Universe Scientific Publishing

ORIGINAL ARTICLE

REplication Jumping Duration PCR Amplification,Closing and Sanger Sequencing of A Fragment of the Temptation Gene in Black-spotted frog1

Xindi Cui

Inner Mongol University of Technology, Shijiazhuang, Hebei,Province

Abstract: During in vitro replication of DNA by DNA Polymerase,The diverse palindromic StructuresOn templates of DNA can lead to replication jumping and cause fragments to be missing from daughter strands.We recorded replication jumping in which a 564 bp fragment was missing from daughter DNA.The original template was a 1126 bp fragment of the introduction of the temporary gene of black-spotted frog(Pelophylax nigromaculatus).The replication jump was recorded after amplifica-tion of this template using polymerase chainREaction,Closing Using Vector,And sequencing using the Sanger Method.The replication jump was elevated to rise from this series of events:

Keywords: Reloading DNA polymerase; Replication; vitro processes; fragment; replication jump

Polymerase Chain Reaction(Polymerase ChainREactionPCR)Is refers to inDNAPolymerase of catalytic under to mother chainDNAFor template to specific primers for extension starting point by degeneration,Annealing,Extension and steps in vitro copy the and mother chain TemplateDNAComplementary of sub-chain DNAOf process is a can fast,Specifically in vitro amplification objectiveDNAOf technology is biological research in application the most widely [2-4] Laboratory Technology one of PCRProduct often need to After cloning after the sequencing or other Analysis.Cloning an arcane willPCRProduct and specific carrierDNAMolecular connection formation recombinantDNAMolecular into competent cells in by cells and Carrier of emerging suchT-Carrier and in very big degree on the simplified.PCRProduct of cloning process at the same time also improve the cloning Effect [8-10] Rate But,PCRNot always completely faithful of In addition to primers and template of non-specific binding was to produce non-specific opposite sex amplification product outside because template of superior structure,Polymerase of some properties such as also often will mediated-DNAOf recombinant(Recombination),Copy sliding(Slippage)And

Recombinant isDNAPolymerase in In accordance with template synthesis new chain of when because the other a and template sequence height similar of fragment and template in space on the close, polymerase from original of template chain on the move to the fragment on the continue to synthesis New Chain Final makePCRProduct become two different template chain[12]

The recombinant product.Copy sliding in microsatellite and short tandem repeat sequence of amplification in very common,DNAPolymerase in synthesis new chain more. A or a few repeat unit causedPCRProduct than template chain shorten[13].

Repeat unit integer times Jump amplification is when templateDNATheir own because the original sequence formation more stability of ring tongue local secondary structure New Chain Synthesis whenDNAPolymerase move to the

regional after it can span this ring tongue area continue to down synthesis new chain to makePCRProduct is template lost the

Amplification people18 S rDNAGene when found the middle missing54 BPOf abnormal.

Is big fragment of jump more difficult to happen in experimental also very rare.Because so,PCROf jump problem often be ignored caused error results.

PCRProduct of cloning is use bacteria inDNAPolymerase and copy system will targetDNAFragment the in body copy.Because bacteria Copy System of faithful of much higher than in generalTaq DNAPolymerase(No3'-5'Enzyme Activity)Molecular cloning using plasmid replication is generally considered to be very accurate..However, in special cases, such as when the target molecule contains some special palindrome structure, the bacterial plasmid.

Jumps or slides during replicatio.

We're studying the black-spotted frog.(Pelophylax nigromacula-Tus)Antimicrobial PeptidesTemporinWhen it comes to gene structurePCRAmplification and amplification productsT-AJumps occur simultaneously during cloning and cause up

BPThe fragment is missing and found with a segment6 BPAbout the repeat unit.We reported the results of this experiment to provide a reference for more research..

1. Materials and Methods

1.1 Sample

Chuan,Xi'an,Taiyuan, etc.20Adult black spotted lateral pleats collected in different regions, and each region5.Only, the hind leg muscle samples were taken immediately after double-damaged pulp was executed in the wild and preserved in liquid nitrogen, and then transferred-80Bao in the refrigerator Save.

1.2 GeneralDNAExtraction

20 mgAdd muscle tissue after being cut25μL protein-ase K(10 mg/ml),35μL SDS(10%),295μL tne Buffer(PH = 8), Fully mix and then centrifuge,56Water bath digestion

H.Digestive JuiceAxyprepGenomeDNAExtraction kit(Axyprep, Hangzhou)Total ExtractionDNA, Operation in accordance with the instructions.

As shown in figure1PCRProduct by agarose electrophoresis found big about 73%Of individual in1200 BPAnd550 BPThe at the same time appear clear of two,18%Individual short of strip signal weaker,9%The individual only1200 BPOf Strip.Will two the bands respectively recovery after and sequencing results display is long of bands1126 BPShort of bands562 BPAfter is in the former198 bpThe start missing564 bpMade.ference for two different of allele gene.

But in the long fragmentSangerSequencing when found a kind of abnormal phenomenon template actual length1126 BPBut sequencing results in sometimes will only get562 BPOf Short Sequence.The short sequence is long fragment in BPThe start missing564 bpMade.This show that short very may isPCRCause of a kind of illusion the in amplification reaction in the fragment missing.

With recovery of long fragment as an Template PCRAmplification agarose gel electrophoresis detection PCRProduct when found 3A situation A is only get1126 BPThe long fragment A is only get562 BPOf Short Fur ther more two fragment at the same time (Figure2) OnPCRProduct sequencing also prove that the this a little.

Template pollution of possibility we the long fragment purification recovery after.T-ACloning.Extraction positive cloning of plasmid after with restriction enzymePst IAndNot IToPCRProduct from carrier on cut down again by agarose electrophoresis detection found positive cloning in containing two kind of cloning fragment A is containing and long fragment completely same of cloning Fragment, A kind of IS and short completely same of cloning fragment (figure 3).That is,1126 BPLong of objective fragment in different colony in become size two of Fragment.Sequencing show that short remain is long fragment 198 bpThe missing564 bpMade.This show that this1126 BP Long of fragment not only inPCRAmplification process in can happen missing and in Escherichia coli of in of plasmid copy process also can

occur missing this a kind of phenomenon is very rare.

Use softwareREpeataround 2. 1Okay1126 BPOf fragment the repeat sequence analysis results display the sequence has the same direction repeat sequence (Direct repeats) 70Of,Reverse repeat sequence (Inverse re-peats) 13Of,Mirror repeat sequence (Mirror repeats) 21 Of and complementary repeat sequence (Complementary repeats) 21 Of (Table1). Will1126 BPAnd526 BPThe sequence of the two fragments was compared and found that the two sequences at the upstream and end of the missing fragment have one6 BPSame-direction repeat sequence5'-AGACCT-3'(Figure4.)Is where the sequence deletion occurred, so far we speculateDNAA jump occurred while copying to the second repeat sequence (PCRJumping).

2. Discussion

DNAA sequence can be repeated in the same direction, in the same direction[20] Jump or slide.In this study1126 BPDetected in Fragment Different repetitive sequences (Table1.), But repeated amplification, only in194~199 BPLocation and first757~762 BPRepeat sequence that appears on the location5'-AGACCT-3'A jump occurred at the site, resulting in the deletion of the amplified product564 bp.Surprisingly, by this pair of repetitions The jump will occur in vitro and in vivo[21.]

CanceillTwo replication-hopping models are proposed..One is related to the double strand hairpin structure of the parent..The strand synthesis is closed at the base that forms the hairpin structure, and then throughDNAPolymerase Chain replacement activity continued and finally slid over the hairpin Structure.However, the parental double helix model does not seem to be applicable to our replication Jump Events because in our study, a repeat sequence is missing in the sub chain.This indicates that one of the repeat sequences is incorporated into the hairpin structure,

Yes, in our study, the sequence after the first repeat or before the second repeat cannot form a stable hairpin structure with the repeat sequence (Figure5AAnd5b).

The other model is the heterohelix model, which blocks the synthesis of the chain at the first repeat, whileDNAPolymerase and sub-chain are detached from the template. In the next cycle, the new chain acts as a complementary chain, matching the second repeat unit.,Annealed and became primers,DNAPolymerase rebinds, starting downstream of the second repeat unit to complete the new chain

Because in this case a repeat sequence is missing in the sub chain, which is consistent with our case..The only difference between our results and the model is the weak double chain body at the bottom of the hairpin.It is reported that,DNAPolymerase shedding and chain extension blocking may not be caused by hairpin, butDNA

This structure appears when the copy occurs, which leads to the occurrence of jumps (Figure5C).We were unable to test this prediction further, but we speculated that both high-level structural-induced strand synthesis blocking and Polymerase shedding were associated with repeat sequences, and this happens both in vivo and in vitro..

DNAThe ability of Polymerase to produce jumping is related to its chain replacement activity.When plasmid replication is done in bacteria,DNAPolymeraseIII(Early replication, sometimesDNAPolymeraseI)[24]When making a new chain extension, because of its chain[25],

Replacement activity is not highAppropriate advanced knot on encountered Template After shedding, the new chain can be reannealed with another homologous sequence (the second repeat unit in this study, and finish the chain extension and jump.Single clone in this studyDNAThis is probably the case when the fragment is cloned again.(Figure5C)In factPCR,Sanger Sequenced or Molecular Cloned DNA The type of PolymeraseTaq DNA Poly merase,DNA Poly meraseII,T4Polymerase, etc.

A high structure that is easy to jump and is also prone to replication jumps when these enzymes are involved in replication.

The replication jump encountered by this research not only happens inTemporinThe two-way amplification of the gene intron also occurs inSangerIn the process of sequencing, it even happened in the Process of plasmid replication in bacteria..Based on our confirmation experiment, the purified1126 BPThe fragment was used as a template for

reamplification and the positive plasmid was digested with double enzymes, which led to this conclusion; otherwise, jumping may be misunderstood as an independent allele..So, we suggest that when we use Fragment Size for diploid or polyploid genotyping, we should first select the allele with larger fragment

References

1. Li meiying.DNAIn vitro amplification technology---Polymerase Chain Reaction (PCR)J. .Shen Journal of Yang Normal University: Natural Science,2000,18(4.):40-44.
2. Guo yulian,PCRPrinciple and Application of Technology J. .Wisdom,2008(18):230,168.
3. Lynch J.PCRTechnology: Principles and Applications for DNA ampli-FificationJ. .Journal of Medical Genetics,1990,27(8.):536.
4. Beroldingen c h v,Blake e t,HiguchiR,Et al.Applications of PCRTo the analysis of biological evidenceM. /PCRTechnology.London:Palgrave Macmillan,1989:209-223.
5. Xu Yishan, He Jingjing, Chen wanjuan, *et al*.TaThe Principle and Application of cloning technology J. Technology Outlook,2015(6.):89-90.
6. Sambrook J,RUssell D W.Molecular cloning experiment GuideM. .Huang Pei Hall Translation., Beijing:Chemical Industrial Press,2008.
7. Xue Fen ground Xu Qing Wei Hua and.OnlyPCRThe Molecular Cloning of Methods exploreJ. .Capital Medical University of Journal,201435(6):785-789.
8. ROBL J M.Application. cloning technology. production. Human polyclonal antibodies. cattleJ. .Cloning, Stem Cells200791):12-16.
9. Yellow yuan Tao Ying Zhang Wen dew and.A kind of newDNAMolecular cloning methods J, China Science: Life Science,201141(9):722-729.
10. Lewis I MPeura TTrounson A O.Large-scale applications.Cloning technologies. agriculture:An industry perspectiveJ. .REproductionFertility, development199810(8):677-682.
11. Luo Rui.ContinuousPCRAmplification process in abnormal phenomenon of Mechanism Research D. .North Beijing: , China Academy of Sciences Plant Institute,2006.
12. SaikiRKGelfand D HStoffel SEt al.Primer-directed enzymat-ic amplification DNA. a thermostable DNA PolymeraseJ. .Science1988239(4839):487-491.
13. Hartenstine M JGoodman M FPetruska J.Base stacking and e-ven/odd behavior of hairpin loops in DNA triple repeat slipage and expansion with DNA PolymeraseJ. .The Journal of Biological Chemistry,2000,275(24):18382-18390.