Data Article

Data on multimerization efficiency for short linear DNA templates and phosphoryl guanidine primers during isothermal amplification with Bst exo- DNA polymerase

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This article reports experimental data related to the research article entitled “Prevention of DNA multimerization using phosphoryl guanidine primers during isothermal amplification with Bst exo- DNA polymerase” (R.R. Garafutdinov, A.R. Sakhabutdinova, M.S. Kupryushkin, D.V. Pyshnyi, 2020) [1]. Here, multimerization efficiency in terms of Tt (time-to-threshold) values obtained for artificial DNA templates with the different nucleotide sequences during isothermal amplification with Bst exo- DNA polymerase is given. Data on the influence of phosphoryl guanidine primers (PGO) on multimerization for the LTc template which has shown high efficiency of multimerization are presented as well.

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DNA multimerization is a by-side amplification reaction that occurs under isothermal conditions on short single-stranded DNA templates using Bst exo- DNA polymerase [2] through cycle-like structure formation [3]. The products of multimerization appear as a ladder on electrophoretic gels and represent tandem repeats that correlate with nucleotide sequence of initial template [1–3]. Here, we present data on multimerization using short (51 nucleotides) DNA templates (LTa–LTf) with different nucleotide sequences and corresponding natural and modified primers (F/R primer pairs) with one, two or three internucleosidic phosphates containing 1,3-dimethyl-2-imino-imidazolidine moieties (phosphoryl guanidine (PG) groups). Primers with three options of PG position were designed: near the 3'-end (Fc1/Rc1, Fc4/Rc4 and Fc6/Rc6 pairs), in the middle (Fc2/Rc2, Fc5/Rc5, Fc7/Rc7 and Fc8/Rc8 pairs) and near the 5'-end (Fc3/Rc3 and Fc9/Rc9 pairs) of the primers. In primers containing two or three PG groups, modifications were separated by one nucleoside with the exception of the Fc8/Rc8 pair where PG groups were separated by two and three nucleosides.

Table 1 represents time-to-threshold (Tt) values obtained for amplification of linear (LT) and circular (CT) forms of different DNA templates using unmodified primers. Sequences and molecular masses of modified primers are given in Tables 2 and 3 represents time-to-threshold (Tt) values obtained for amplification of linear LTc and circular CTc templates using modified primers. Raw data are provided in Supplementary file.

## Value of the Data
- The data presented indicate that multimerization proceeds efficiently during isothermal amplification using Bst exo- polymerase and slightly depends on the nucleotide sequence of the DNA templates.
- Three contiguous phosphoryl guanidine (PG) groups in the middle of the both primers are enough and the most appropriate for prevention of multimerization.
- The obtained results allow to design of improved primers for isothermal amplification with Bst exo- polymerase that could provide accurate and reliable DNA diagnostics.

## 1. Data

DNA multimerization is a by-side amplification reaction that occurs under isothermal conditions on short single-stranded DNA templates using Bst exo- DNA polymerase [2] through cycle-like structure formation [3]. The products of multimerization appear as a ladder on electrophoretic gels and represent tandem repeats that correlate with nucleotide sequence of initial template [1–3]. Here, we present data on multimerization using short (51 nucleotides) DNA templates (LTa–LTf) with different nucleotide sequences and corresponding natural and modified primers (F/R primer pairs) with one, two or three internucleosidic phosphates containing 1,3-dimethyl-2-imino-imidazolidine moieties (phosphoryl guanidine (PG) groups). Primers with three options of PG position were designed: near the 3'-end (Fc1/Rc1, Fc4/Rc4 and Fc6/Rc6 pairs), in the middle (Fc2/Rc2, Fc5/Rc5, Fc7/Rc7 and Fc8/Rc8 pairs) and near the 5'-end (Fc3/Rc3 and Fc9/Rc9 pairs) of the primers. In primers containing two or three PG groups, modifications were separated by one nucleoside with the exception of the Fc8/Rc8 pair where PG groups were separated by two and three nucleosides. Table 1 represents time-to-threshold (Tt) values obtained for amplification of linear (LT) and circular (CT) forms of different DNA templates using unmodified primers. Sequences and molecular masses of modified primers are given in Tables 2 and 3 represents time-to-threshold (Tt) values obtained for amplification of linear LTc and circular CTc templates using modified primers. Raw data are provided in Supplementary file.
2. Experimental design, materials, and methods

2.1. Materials

The following reagents were used: Bst 2.0 DNA polymerase and Isothermal buffer (New England Biolabs); T4 DNA ligase, exonuclease I, T4 polynucleotide kinase, dNTP (Thermo Fisher Scientific); SYBR Green I (Lumiprobe); tetrahydrofuran for DNA synthesis (Panreac); 2-cyanoethyl deoxynucleoside phosphoramidites and CPG solid supports for DNA synthesis (Glen Research). All solutions were prepared with highly purified water (>18 MOm) (Millipore).

2.2. Oligonucleotides

Linear DNA templates LTa-LTf, unmodified oligonucleotide primers Fa-Ff and Ra-Rf and splint probes Sa-Sf were designed using an OligoAnalyzer tool (Integrated DNA Technologies) and purchased from Syntol (Russia). Oligonucleotides Fc1-Fc9 and Rc1-Rc9 with internucleosidic phosphoryl 1,3-dimethyl-2-imino-imidazolidine groups (phosphoryl guanidine oligonucleotides (PGO)) were synthesized as described in Refs. [4,5]. PGO were isolated by reverse-phase HPLC on an Agilent 1200 HPLC system (USA) using a Zorbax SB-C18 (4.6 × 150 mm) column with a linear gradient of elution buffer.

**Table 1**
The mean Tt (time-to-threshold) values (minutes) for amplification of linear (LT) and circular (CT) DNA templates (unmodified primers were used).

| Templates | Nucleotide sequence, 5’→3’ | Linear form (LT) | Circular form (CT) |
|-----------|--------------------------|----------------|------------------|
| LTa       | GTCAGCTGCTTCGCTTGCTAGTGCTACAGCTACCGCACTTGTCGTCAGACCGCACTATGGCAGAGAAGAAGACAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGAGA...
masses of phosphoryl guanidine oligonucleotides were calculated using experimental LC-MS/MS ESI MS on an Agilent G6410A mass spectrometer (USA) in a negative ion mode. Molecular spectra recorded on a refrigerated acetone and desiccation under vacuum. PGO structures were confirmed by MALDI-TOF mass spectrometry (Bruker) using 3-hydroxypicolinic acid or 50% acetonitrile in 20 mM triethylammonium acetate, pH 7.0, flow rate 2 ml/min). Purified oligonucleotides were concentrated followed by precipitation with 2% LiClO₄ in acetone, washing with 2% LiClO₄ and desiccation in a vacuum. PGO solutions of oligonucleotides were prepared by dilution of precipitates in deionized water. Sequences of corresponding splint probe Sa-Sf and 2 µl of 2.5 mM ATP and 5 U of T4 DNA ligase were added. The mixtures were incubated for 18 h at 8°C for inactivation at 85°C. The reaction mixtures were put in T100 thermal cycler (Bio-Rad Laboratories) for DNA strands annealing. The temperature was slowly decreased from 80 to 25°C within 1 hour. After the end of annealing, 2 µl of 10 mM ATP and 5 U of T4 DNA ligase were added. The mixtures were incubated for 18 h at 8°C, after which the ligase was inactivated at 75°C for 15 min. Then, 1 U of exonuclease I was added in each sample, and the reaction mixtures were incubated for 2 h at 37°C and then for 1 h at 45°C followed by enzyme inactivation at 85°C for 15 min. The circular DNA templates were diluted up to 10⁷ molecules/ul and used for further amplification reactions without additional purification.

### Table 3

The mean Tt (time-to-threshold) values for amplification of linear LCt and circular CTc DNA templates using phosphoryl guanidine primers (minutes).^a^

| Fc  | Fe1  | Fe2  | Fe3  | Fe4  | Fe5  | Fe6  | Fe7  | Fe8  | Fe9  |
|-----|------|------|------|------|------|------|------|------|------|
| Rc  | 15.2 ± 1.5 | 13.4 ± 1.4 | 14.6 ± 1.6 | 15.1 ± 2.2 | 50.6 ± 1.5 | 13.2 ± 1.9 | N/A 53.6 ± 1.4 | 13.8 ± 0.9 | 14.3 ± 1.4 |
| Rc1 | 17.3 ± 1.8 | 27.4 ± 2.1 | 16.5 ± 1.6 | 15.4 ± 2.1 | 33.5 ± 1.6 | 17.7 ± 1.5 | N/A 53.5 ± 2.0 | 17.5 ± 1.4 | 13.7 ± 1.1 |
| Rc2 | 14.5 ± 1.7 | 13.1 ± 1.2 | 13.5 ± 1.4 | 14.6 ± 1.5 | 44.5 ± 1.7 | 15.8 ± 1.3 | N/A 34.6 ± 1.3 | 14.3 ± 1.5 | 15.1 ± 1.6 |
| Rc3 | 15.8 ± 1.1 | 14.1 ± 1.2 | 18.3 ± 1.7 | 15.7 ± 1.8 | 40.8 ± 1.3 | 15.7 ± 1.5 | N/A 23.6 ± 1.5 | 14.6 ± 1.6 | 15.0 ± 1.5 |
| Rc4 | 60.5 ± 2.2 | 36.6 ± 1.6 | 44.7 ± 2.5 | 49.4 ± 2.0 | 60.4 ± 1.7 | 27.4 ± 1.5 | N/A 60.2 ± 2.1 | 71.5 ± 2.7 | 44.3 ± 1.9 |
| Rc5 | 47.5 ± 1.7 | 272 ± 1.4 | 352 ± 2.0 | 252 ± 1.2 | 33.6 ± 1.8 | 13.9 ± 1.6 | N/A 29.7 ± 1.4 | 34.4 ± 1.5 | 25.5 ± 1.3 |
| Rc6 | N/A  | N/A  | N/A  | N/A  | N/A  | N/A  | N/A  | N/A  | N/A  |
| Rc7 | 53.4 ± 1.4 | 60.4 ± 1.9 | 54.8 ± 3.3 | 55.3 ± 1.4 | 50.5 ± 1.1 | 86.4 ± 2.5 | N/A 11.6 ± 1.5 | 15.0 ± 1.5 | 33.3 ± 1.5 |
| Rc8 | 15.6 ± 0.9 | 156 ± 1.7 | 165 ± 1.4 | 154 ± 1.2 | 46.2 ± 2.0 | 15.4 ± 1.3 | N/A 17.8 ± 1.0 | 44.2 ± 1.8 | 17.5 ± 1.6 |
| Rc9 | 13.7 ± 2.5 | 155 ± 2.0 | 148 ± 1.2 | 151 ± 1.2 | 38.7 ± 1.1 | 23.7 ± 2.6 | N/A 19.1 ± 1.5 | 21.0 ± 1.7 | 11.2 ± 1.3 |

^a^ N/A — no amplification occurs.

(0–50% acetonitrile in 20 mM triethylammonium acetate, pH 7.0, flow rate 2 ml/min). The circular DNA templates was prepared as follows. One pmol of each linear oligonucleotides LTa-LTf was routinely phosphorylated by T4 polynucleotide kinase in a 10 µl reaction mixture. Then, 5 pmol of corresponding splint probe Sa-Sf and 2 µl of T4 DNA ligase buffer were added, and the reaction mixtures were put in T100 thermal cycler (Bio-Rad Laboratories) for DNA strands annealing. The temperature was slowly decreased from 80 to 25°C within 1 hour. After the end of annealing, 2 µl of 10 mM ATP and 5 U of T4 DNA ligase were added. The mixtures were incubated for 18 h at 8°C, after which the ligase was inactivated at 75°C for 15 min. Then, 1 U of exonuclease I was added in each sample, and the reaction mixtures were incubated for 2 h at 37°C and then for 1 h at 45°C followed by enzyme inactivation at 85°C for 15 min. The circular DNA templates were diluted up to 10⁷ molecules/ul and used for further amplification reactions without additional purification.

### 2.3. DNA circularization

The circular DNA templates was prepared as follows. One pmol of each linear oligonucleotides LTa-LTf was routinely phosphorylated by T4 polynucleotide kinase in a 10 µl reaction mixture. Then, 5 pmol of corresponding splint probe Sa-Sf and 2 µl of T4 DNA ligase buffer were added, and the reaction mixtures were put in T100 thermal cycler (Bio-Rad Laboratories) for DNA strands annealing. The temperature was slowly decreased from 80 to 25°C within 1 hour. After the end of annealing, 2 µl of 10 mM ATP and 5 U of T4 DNA ligase were added. The mixtures were incubated for 18 h at 8°C, after which the ligase was inactivated at 75°C for 15 min. Then, 1 U of exonuclease I was added in each sample, and the reaction mixtures were incubated for 2 h at 37°C and then for 1 h at 45°C followed by enzyme inactivation at 85°C for 15 min. The circular DNA templates were diluted up to 10⁷ molecules/ul and used for further amplification reactions without additional purification.

### 2.4. Isothermal DNA amplification

All amplification samples were prepared in an UVC/T-M-AR PCR box (Biosan). The working space, dispensers, and plastic ware were preliminarily irradiated with ultraviolet for 20 min. Amplification was carried out in an iQ5 thermal cycler (Bio-Rad Laboratories) in 10 µl of reaction mixture containing 10⁷ linear or 10⁷ circular DNA target copies per reaction, 5 pmol of each primer, 1 µl of 2.5 mM dNTP,
1 × Isothermal buffer, 0.1 × SYBR Green I intercalating dye and 1.5 U of Bst 2.0 polymerase. Each sample was represented in three repeats. The program of amplification consisted of the following steps: 1) 70°C – 30 s, 2) 60°C – 3 h.

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Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.dib.2020.105188.

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