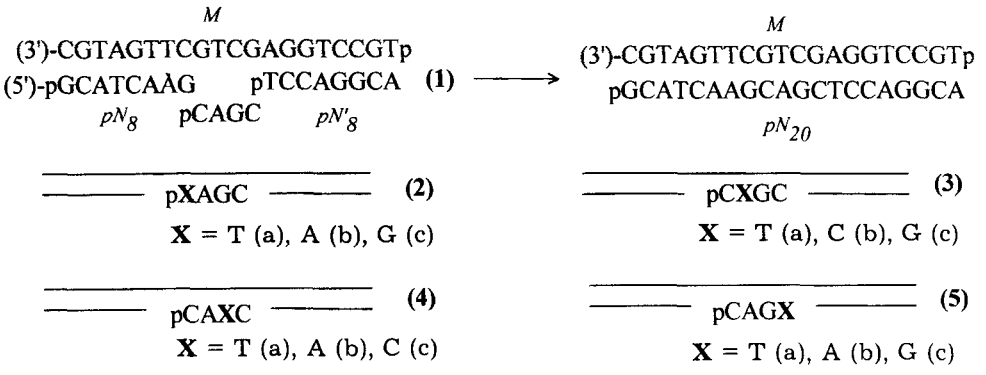
The main part of our investigation was carried out with the use of 20-mer DNA (*M*) as template, two octanucleotides (pN_8 and pN'_8) and tetranucleotides pXAGC, pCXGC, pCAXC, and CAGX depicted in Scheme 1.

The first we studied the ligation of the "correct" tandem pN_8 + pCAGC + pN'_8 and found the optimum condition for the reaction. The ligation of pN_8 + pCAGC + pN'_8 in the presence of 50 units of T4 DNA ligase results in formation of the full length product pN_{20} with the yield of 40-45% when all oligonucleotide components are used in equimolar concentration (FIG. 1). If any of the oligonucleotides is absence, no products of ligation are observed (FIG. 1). A decrease of the concentration of any component of tandem pN_8 + pN_4 + pN'_8, which was labeled by ^{32}P (*p), leads practically to its quantitative inclusion into the product $*pN_{20}$.

Since for the application of oligonucleotide probe in diagnostic medicine it is necessary to use nonradioactive report group, we have studied the ligation of tandems (RL')pN_8 + pN_4 + pN'_8p(LR) where R is fluorescence group as depicted in Scheme 2. In all cases the single registered product was found to be the full length 20-mer (RL')pNp_{20}(LR) (FIG. 1). Thus, the quantitative and high specific ligation of tetranucleotide and flanking pair of octanucleotides or their derivatives bearing nonradioactive report groups was demonstrated.

Then we investigated the ligation of "incorrect" tandems in complexes 2-5. The efficacy of discrimination of a mismatch in the complex of DNA template and oligonucleotide probes is estimated as the ration of a yield of the reaction in the correct complex to a yield of the reaction in the mismatched complex (factor of discrimination, FD) [7]. The higher discrimination factor is the more precisely the base substitution in the sequence of DNA template or oligonucleotide probe is revealed.

The reactions were carried out at 37 °C for 30 min using equimolar concentrations of oligonucleotide components. Under these conditions the ligation of correct tandem in complex 1 was occurred with 42% yield (FIG. 2). Among incorrect tetramers only pCCGC in the complex 3b is transformed into 20-mer with a low yield (4%) (FD = 10). In the other complexes the products of reaction were not observed (FIG. 2). Taking into account the accuracy of measurement of ^{32}P-radioactivity, the yield of ligation could not exceed 0.1 %, and FD was estimated to be more than 400 in this case. The efficacy of discrimination of the mismatched complexes may be enhanced by the use of the buffer containing 0.3 M NaCl and



Scheme 1

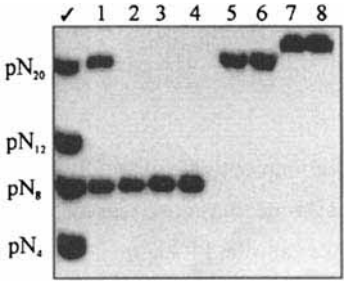
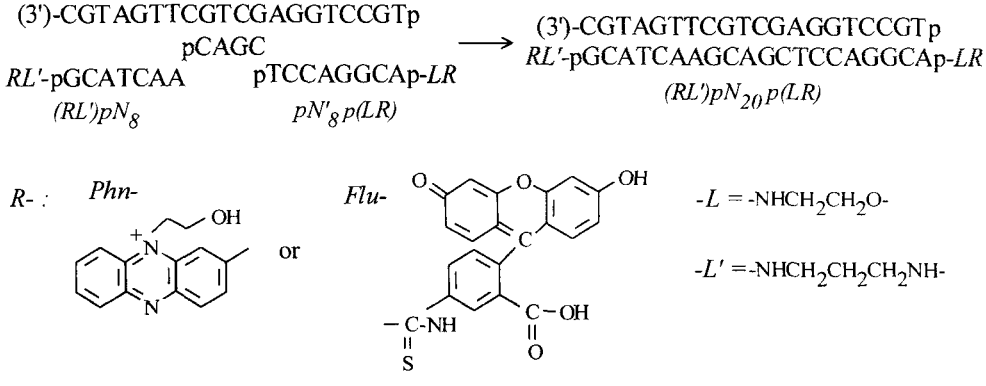


FIG. 1. Autoradiogram of the 20% denaturating PAGE of the product of ligation M+*pN_8+pN_4+pN'_8 (**1**), M+*pN_8+pN_4 (**2**), M+ pN_4+*pN'_8 (**3**), *pN_8+pN_4+pN'_8 (**4**), [M]=[pN_8]=[pN_4]=[pN'_8]=10^{-5}M; M+pN_8+*pN_4+pN'_8: [pN_4]=10^{-7} M (**5**), M+pN_8+pN_4+*pN'_8: [pN'_8]=10^{-7} M (**6**); M+(PhnL')pN_8+*pN_4+pN'_8p(LPhn) (**7**), M+(FluL')pN_8+*pN_4+pN'_8p(LFlu) (**8**) [pN_4]=10^{-7} M at 37°C, 30 min, 50 units of T4 DNA ligase in buffer 20 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 8% PEG, 10 mM $MgCl_2$. ✓ - markers of the length



Scheme 2

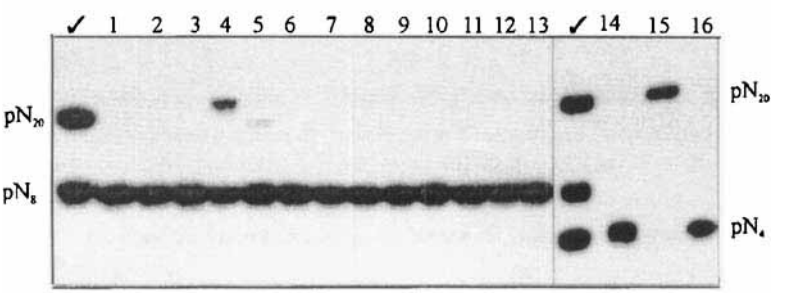


FIG. 2. Autoradiogram of the 20% denaturating PAGE of the product of ligation in complexes: 2b (1), 2c (2), 2a (3), 1 (4), 3b (5), 3a (6), 3c (7), 4c (8), 4a (9), 4b (10), 5a (11), 5c (12), 5b (13), 3b (14), 1 (15), 6 (16) at 37 °C during 30 min, 50 units of ligase. The concentration of oligonucleotide components: [DNA template] = [*pN$_8$] = [pN'$_8$] = 10^{-5} M, [pN$_4$] = 10^{-5} M (1-13), [*pN$_4$]= 10^{-7} M (14-16). The buffer 20 mM Tris-HCl (pH 7.5), 8% PEG, 10 mM $MgCl_2$, NaCl: 0.1 M (1-13), 0.3 M (14-16). ✓ - markers of the length.

lower concentration of any oligonucleotide probe. Under these conditions in all incorrect complexes 2-5 the formation of 20-mer was not observed while the ligation in complex 1 was occurred quantitatively (see for example, FIG. 2).

The confirmation of the possibility of discrimination of a point mutation in DNA is absence of the product of ligation of the tandem in complex 6 of the "wrong" DNA template in which one base in the binding site of the tetramer was replaced (FIG. 2).

complex 6

(3')-CGTAGTTCGTCTAGGTCCGTp

pCAGC

(5')-pGCATCAAG pTCCAGGCA

Thus, the ligation of tetranucleotide and flanking its pair of octanucleotides on DNA template is high specific and allow all possible base substitution in tetramer binding site to be discriminated.

ACKNOWLEDGMENT

This work was made possible by Grants from Russian State Programs "The new methods of bioengineering" and "Genom".

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