2nd ICST 2017

by Muhamad Ali

Submission date: 13-Oct-2020 02:27PM (UTC+0700)
Submission ID: 1413762997
File name: 2nd_ICST_2017.pdf (1.23M)
Word count: 4234
Character count: 22917

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Joint International Conference on Science and Technology in The Tropic

Organized by:
University of Mataram, Indonesia and University of Malaya, Malaysia

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ISBN : 978-602-61265-1-1

UNIVERSITI MALAYA

PROCEEDINGS

The 2nd International Conference on Science and Technology 2017

"Joint International Conference on Science and Technology in The Tropic"

Mataram, August, 23th-24th 2017

PUBLISHED BY :

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ISBN : 978 - 602 - 61265 - 1 - 1

Cetakan pertama : Agustus 2017

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TABLE OF CONTENTS

Modification of Nucleotide Sequences at Upstream and Downstream of the Shine-Dalgarno Sequence to increase DNA Expression in Cell Free System

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Abstract

This research aimed at investigating the effect of nucleotide-sequence modification of upstream and downstream at the Shine-Dalgarno sequence on the yield of DNA expression in an *Escherichia coli* cell-free expression system. An enzyme derived from *E.coli*, Chloramphenicol Acetyltransferase (CAT), was used as a reporter protein. A fragment of T7 promoter (T7P) was amplified from pK7.CAT plasmid using T7P forward (T7Pf) and three homotail T7P reverse primers (T7Pr-A1, T7Pr-A2, T7Pr-A3). The primers contained different sequences of upstream and downstream at the Shine-Dalgarno (SD) sequence to generate three T7P fragments. Then, three CAT fragments containing a different homotail sequence complementary to the upstream and downstream of the SD sequence were generated using three homotail primers (A1-CAT, A2-CAT, A3-CAT). Three expression cassettes (T7P-A1-CAT, T7P-A2-CAT, T7P-A3-CAT) were constructed and expressed in *E. coli* cell-free expression system using an overlapping PCR technology. The expression of targeted protein was determined using a CAT assay without purification. Result of this experiment showed that the expression level of the reporter protein was highly dependent on the sequence upstream and downstream of the SD sequence. This result suggest that insertion of CCTCTTT sequence in upstream region and GATTAGACA sequence in downstream part of SD sequence is a potential technique to improve expression level of recombinant protein in the cell-free expression system.

***Keywords**: DNA expression, Shine-Dalgarno sequence, Chloramphenicol Acetyltransferase (CAT), cell-free expression system, proteomics assay*

1. Introduction

DNA expression is a thriving field and the most important steps for the production of recombinant proteins in functional proteomics, biotechnology, and industry. It is because not only to fulfill huge diversity of functional proteins required for therapeutic entities but also to annotate the function, structure, and interaction network of proteomes which become the next challenge after post-genome projects (Ali *et al*., 2015). Therefore, some efforts have been made to improve the expression level of the target gene.

Despite great achievements have been obtained in DNA expression technologies, high protein yields are still difficult to obtain in research (Ali *et al*., 2005) and industries. For this reason, many strategies and molecular tools have been developed to get a high-throughput and robust expression of heterologous proteins in a cell or in cell-free expression system (Yamane *et al*., 2005).

DNA expression was aaturally regulated by either the transcription step, post-transcription stage, or translation fate. A series of cis-acting elements located in the DNA, such as promoters, enhancers, silencers, and locus control elements are transciption factors which mediated the transcriptional control. The 5'-untranslated

region (UTR) is one of the tripartite structures of mature mRNA which affects both mRNA synthesis and protein expression levels (Be[7] *et al*., 2009). Mignone *et al*. (2002) reported that the UTR has pivotal roles in a transport of matur[2]mRNAs out of the nucleus, and efficiency of translation of the mRNA into protein. A cis elem[23] upstream of the initiation codon, the Shine-Dalgarno (SD) sequence, facilitates the translation initiation of *E. coli* mRNAs (Etchegaray and Inoue, 1999; Bivona *et al*., 2010).

Recent studies [16]rformed by Kudla *et al*., (2009) and Goodman *et al*., (2013) revealed that reduced free energy of the mRNA [19]ucture near the start codon highly correlated with higher protein expression level. In addition, Goodman *et al*., (2013) reported that the expression levels of superfolder green fluorescent protein in *E. coli* were associated with nucleotide changes in the +10 region from the start codon of the protein. In addition, Boel *et al*. (2016) documented that mRNA folding was strongly influenced by the initial 16 codons which then affected mRNA stability and translation efficiency.

Enzyme d[14]ived from *E. coli*, Chloramphenicol Acetyltransferase (CAT) which catalyze a transfer of the acetyl group from acetyl-coenzyme A (acetyl-CoA) to chloramphenicol, was used as a reporter gene because of easily expressed and asseyed. Since founded as the first reported gene reporter, CAT has been extensively utilized for monitoring a transcriptional regulation in mammalian cells and transgene expression (Jiang *et al*., 2008). In addition, the enzyme was also used for monitoring the delivery, location and pattern of transgene expression in some disease models such as hepatitis B, heart disease, and drug resistance in bacteria (Selbert *et al*., 2002, Arnone *et al*., 2004; Rajamanickam and Jeejab[15], 2005). Therefore, the protein has been frequently used a[15] reporter protein in *E. coli* cell-free expression system (Ali *et al*., 2005; Ali *et al*., 2006).

Cell-free protein synthesis systems are fruitfully used in studies of basic molecular mechanisms of transciption and translation of the protein biosynthesis. Attention of biotechnologists was attracted to use the expression system for gene expression since capable of functioning for many hours and producing protein in short time and high yield (Sabrina and Ali, 2013; Ali *et al*., 2005).

In this research, the effect of a functional nucleotide sequence upstream and downstream of the Shine[4]algarno (SD) sequence on CAT-encoding gene expression was investigated in an *E. coli* cell-free protein synthesis system. For the purpose, three CAT fragments containing different homotail sequence complementary to the upstream and downstream of the SD sequence were generated using three homotail primers (A1-CAT, A2-CAT, A3-CAT). By using overlapping PCR technology, three expression cassettes (T7P-A1-CAT, T7P-A2-CAT, T7P-A3-CAT) were constructed using three co[21]les of T7P and CAT fragments as template and subsequently expressed in *E. coli* cell-free expression system. The level of target protein expression was clearly determined by CAT assay without purification. Result of this experiment showed that the expression level of the reporter protein depended on the sequence upstream and downstream of the SD sequence. The use of CCTCTTT in upstream and GATTAGACA in downstream of SD sequences gave higher expression than the others sequences. This result would be useful for creating the functional PCR product for high-throughput functional proteomics assay.

2. Materials and Methods

2.1. Primers Design

Primers (the T7 promoter (T7P) fragment, T7P forward (T7Pf) and 3 T7P reverse (T7Pr)) containing homotail with different sequence upstream and downstream of SD sequence were designed. At the same time, CAT reverse (CAT-R) and 3 homotail primers (A1-CAT, A2-CAT, A3-CAT) with different sequences upstream and downstream on the SD sequence were directly added before CAT coding region, to amplify CAT fragment. List of primers used in this study are shown in the Table 1.

2.2. Construction T7 Promoter Fragment

PCR mixtures contained the following component: 10x *Ex Taq* buffer 2.0 µl, 2.5 mM dNTPs mix: 3.0 µl, 0.86 ng/ µl pK7.CAT plasmid 1.0 µl, 10 mM T7Pf 1.5 µl, 10 mM T7Pr-A1/A2/A3 1.5 µl, *Ex Taq* 1.0 µl and SW 10.5 µl. The PCR was run under this program: 5 min at 94°C; 25 cycles of 30 s at 94°C, 30 s at 55°C, 30 s at 72°C, and 7 min at 72°C. PCR product (the whole of each DNA sample) was electrophoresed on 1 % agarose gel, extracted, and purified using a Agarose Gel Extraction Kit (Qiagen), ethanol precipitated, and resuspended in TE buffer.

Table 1. Primers used in this study

Description	Sequence (5'-3')
Primers for T7 promoter amplification	
T7Pf	CGCCTGGTATCTTTATAGTCCTGT
T7Pr-A1	GTTATC*TCCTTCTT*ACGTTGTAACAAAATTATTTCTAGAGGGAAACCG
T7Pr-A2	CTAATC*TCCTTCTT*AAAGAGGAACAAAATTATTTCTAGAGGGAAACCG
T7Pr-A3	ACTATC*TCCTTCTT*AATCCGAAACAAAATTATTTCTAGAGGGAAACCG
Primers for CAT amplification	
A1-CAT	ACAACGT*AAGAAGGA*GATAACGTAATGGAGAAAAAAATCACTGGATATACCACC
A2-CAT	CCTCTTT*AAGAAGGA*GATTAGACAATGGAGAAAAAAATCACTGGATATACCACC
A3-CAT	TCGGATT*AAGAAGGA*GATAGTGTAATGGAGAAAAAAATCACTGGATATACCACC
CAT-R	GCCTGCAGCTCGAGGTTATCC

Underlined sequences: homotail sequences of primers used in this study

2.3. Construction of CAT Cassettes

PCR mixtures for CAT cassettes consisted of: 10x *Ex Taq* buffer 2.0 µl, 2.5 mM dNTPs mix 3.0 µl, 0.86 ng/ µl pK7.CAT plasmid 1.0 µl, 10 mM A1/A2/A3-CAT 1.5 µl, 10 mM CAT-R 1.5 µl, *Ex Taq* µl and SW 10.5 µl. The PCR program used was as follows: 5 min at 94°C; 25 cycles of 30 s at 94°C, 30 s at 55°C,

30 s at 72°C, and 7 min at 72°C. For the purification of CAT cassettes, agarose gel extraction was performed after PCR as T7 promoter fragment purification.

2.4. Construction of Expression Cassettes

Each purified PCR product (0.15 μl) was added to a tube containing 10x *Ex Taq* buffer 1.5 μl, 2.5 mM dNTPs mix, *Ex Taq* 0.15 μl and SW 11.85 μl. The overlapping PCR program consisted of: 5 min at 94°C; 10 cycles of 30 s at 94°C, 20 s at 48°C, 1.0 min 30 s at 72°C. The second PCR was run using the following program: 5 min at 94°C; 25 cycles of 10 s at 94°C, 30 s at 55°C, 1.0 min 30 s at 72°C, and 7 min at 72°C. The PCR products were confirmed in agarose gel electrophoresis.

2.5. Cell-Free Expression

The obtained dsDNA fragments in which T7 promoter was located upstream of the CAT genes were used for the expression of CAT. The cell-free reaction in *E. coli* system was adopted from previous report (Ali *et al*., 2005). The detailed composition of cell-free reaction was 5 M KoAc: 0.3 μl, 0.2 M Mg(OAc)2: 0.75 μl, 0.1 mg/ml Rifampicin: 1.5 μl, LM (+DTT): 3.75 μl, 0.485 mg/ml pK7-CAT: 0.41 μl or overlapping PCR product: 3.0 μl, 2.3 mg/ml T7RNA polymerase: 0.07 μl, 10 mg/ml creatine kynase: 0.23 μl, and *E. coli* E30 extract: 4.25 μl, and steril water: 3.74 μl. The mixtures were incubated at 37°C for 90 min and continued for CAT assay.

2.6. CAT Assay

Expression of the reporter gene was measured using CAT activity. Then, the CAT activity was assayed according to a protocol developed by Yamane *et al*. (2005). An automatic 96-well plate reader (Molecular Devices Inc., CA, model Spectra MAX 250) was used to measure the absorbance of the assay mixture at 412 nm. The absorbent of the reaction was read using a spectrofotometer**.** All experiments were performed in triplicates.

3. Results and Discussion

This research was started by preparing three DNA expression cassettes having a 24-nt homotail sequences upstream of a CAT coding sequence which could be transcribed under the control of a T7 RNA polymerase promoter. T7 promoter fragments were constructed by PCR using T7Pf and T7Pr primers containing homotail sequences, Fig. 1 (A). The results were short fragments of T7 promoter containing homotail sequences in the down-stream regions, Fig. 2 column 1.

In addition, CAT-encoding gene fragments with the same sequences in the up-stream region with the T7 promoter fragment were produced by amplification of CAT gene using a T7.CAT plasmid as a template with homotail and CAT-R primers. As shown in the Fig. 1 (B), this step produced CAT fragments containing homotail sequences in the up-stream regions, Fig. 2 column 2.

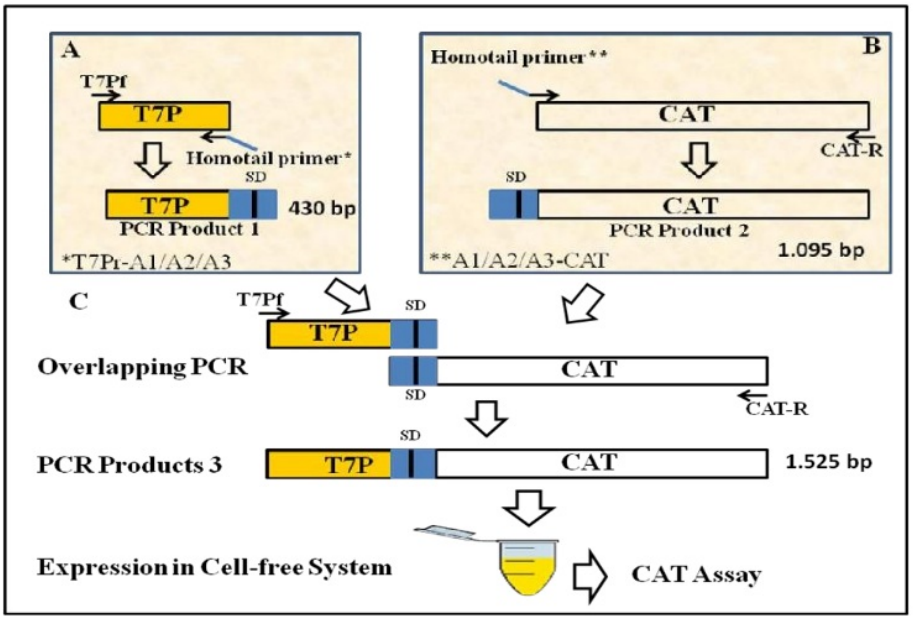

Fig. 1. Schematic workflow summarizing the construction of the T7 Promoter fragment (A), CAT cassettes (B), and cell-free expression cassettes (C). SD = Shine-Dalgarno Sequence.

To generate the expression cassettes suitable for cell-free expression system, overlapping PCR to fuse the T7 promoter and CAT fragments was conducted, Fig. 1C. In this step, the homolog sequences of T7 promoter and CAT were overlapped to produce the expression cassettes which have all expression requirements such as promoter, Shine-Dalgarno sequence as ribosom binding site, start codon, and reporter gene.

All fragments produced during generation of T7 promoter fragment, CAT fragment, and expression cassettes were presented in Fig. 2 (Column 3, 6, and 9). A ban of short T7 promoter (~ 430 bp) was obtained, Fig. 2 (1), followed by larger ban (~1.095 bp) of CAT fragment. Overlapping PCR between T7 promoter fragment (shorter ban) with CAT fragment (larger ban) produced the largest ban (~1.525 bp) of expression cassettes.

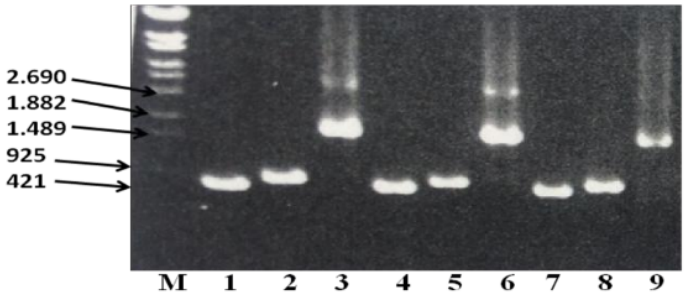

Fig. 2. Amplification results of T7 promoter, CAT fragment and overlapping PCR. A = λ DNA marker (M), 3 fragments of T7 promoter (1, 4, 7), 3 fragments of CAT (2, 5, 8), and 3 fragments of expression cassettes (3, 6, 9).

Three frements of expression cassettes generated by overlapping PCR contain all required elements for the control of transcription and translation processes for generation of active chlorampenicol acetyl transferase in cell-free protein synthesis. As shown in Fig. 3, the expression cassettes contain T7 promoter (yellow part), Shine-Dalgarno sequences as a ribosom binding site, and CAT gene. T7 promoter element is extremely popular and a strong phage promoter for recombinant CAT expression. Shine-Dalgarno (SD) sequences is a cis-element upstream of the initiation codon of CAT which facilitate the translation initiation in *E. coli* mRNAs. Etchegaray and Inouye (1999) stated that the SD sequence complementary to the 3'-end of 16 S rRNA enhances the translation initiation complexes formation between the 30 S ribosomal subunit with mRNAs.

All PCR-generated expression cassettes with variation sequences upstream and downstream of SD sequence (Fig. 3) were expressed in *E. coli* cell-free system with T7.CAT plasmid as a control. At the theoretical level, expression of T7.CAT plasmid would generate higher yields comparing to the use of PCR product. It is because the plasmid is more stable than PCR product as a expression template in cell-free expression system.

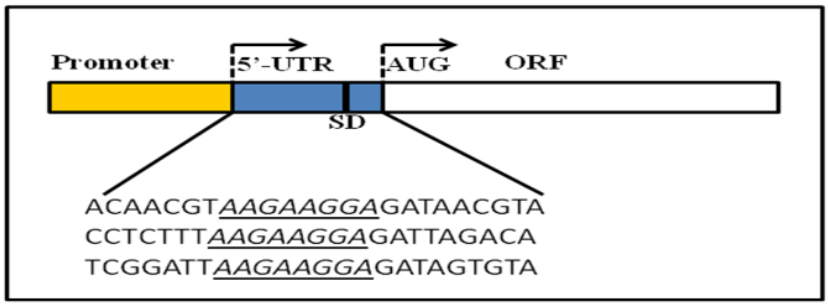

Fig. 3. Schematic representation of expression construct which contain promoter (T7 promoter), 5'-UTR region, Shine-Dalgarno (SD) sequence (underline), and start codon of CAT.

However, as shown in Fig. 4, the highest expression result of CAT was obtained by expression of PCR product generated by T7Pr-A2 and A2-CAT primers.

The difference between the PCR product is sequence in the upstream and downstream of SD sequence. The highest expression product was produced by expression cassettes containing CCTCTTT*AAGAAGGA*GATTAGACA sequences. Interestingly, the expression result of the PCR product was higher than the expression result of CAT-encoding plasmid which assumed more stable than the PCR product during the expression process. The others generated expression cassettes, ACAACGT*AAGAAGGA*GATAACGTA and TCGGATT*AAGAAGGA*GA TAGTGTA sequences, produced lower protein than produced by the p7.CAT plasmid in cell-free expression system.

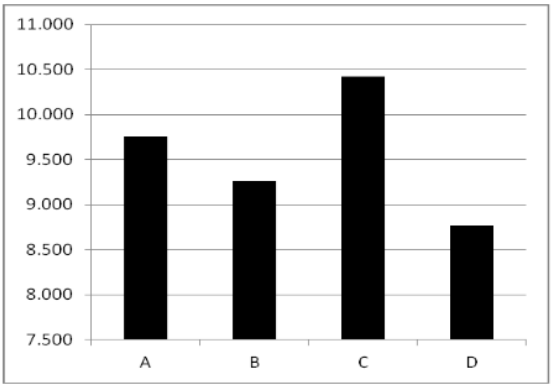

Fig. 4. Results of CAT Assay on expresion of plasmid pK7-CAT as a Control(A), and three homotail PCR products expression (B-D).

Results of this research indicates that the difference in some sequences of upstream and downstream of SD sequence (underline) gave significant effect on the yields of protein expressions. As previously described by Spregart *at al*. (1996), the sequence located at downstream of the initiation codon served as an indipendent translation signal for the protein expression. Their interactions with several 16S rRNA sequences is responsible for translational enhancement. Therefore, this result would be useful for preparing the PCR product for high-throughput functional proteomics assay.

4. Conclusion

The expression level of the reporter protein highly depends on the sequence at upstream and downstream of SD. Sequence insertion of CCTCTTT sequence in upstream region and GATTAGACA sequence in downstream part of SD sequence had higher expression result than the other. This result would be useful for preparing the PCR product template for high-throughput functional proteomics assay.

5. Acknowledgements

This work was financially supported by a grant from the Hibah Kompetensi, Directorate General of Higher Education, Indonesian Ministry of Research,

Technology, and Higher Education. The authors are grateful to Prof. Hideo NAKANO (Laboratory of Molecular Biotechnology, Nagoya University, Japan) for providing devices and fruitful discussion.

6. References

[1] Ali M., Ismaini, Depamede S.D., Setyono BDH., Mukhlis A., Amin M., and Ashari M. 2015. Stirred bioreactor for the robustness production of recombinant GST.VP28 in fed-batched cultivation of *Escherichia coli*. Scientific Study & Research, 16, 245-252.

[2] Ali M., Nakano H., Hitomi K., 2006. Generation of monoclonal antibodies using simplified single-cell reverse transcription-polymerase chain reaction and cell free protein synthesis. J. Biosci. Bioeng. 101, 284-286.

[3] Ali M., Suzuki H., Fukuba T., Jiang X., Nakano H., Yamane T., 2005. Improvements in the cell-free production of functional antibodies using cell extract from protease-deficient *Escherichia coli* mutant. Journal of bioscience and bioengineering. 99, 181-186.

[4] Arnone MI., Dmochowski IJ., Gache C. 2004. Using reporter genes to study cis-regulatory elements. Methods Cell Biol. 74:621-52.

[5] Berg L., Lale R., Bakke I., Burroughs N., and Valla S. 2009. The expression of recombinant genes in *Escherichia coli* can be strongly stimulated at the transcript production level by mutation the DNA-region corresponding to the 5'-untranslated part og mRNA. Microb. Biotechnol, 2, 379-389.

[6] Bivona L., Zou Z., Stutzman N., and Sun PD. 2010. Influence of second amino acid on recombinant protein expression. Protein Expr. Purif., 74, 248-256.

[7] Boel G., Letso R., Neely H., Price WN., Wong KH., Su M., Luff J., Valecha M., Everett JK., Acton TB., Xiao R., Montelione GT., Aalberts DP., Hunt JF.2016. Codon influence on protein expression in E. coli correlates with mRNA levels. Nature. 21;529(7586):358-363.

[8] Etchegaray JP., and and Inoue M. 1999. Translational enhancement by an element downstream of the initiation codon in *E. coli*. J. Biol. Chem., 274, 10079-10085.

[9] Goodman DB., Church GM., and Kosuri S. 2013. Causes and effects of N-terminal codon bias in bacterial genes. Science, 342, 475-479.

[10] Jiang T., Xing B., Rao J. 2008. Recent Developments of Biological Reporter Technology for Detecting Gene Expression. Biotech. Gen. Engin. Rev. 25, 41-76.

[11] Kudla C., Murray AW., Tollevey D., and Plotkin JB. 2009. Coding-sequence determinants of gene expression in *Escherichia coli*. Science, 324, 255-258.

[12] Mignone F., Gissi C., Liuni S., and Pesole G. 2002. Untranslated regions of mRNAs. Genome Biology, 3, 1-8.

[13] Rajamanickam C., Jeejabai R. 2005. Delineation of sequences essential for specific promoter activation during pressure overloaded hypertrophy or factor-induced hypertrophy. Methods Mol Med.,112:251-60.

[14] Sabrina Y. And Ali, M. 2013. Cloning, expression, and functional assay of monoclonal antibody fab fragment against merozoite surface protein 1 (msp1) of *Plasmodium falciparum*. Bioscience Research,10(2):71-77.

[15] Seibert E., Ross JB., Osman R. 2002. Role of DNA flexibility in sequence-dependent activity of uracil DNA glycosylase. Biochemistry., 10;41(36):10976-84.

[16] Sprengart ML., Fuchs E., Porter AG. 1996. The downstream box: an efficient and independent translation initiation signal in *Escherichia coli*. EMBO J. 1;15(3):665-74.

[17] Yamane T, Ikeda Y, Nagasaka T, Nakano H. 2005. Enhanced cell-free protein synthesis using a S30 extract from Escherichia coli grown rapidly at 42 degrees C in an amino acid enriched medium. Biotechnol. Prog., 21(2):608-13.

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