A simple, rapid and very efficient protocol for DNA isolation from mosquito species

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Abstract

We represent a simplified and rapid version of DNA extraction protocol from different mosquito species which is suitable for polymerase chain reaction\(PCR) and other molecular biology works. The protocol involves three steps like lysis, phenol : chloroform \(1:1) extraction and two fold isopropanol precipitation at -20 degree celcius using 1X STE buffer \(50mM NaCl, 50mM Tris-Hcl,100mM EDTA,PH 8.0).The proposed extraction protocol has an advantage of DNA extraction from mosquitoes using 1X STE buffer at 37ºcelcius which prevent DNA degradation at higher temperature and kept DNA stability in long term storage . The protocol proved by three different mosquito species and removing for potential contamination showed that the protocol yields good quantity and quality DNA, typically better than commercial kits .The protocol was evaluated by polymerase chain reaction \(PCR). The protocol of DNA extraction is a time saving as well as economies with available laboratory chemicals, consumables and basic equipments. The protocol is adoptable for laboratories in external and internal funded research projects with large numbers of mosquito samples in a small period .The protocol minimize the time for other DNA extraction protocols in two to three days and provides a workflow for mapping of malaria vectors ,their vectorial transmission in the area of translational research .

Introduction

Insect research including population genetics ,taxonomic and evolutionary field always invite molecular techniques .Molecular techniques followed good quality protocols like DNA extraction from a large number of samples in terms of time , money and equipment access. Several methods of DNA extraction have been published using Chelex-100, Sarcosil with Proteinase K, CTAB, Polyvinyal pyrolidone and mercaptoethanol ,Only CTAB ,CTAB with mercaptoethanol ,CTAB with SDS, DIGSOL buffer etc.1,2,3. A non destructive DNA extraction procedure have great potential on insect specimen rather than a destructive and time consuming protocol 4 .A universal and rapid salt of high quality genomic DNA extraction method was used to perform hundreds of pcr based reactions 5 . DNA extraction at room temperature prevent DNA degradation and is suitable for polymerase chain reaction using EDTA , Proteinase K and Guanidinium thiocyanate 6 . Most commercial insect DNA extraction kits \(Merk , prep G, DNeasy kit, Qiagen, Germany EM, Charge Switch and Aquapur DNA extraction kits) are not cost effective and involve use of expensive laboratory equipments. We developed a simple and very efficient DNA isolation protocol from mosquito species for pcr related works in our laboratory.

Reagents

The reagents which were used to make the STE\(Sodium Chloride ,Tris –HCl, EDTA, Ph. 8.0) buffer acting as a lysis buffer in this protocol. • Sodium Chloride • Tris-HCl • Ethylene Diamine Tetraacetic Acid\(EDTA) • Sucrose • Sodium Dodesyl Sulphate\(SDS) • Triton-X • Proteinase-K • Rnase A solution • Phenol:Chloroform\(1:1) • Chloroform:Isoamyl alcohol\(24:1) • Isopropanol • Ethanol • Tris • Borate • Agarose

Equipment

Equipments: • Incubator • Centrifuge • Vortexer • Freezer\(-20 º celcius) • Nanodrop or Spectrophotometer • Electrophoresis apparatus

Procedure

1.Wash preserved mosquitoes in sterile distilled water or phosphate buffer saline\(PBS) to remove excess alcohol. Fresh mosquitoes can be ground directly. 2.Grind mosquitoes in 1.5 ml eppendorf tube with micropistle in 50-100 µl 1X STE buffer \(50mM Nacl,50mM Tris- HCL,100mM EDTA, Ph 8.0) along with 100mM sucrose. Add 1X STE buffer to a total volume of 300-500 µl for a single mosquito and 1 ml for mosquito pool like 4,6,8,10 numbers. Then add 1% SDS ,1% Triton -X , 10 µl/ ml Rnase A \(20mg/ml), 20 µl/ ml Proteinase K \(20mg/ml) and mix it. 3.Lyse for 1 hour 30 minutes at 37º celcius. Gently mix the tube by inverting every 15 minutes. 4.Centrifuge at 12,000g for 10 minutes at 4º celcius .Transfer the supernatant to a fresh tube. 5.Add equal volume of phenol:chloroform\(1:1) ,shake the tube well for 5 minutes and centrifuge at 12,000g for 10 minutes at 4º celcius . 6.Repeat the above step\(5) , then add chloroform:isoamyl alcohol\(24:1) and centrifuge at 12,000g for 10 minutes at 4º celcius . 7.Transfer the very clear supernatant to a fresh tube , add two fold volume cold isopropanol and keep it for 1 hour at -20º celcius . 8.Centrifuge at 12,000g for 30 minutes at 4º celcius and then remove the supernatant . 9. Wash the pellet with 70% ethanol . 10. Keep the pellet at 37 º celcius for 10 minutes. 11. Dissolve the dry pellet in nuclease free water or TE buffer \(Ph 8.0). Store DNA at 4 º celcius or -20 º celcius .

Timing

This protocol consists of three phases like\(1)Incubation phase \(2)Washing /Extraction phase \(3) Precipitation phase. \(1)The incubation phase is a lysis step where maximum cells were disrupted. This phase limits for 2 hours. \(2)Washing /Extraction phase is a removal of lipids , proteins and salt complexes from mosquito materials. This phase limits for 40 minutes. \(3)Precipitation phase is the DNA precipitation in isopropanol organic solution happened at -20º celcius .This phase limits for 1 hour. The end of DNA isolation is a 70% ethanol washing step contribute to removal of proteins , Then dissolved in nuclease free water or TE buffer \(Ph 8.0). The total DNA isolation protocol completed within 4 hours 30 minutes for a single mosquito but two to three days in case of other published protocols

Troubleshooting

The protocol described here offers a rapid and efficient DNA extraction suitable for polymerase chain reaction. The protocol is used for identification of mosquito species in a multiplex polymerase chain reaction. Steps Problems with Possible reason Solution Phase-1,Lysis Lower incubation at 37ºcelcius prevents DNA degradation. EDTA nullyfy the action of DNAse and break down the nuclear envelope in order to release the DNA.Higher temperature like more than 50º celcius is not good for DNA isolation. Solution:The good lysis buffer \(1X STE) composed of EDTA and SDS suitable for mosquito cells lysis.

Phase-2, Extraction:Phenol, Chloroform and Isoamyl alcohol remove protein impurities, prevent shearing of DNA during isolation and reduce foaming during phenol- chloroform addition. Solution:Phenol:Chloroform:Isoamyl alcohol are good organic solvents for DNA isolation. Phase-3, Precipitation: Isopropanol is to precipitate DNA by capturing water molecule in solution. Solution:Isopropanol is a better organic solvent is used in precipitation than ethanol.

Anticipated Results

In this protocol, the quality and quantity of DNA is generally good. The purity value of DNA samples ranges from >1.8 and is suitable for polymerase chain reaction . The amount of DNA ranges from 50-150ng from a single mosquito.The protocol was proved for mosquito species like Anopheles, Culex and Aedes using a pair of D3 primers\(D3 forward 5'-GACCCGTCTTGAAACACGGA-3'and D3 reverse 5' TCGGAAGGAACCAGCTACTA-3') in the 28s rDNA region 7 . The PCR conditions were 1X PCR buffer ,2mM MgCl2,500mM dNTP ,1mM of each primer\(20pico mole) and 1 unit of Taq DNA polymerase per 10 µl reaction volume. \(MP biomedicals).The DNA template 1 µl was used for this pcr reaction mix. The reaction conditions were 94º celcius for 5 minutes before 35 cycles of amplification,94ºcelcius 30 sec,50º celcius for 45 sec and 72 º celcius for 1 minute, followed by a final extension 8 minutes. The 10 microliter PCR amplification product was subjected to electrophoresis in a 1% agarose\(Bangalore genei) stained with ethidium bromide in 1X TBE buffer

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