Wristwatch PCR: A Versatile and Efficient Genome Walking Strategy

Lingqin Wang¹,², Mengya Jia¹,², Zhaoqin Li³, Xiaohua Liu¹,², Tianyi Sun¹,²,⁴, Jinfeng Pei¹,², Cheng Wei¹,², Zhiyu Lin¹,²,⁴ and Haixing Li¹,²*

¹State Key Laboratory of Food Science and Technology, Nanchang University, Nanchang, China, ²Sino-German Joint Research Institute, Nanchang University, Nanchang, China, ³Charles W. Davidson College of Engineering, San Jose State University, San Jose, CA, United States, ⁴Key Laboratory of Poyang Lake Environment and Resource Utilization, Ministry of Education, School of Environmental and Chemical Engineering, Nanchang University, Nanchang, China

Genome walking is a method used to retrieve unknown flanking DNA. Here, we reported wristwatch (WW) PCR, an efficient genome walking technique mediated by WW primers (WWPs). WWPs feature 5′- and 3′-overlap and a heterologous interval. Therefore, a wristwatch-like structure can be formed between WWPs under relatively low temperatures. Each WW-PCR set is composed of three nested (primary, secondary, and tertiary) PCRs individually performed by three WWPs. The WWP is arbitrarily annealed somewhere on the genome in the one low-stringency cycle of the primary PCR, or directionally to the previous WWP site in one reduced-stringency cycle of the secondary/tertiary PCR, producing a pool of single-stranded DNAs (ssDNAs). A target ssDNA incorporates a gene-specific primer (GSP) complementary at the 3′-end and the WWP at the 5′-end and thus can be exponentially amplified in the next high-stringency cycles. Nevertheless, a non-target ssDNA cannot be amplified as it lacks a perfect binding site for any primers. The practicability of the WW-PCR was validated by successfully accessing unknown regions flanking Lactobacillus brevis glutamate decarboxylase gene and the hygromycin gene of rice. The WW-PCR is an attractive alternative to the existing genome walking techniques.

Keywords: wristwatch primer, partially annealing, wristwatch-like DNA, wristwatch PCR, genome walking

INTRODUCTION

Genome walking refers to a cluster of molecular technologies that are used to capture the full-length sequence of a target gene or identify unknown regions adjacent to a known sequence. Genome walking is particularly useful when the genetic information available for biological sequence analysis is limited (Leoni et al., 2011; Ashrafmansouri et al., 2020). Genome walking relies on genome library screening or the PCR. However, the construction and screening of genomic DNA libraries are cumbersome and labor-intensive (Li et al., 2015; Zeng et al., 2020). Hence, PCR-based methods are currently promising as they are considered simple and rapid. Although numerous, the reported PCR-based walking techniques can be classified into three types according to the involved rationales (Wang et al., 2007; Chang et al., 2018; Alquezar-Planas et al., 2020): 1) inverse PCR (Ochman et al., 1988), 2) cleavage-ligation-mediated PCR (Mueller et al., 1989; Rosenthal et al., 1990; Jones et al., 1992), and 3) randomly primed PCR (Liu et al., 1995; Tan et al., 2005; Wang et al., 2013).

In the inverse PCR, a circularized target DNA must be generated by digesting genomic DNA, followed by intramolecular ligation. Then, the fragments of interest upstream and downstream from a known sequence are amplified by two GSPs with inverse extension directions (Ochman et al., 1988;...
stringency cycle restricts partial annealing of any primer to only one. The feasibility of this method was verified by isolating flanks of the glutamate decarboxylase (gcdA) locus and hygromycin gene (hyg). The WW-PCR can be used to probe unknown DNA flanks, identify transgene integration sites, and obtain new genes from environmental DNA.

**MATERIALS AND METHODS**

**Genomic DNA Isolation**

The genomic DNA of *Lactobacillus brevis* CD0817 was extracted with the Bacterial Genomic DNA Isolation Kit (TIANGEN Biotech Co., Ltd., Beijing, China), according to the manufacturer’s guidance. Rice genomic DNA was kindly supplied by Dr. Xiaojue Peng (Nanchang University).

**Primers**

The oligonucleotide sequences of all WWPs are completely random and are 25 nucleotides (nt) in length comprising the identical 5′- (12 nt) and 3′-part (3 nt) and a heterologous spacer (10 nt) (underlined). WWP: wristwatch primer.

**PCR Procedures**

Three permutations were produced from the three WWPs. A WWP permutation is shown in the same column. The WW-PCR can be used to probe unknown DNA flanks, identify transgene integration sites, and obtain new genes from environmental DNA.
(Mg²⁺ plus), 8 μL of dNTP mixture (2.5 mM each), 1 μL of each primer (10 μM each), 1 μL of the former PCR product, and 0.5 μL of TaKaRa LA Taq polymerase (5 U/μL). Each round of PCR consisted of three annealing stages: stage 1, five high-stringency (65°C) cycles (HSC); stage 2, one low-stringency (25°C) cycle (LSC) in the primary PCR or one reduced-stringency (40°C) cycle (RSC) in secondary/tertiary PCR; and stage 3, 25 HSCs (65°C). The detailed thermal cycling parameters for the WW-PCR are presented in Table 3.
DNA Manipulation and Sequencing

PCR products were purified using the TaKaRa MiniBEST Agarose Gel DNA Extraction Kit Ver.4.0 (Dalian, China). A purified fragment was ligated to pMD19-T simple vector using the T-vector Kit (TaKaRa). Then, the recombinant plasmids were transformed into *E. coli* DH5α cells in accordance with the instruction of TaKaRa. Several selected positive colonies were then sequenced by Shanghai Sangon Biotech Co., Ltd. (Shanghai, China).

RESULTS

Overview of Wristwatch-PCR

Each walking includes three parallel sets of WW-PCRs individually performed by the three WWP permutations WWP1-WWP2-WWP3, WWP2-WWP3-WWP1, and WWP3-WWP1-WWP2 (Table 2). Each WW-PCR set consists of three successive rounds (primary, secondary, and tertiary) of nested PCRs. For convenience and clarity, only permutation WWP1-WWP2-WWP3 is employed to illustrate the rationale and process of the WW-PCR (Figure 1).

In the primary PCR, the first five HSCs only allow GSP1 to anneal to its complementary site on a known region, thus authentically increasing copies of target single-stranded DNA (ssDNA). The following one LSC makes WWP1 arbitrarily anneal to a certain place(s) on an unknown region of ssDNA and extends toward GSP1 and other loci. As a result, an ssDNA pool comprising target and non-target molecules is newly generated. It should be emphasized that the 10 nt internal mismatch allows the WWPs anneal to distinctive loci on the unknown flanking region. If more than one WWP is used in parallel, at least one will successfully anneal to the flanking region. In the next one HSC, a nascent target ssDNA is converted into double-stranded DNA (dsDNA) defined by GSP1 and WWP1 as it has an exact binding site for GSP1 at the 3′-end; this dsDNA can be exponentially enriched in the remaining HSCs. A non-target ssDNA, however, cannot be converted into dsDNA in the HSCs because it lacks perfect binding sites for any primers and thereafter is diluted.

In the secondary PCR, the first five HSCs only permit GSP2 to hybridize to its complement on known regions and extend toward WWP1, thus accumulating the ssDNA of interest. In the next one RSC, WWP2 directionally anneals to the WWP1 locus to form a wristwatch-like structure (if WWP2 matches some site(s) internal to the WWP1 well, WWP2 annealing to this site cannot be ruled out) and then initiates DNA elongation (Figure 1). As a result, a pool of ssDNAs is newly produced. The nascent target ssDNA has WWP2 at the 5′-end and the GSP2 complement at the 3′-end, which is converted into dsDNA driven by GSP2 in the next one HSC. This dsDNA is exponentially amplified in the remaining HSCs. The non-target ssDNA, however, cannot be converted into the double-stranded form in the HSCs due to the absence of a perfect binding site for any primers. This non-target ssDNA is hence removed.

The tertiary PCR driven by GSP3 and WWP3 is used to further eliminate non-target products; the involved mechanism and process are the same as those of the secondary PCR. Eventually, the target molecule becomes predominant.

**Genome Walking of gadA and hyg**

To verify the feasibility of the WW-PCR, we applied this method to isolate lateral segments of *L. brevis* CD0817 gadA and rice *hyg*. The *L. brevis* CD0817 genome (AYM03982.1), as well as rice *hyg* (KF206149.1), and its surrounding region were deposited in the GenBank database. A portion of gadA or *hyg* was designated as a known sequence for designing nested GSPs, and the region adjacent to the known DNA was assumed to be an “unknown sequence”, which are herein collectively referred to as the reference sequence. The three WWP permutations (WWP1-WWP2-WWP3, WWP2-WWP3-WWP1, and WWP3-WWP1-WWP2) were used to perform three parallel sets of WW-PCRs in each walking, by pairing with a GSP set (Table 2), respectively.

The PCR products were separated by agarose electrophoresis. As shown in Figure 2, each WW-PCR released discrete DNA fragments.
band(s) after two or three rounds of reactions. The distinct bands in secondary and tertiary PCRs were recovered for T-cloning and sequencing. Sequence alignment was conducted using the MegAlign tool in Lasergene software. The results demonstrated that all of the bands belong to target products as they are identical to the corresponding reference sequence (Supplementary Figure S1). In most cases, more than one clear DNA band appeared in a PCR (secondary/tertiary). Figure 2 also demonstrates that each WW-PCR in the set exhibited a distinctive electrophoretic pattern, and the longest amplicon ranged from 1 to 3 kb in size. Specifically, the largest fragments walked for gadA by the three WW-PCRs were 2.8 (GT1), 3.0 (GT4), and 3.3 kb (GT10), respectively (Figure 2A), and for hgy were 0.6 (HT1), 0.7 (HT2), and 3.1 kb (HT3), respectively (Figure 2B).

**DISCUSSION**

In this study, the WW-PCR, a new tool for determining unknown flanking DNA, has been established. The key to WW-PCR is the use of WWPs characterized by identical 5’-part and 3’-part and heterologous spacers. The current technique was termed WW-PCR due to the formation of wristwatch-like structures between WWPs. We have illustrated how the WW-PCR can be used to efficiently obtain unknown flanking regions, starting from a known DNA sequence (Figure 1). A WWP used in primary PCR determines the annealing pattern, while the one used in the secondary or tertiary PCR is responsible for eliminating non-target products.

The 3’-overlap ensures that the WWP initiates DNA extension once it anneals to the former WWP complement, with the 5’-overlap stabilizing the wristwatch-like structure (Tan et al., 2005). In general, functional priming requires at least a 2-nt accurate match at the 3’-end (Parker et al., 1991; Parks et al., 1991). A longer identical 3’ end (five or more bases), however, may weaken individualized random annealing of the WWPs in the primary PCR (Parks et al., 1991). Comprehensively, a 3’-overlap of 3 nt was assigned to the WWPs in this study. The overall difference in the sequence, due to the 10 nt mismatch, facilitates personalized annealing of the WWPs in the primary PCR, providing a guarantee for the success and efficiency of the WW-PCR. It can be expected that if more than one WWP permutation is used, at least one would give positive results, and some may produce satisfactory amplicon(s). In this work, each WW-PCR yielded positive outcomes (Figure 2), suggesting a high success rate of the WW-PCR. The longest product from each walking was approximately 4 kb (Figure 2), verifying the high efficiency of the WW-PCR. In most cases, multiple bands were observed in a secondary/tertiary PCR (Figure 2). This is common in PCR-based genome walking as the primary walking primer has multiple annealing sites on the flank of interest (Chang et al., 2018; Lo et al., 2018). We also noticed that a DNA band of the secondary PCR was slightly larger than that of the corresponding tertiary PCR (Figure 2), which is attributed to the mutual position relationship between the nested GSPs used (Liu et al., 2007).

In the RSC of the secondary/tertiary PCR, in the case that the WWP is well-matched with some site(s) internal to the former WWP locus on an unknown region, the WWP may anneal to the site and prime DNA elongation. If this annealing occurs on the DNA of interest, an extra shorter target product may be generated. If this annealing occurs on the non-target DNA, the resultant shorter one cannot be further amplified in the subsequent HSCs because it lacks a perfect binding site for any primer (Figure 1). Therefore, the internal annealing of the WWP contributes to the multi-band phenomenon while not affecting the specificity of the PCR. It should be pointed out that the DNA band pattern of any tertiary PCR resembles with that of the corresponding secondary PCR (Figure 2), implying that internal partial annealing is rare.

It is worth emphasizing that the Tm between WWPs should be at least 20°C lower than that of any WWP itself (Li et al., 2015; Chang et al., 2018) so that the WWP anneals to the former WWP locus only in the one RSC of the secondary/tertiary PCR (Figure 1). Here, the Tm between the WWPs was as low as around 40°C, while that of any WWP itself is rather high (60–65°C) (Table 1). The sequences of WWPs are variable, as long as they can form a wristwatch-like structure under expected temperature. Moreover, users can devise x WWPs as their wish to
perform x sets of WW-PCRs. The three WWPs (Table 1) presented here have been validated. Users need to design their nested GSPs according to known sequences. The sequences of our WWPs are completely random and thus should be universal for any genomes.

Non-target amplification is a big problem in PCR-based walking strategies (Kilstrup et al., 2000). Three types of non-target products are usually produced: I) primed by GSP alone, II) primed by GSP and random primer (here referred to as WWP), and III) primed by the random primer alone (Arnold et al., 1991; Bae et al., 2010; Wang et al., 2011), as shown in Figure 1. Types I and II could be easily diluted in the next round of the PCR as there is a lack of an authentic binding site for the inner GSP. The real challenge presented is the elimination of type III products (Thirulogachandar et al., 2011; Zhu et al., 2016). The WW-PCR can effectively inhibit the amplification of type III. In each WW-PCR, the partial annealing of the WWP in the one LSC/RSC directs the synthesis of a new non-target ssDNA. This nascent ssDNA and its template, however, cannot be further amplified in the following HSCs due to their lack of complementary sites for any primers. Our results confirmed the high specificity of WW-PCR, as all the clear bands in the secondary or tertiary PCR were correct (Figure 2).

For the inverse PCR or cleavage-ligation-mediated PCR, extra operations are compulsory prior to the amplification reaction, sequentially including restriction digestion, self-cyclization, or ligation of the adapter/linker/cassette to target DNA. These steps are time-consuming, expensive, and are always accompanied by a strong background (Leoni et al., 2008; Reddy et al., 2008; Deng et al., 2010). The randomly primed strategy does avert these extra steps prior to the PCR. The reproducibility, efficiency, or universality of the available randomly primed PCR, however, has been unsatisfactory (Wang et al., 2007; Zhou et al., 2012; Wang et al., 2013). Compared to the routine randomly primed methods, WW-PCR may have at least one of the following advantages: 1) great simplicity and efficiency with more than one set of the WW-PCR that can be set up by simply varying the use order of the WWPs, increasing the success rate and efficiency of DNA walking; 2) superior versatility with the WWPs being universal for any genomes as they are completely random; and 3) high specificity with the WW-PCR selectively accumulating target DNA while removing non-target species as any primers partially anneal to the DNA template once only. A detailed comparison of the WW-PCR to the existing classical walking methods is shown in Table 4.

The targeted long-read sequencing method has gained substantial interest as an emerging technology (Bethune et al., 2020; Ebert et al., 2021). The WW-PCR is currently more adaptable for a general laboratory, given its cheapness and high accuracy. Meanwhile, in the future, the WW-PCR may become a supplement to the targeted long-read sequencing.

CONCLUSION

The WW-PCR, an efficient and reliable genome walking tool based on the partial overlap between WWPs, has been described in this work. The concept of the WW-PCR has been validated in the genomes of a microbe and rice. The current method is a promising alternative to the existing genome walking technologies because of its specificity, simplicity, and efficiency.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://www.ncbi.nlm.nih.gov/Genbank/, AYM03982.1 https://www.ncbi.nlm.nih.gov/Genbank/, and KF206149.

AUTHOR CONTRIBUTIONS

LW: project administration, methodology, and writing. MJ and CW: investigation. ZoL and JP: data curation. XL: resources. TS: conceptualization. ZyL: software. HL: funding acquisition and resources.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fbioe.2022.792848/full#supplementary-material

REFERENCES

Alquezar-Planas, D. E., Lober, U., Cui, P., Quedenau, C., Chen, W., Greenwood, A. D., et al. (2020). DNA Sonication Inverse PCR for Genome Scale Analysis of Uncharacterized Flanking Sequences. Methods Ecol. Evol. 12 (1), 182–195. doi:10.1111/2041-210x.13497

Arnold, C., and Hodgson, I. J. (1991). Vectorette PCR: a Novel Approach to Genomic Walking. Genome Res. 1, 39–42. doi:10.1101/gr.1.1.39

Ashrafmansouri, S.-S., Kamaladini, H., Haddadi, F., and Seidi, M. (2020). Simple Innovative Adaptor to Improve Genome Walking with Convenient PCR. J. Genet. Eng. Biotechnol. 18 (1), 64. doi:10.1186/s43141-020-00082-2

Bae, J.-H., and Sohn, J.-H. (2010). Template-blocking PCR: An Advanced PCR Technique for Genome Walking. Anal. Biochem. 398 (1), 112–116. doi:10.1016/j.ab.2009.11.003
Bethune, K., Maric, C., Couderc, M., Scarcelli, N., Santoni, S., Ardisson, M., et al. (2019). Long-fragment Targeted Capture for Long-read Sequencing of Plastomes. Appl. Plant Sci. 7 (5), e1243. doi:10.1002/ap3.1243

Chang, K., Wang, Q., Shi, X., Wang, S., Wu, H., Nie, L., et al. (2018). Stepwise Partially Overlapping Primer-Based PCR for Genome Walking. AMB Expr. 8 (1), 77. doi:10.1186/s13186-018-0610-7

Dawes, J. C., Webster, P., Iadarola, B., Garcia-Diaz, C., Dore, M., Bolt, B. J., et al. (2020). LUMI-PCR: an Illumina Platform Ligation-Mediated PCR Protocol for Integration Site Cloning, Provides Molecular Quantification of Integration Sites. Mobile DNA 11, 7. doi:10.1186/s13130-020-0201-0

Deng, J., Wei, M., Yu, B., and Chen, Y. (2010). Efficient Amplification of Genes Involved in Microbial Secondary Metabolism by an Improved Genome Walking Method. Appl. Microbiol. Biotechnol. 87 (2), 757–764. doi:10.1007/s00253-010-5269-4

Ebert, P., Audano, P. A., Zhu, Q., Rodriguez-Martin, B., Porubsky, D., Bonder, M. J., et al. (2021). Haplotypes-resolved Diverse Human Genomes and Integrated Analysis of Structural Variation. Science 372 (6537). doi:10.1126/science.aba7117

Hu, Z., Wang, L., Shi, Z., Jiang, J., Li, X., Chen, Y., et al. (2019). Customized One-step Preparation of sgRNA Transcription Templates via Overlapping PCR Using Short Primers and its Application In Vitro and In Vivo Gene Editing. Cell Biosci. 9, 87. doi:10.1186/s13578-019-0350-7

Huang, S.-H. (1994). Inverse Polymerase Chain Reaction. FEBS J. 44, 432. doi:10.1111/j.1574-9341.1994.tb04052.x

Jia, X., Lin, X., and Chen, J. (2017). Linear and Exponential TAIL-PCR: a Method for the Amplification of Unknown Genomic Regions Using Long Primer and RAPD Primer. J. Biotechnol. 263 (3), 554–560. doi:10.1007/011680

Kilstrup, M., and Kristiansen, K. N. (2000). Rapid Genome Walking: a Simplified PCR Method for Chromosome Walking. Nucleic Acids Res. 28, 55–60. doi:10.1093/nar/28.1.55

Kotik, M. (2009). Novel Genes Retrieved from Environmental DNA by Polymerase Chain Reaction: Current Genome-Walking Techniques for Future Metagenome Applications. J. Biotechnol. 144 (2), 75–82. doi:10.1016/j.jbiotec.2009.08.034

Leoni, C., Gallerani, R., and Ceci, L. R. (2008). A Genome Walking Strategy for the Identification of Eukaryotic Nucleotide Sequences Adjacent to Known Genes. Biotechniques 44 (2), 229–235. doi:10.2144/00012680

Leoni, C., Volpicella, M., De Leo, F., Gallerani, R., and Ceci, L. R. (2011). Genome Walking in Eukaryotes. FEBS J. 278, 3953–3977. doi:10.1111/j.1742-4658.2011.08307.x

Li, F., Fu, C., and Li, Q. (2019). A Simple Genome Walking Strategy to Isolate Unknown Genomic Regions Using Long Primer and RAPD Primer. Iran J. Biotechn. 17 (2), 89–93. doi:10.21859/ijb.2183

Li, H., Ding, D., Cao, Y., Yu, B., Guo, L., and Liu, X. (2015). Partially Overlapping Primer-Based PCR for Genome Walking. PLoS One 10 (3), e0120139. doi:10.1371/journal.pone.0120139

Liu, Y.-G., and Chen, Y. (2007). High-efficiency thermal Asymmetric Interlaced PCR for Amplification of Unknown Flanking Sequences. Biotechniques 43 (5), 649–656. doi:10.2144/000112601

Liu, Y.-G., and Whittier, R. F. (1995). Thermal Asymmetric Interlaced PCR: Automatable Amplification and Sequencing of Insert End Fragments from PI and YAC Clones for Chromosome Walking. Genomics 25, 674–681. doi:10.1016/0888-7543(95)80010-4

Lo, Y.-T., and Shaw, P.-C. (2018). DNA Barcoding in Concentrated Chinese Medicine Granules Using Adaptor Ligation-Mediated Polymerase Chain Reaction. J. Pharm. Biomed. Anal. 149, 512–516. doi:10.1016/j.jpba.2017.11.048

Mitsuhashi, S., and Matsumoto, N. (2020). Long-read Sequencing for Rare Human Genetic Diseases. J. Hum. Genet. 65 (1), 11–19. doi:10.1038/s10038-019-0671-8

Mueller, P. R., and Wold, B. (1989). In Vivo Footprinting of a Muscle Specific Enhancer by Ligation Mediated PCR. Science 246 (4903), 780–786. doi:10.1126/science.1695049

Ochman, H., Gerber, A. S., and Hartl, D. L. (1988). Genetic Applications of an Inverse Polymerase Chain Reaction. Genetics 120 (3), 621–623. doi:10.1007/BF02728711.1093 genetics/120.3.621

Parker, J. D., Rabinovitch, P. S., and Burmer, G. C. (1991). Targeted Gene Walking and Chromosome Walking. Nucl. Acids Res. 19 (11), 3055–3060. doi:10.1093/ nar/19.11.3055

Parks, C. L., Chen, L.-S., and Shenk, T. (1991). A Polymerase Chain Reaction Mediated by a Single Primer: Cloning of Genomic Adjacent to a Serotonin Receptor Protein Coding Region. Nucl. Acids Res. 19 (25), 7155–7160. doi:10.1093/ nar/19.25.7155

Reddy, P. S., Mahanty, S., Kaul, T., Nair, S., Sopory, S. K., and Reddy, M. K. (2008). A High-Throughput Genome-Walking Method and its Use for Cloning Unknown Flanking Sequences. Anal. Biochem. 381 (2), 248–253. doi:10.1016/j.abb.2008.07.012
Walking or for Isolation of Tagged DNA Ends. Nucleic Acids Res. 31 (12), 68e–68. doi:10.1093/nar/gng068
Zeng, T., Zhang, D., Li, Y., Li, C., Liu, X., Shi, Y., et al. (2020). Identification of Genomic Insertion and Flanking Sequences of the Transgenic Drought-Tolerant maize Line “ShSNAC1-382” Using the Single-Molecule Real-Time (SMRT) Sequencing Method. PLoS One 15 (4), e0226455. doi:10.1371/journal.pone.0226455
Zhang, H., Xu, W., Feng, Z., and Hong, Z. (2018). A Low Degenerate Primer Pool Improved the Efficiency of High-Efficiency thermal Asymmetric Interlaced PCR to Amplify T-DNA Flanking Sequences in Arabidopsis thaliana. 3 Biotech. 8 (1), 14. doi:10.1007/s13205-017-1032-y
Zhou, Z., Ma, H., Qu, L., Xie, F., Ma, Q., and Ren, Z. (2012). Establishment of an Improved High-Efficiency thermal Asymmetric Interlaced PCR for Identification of Genomic Integration Sites Mediated by phiC31 Integrase. World J. Microbiol. Biotechnol. 28 (3), 1295–1299. doi:10.1007/s11274-011-0877-1
Zhu, X.-J., Sun, S., Xie, B., Hu, X., Zhang, Z., Qiu, M., et al. (2016). Guanine-rich Sequences Inhibit Proofreading DNA Polymerases. Sci. Rep. 6, 28769. doi:10.1038/srep28769

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