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Optimizing Phenol-Chloroform Extraction Method for Human Papilloma Virus (HPV) Genomic DNA Isolation from Fresh Cervical Tissue

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Abstract

Our aim was to optimize a cost effective, efficient protocol for genomic DNA extraction of HPV from exfoliated cells of cervix. The final goal was to get a high quality and quantity DNA yield. The DNA obtained was confirmed by horizontal gel assembly and visualizing the same by Gel Doc System. The optimized protocol for genomic DNA extraction of HPV is good for laboratory with low funds or budget.

Keywords: DNA; Extraction; HPV; Cervical Cells.

Introduction

Extraction of DNA is a routine procedure in many biological studies including molecular identification, phylogenetic inference, genetics and genomics. This is used in medical examinations, clinical diagnostics as well in forensic investigations. So there are many methods indentified for to isolate the DNA from biological samples and many expensive DNA extraction kits are also available in the market. In order to get better PCR results, the quality and the quantity of DNA extracted from cell suspension should be high. Different DNA extraction methods have various effects on DNA. An ideal extraction technique should optimize DNA yield, minimize DNA degradation and be efficient in terms of cost, time, labor and supplies. It should be suitable for extraction of multiple samples and results into production of less hazardous waste (Harith AK et al 2016 and Chen H et al 2010).

DNA can be isolated from the variety of human sample sources like whole blood, bloodstains, hairs, urine, tissue samples and buccal epithelial tissues. Teeth were removed from the two individuals who died in the first human plague pandemic to sequence and analyze draft genomes of Yersinia pestis. The DNA was isolated from the teeth using a modified phenol-chloroform method. It was observed that the Y pestis was emerged from rodents (Wagner DM et al 2014). DNA was extracted from the unstained bone marrow smear slides by phenol chloroform method with 74% extraction efficiency (Gari MA et al 2006). DNA samples isolated from blood on filter-paper resulted in the detection and identification of Plasmodium in 165 (44.7%) of the 369 individuals evaluated, while only 62 (16.8%) had positive results using DNA obtained from thick smears, a similar rate observed by the microscopic examination (Kezia KG Scope et al 2004). A rapid, cost-effective and noninvasive method of sample collection and simple DNA extraction was done from buccal swabs, urine and hair using the phenol-chloroform method. The purity and the concentration of the extracted DNA were obtained spectrophotometerically (Ghatak S eta al 2013).

Aim of our Study

Extraction of high quality of HPV DNA with high quantity yield.

Material Methods

The study was approved by the ethical committee of MGIMS, Sevagram. The cervical smear samples

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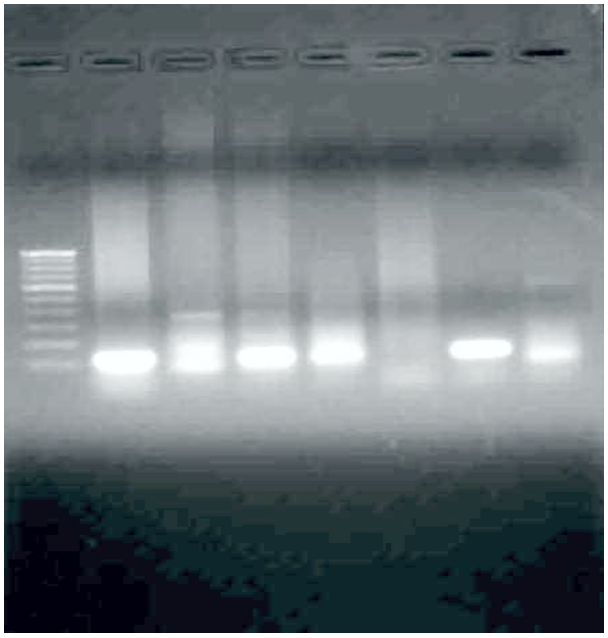
were collected in centrifuge tubes with PBS (phosphate buffer solution) by the trained Gynecologist after the written consent for the DNA extraction and PCR analysis. The exfoliated epithelial cells were the target of DNA extraction from the cervical smears.

The procedure of HPV DNA extraction from cervical smears after modification to achieve our desirable goal was as follows:

1. In the morning hours on the day of DNA extraction, cervical sample tubes which were stored in deep freezer were kept at room temperature to thaw them.
2. Start cooling centrifuge with temperature set at 4°C. Centrifuge the sample tubes at 3000 rpm for 10 minutes.
3. Decant the tubes and add 400 ul of Lysis buffer (3% SDS in 2 X TE, PH 8.1 + 100 X TE+ 10 % SDS + DW). Mix the cell pallet well. Remove the whole solution in the eppendorf's tubes.
4. Add 20 ul of Proteinase K. Put the tubes with sample in the incubator at 37°C for overnight. Shake the tubes 1 hourly for 3 times.
5. Next day take out the phenol out of the refrigerator at room temperature. Start cooling centrifuge at 4°C. Add 600 ul of phenol and shake for 10 minutes.
6. Centrifuge at 4500 rpm at 4°C for 10 minutes. Take supernatant in fresh eppendorf's tubes.
7. Add 600 ul of phenol + Chloroform + Isoamyl alcohol and shake vigorously for 10 minutes. Centrifuge at 4500 rpm at 4°C for 10 minutes. Take supernatant in fresh eppendorf's tubes.
8. Add 600 ul of Chloroform + Isoamyl alcohol and shake vigorously for 10 minutes. Centrifuge at 4500 rpm at 4 °C for 10 minutes. Take supernatant in fresh eppendorf's tubes.
9. Add 6 drops of Sodium acetate and equal volume of chilled alcohol. Shake invertly for 10 minutes. Observe the DNA cotton wool. Centrifuge at 10000 rpm at 4°C for 10 minutes.
10. Discard the supernatant. Add 70% alcohol in DNA pallet up to neck of the tube. Mix it properly. Centrifuge at 10000 rpm at 4°C for 10 minutes.
11. Remove the supernatant without disturbing the DNA pallet and dry alcohol trace in the vials by putting the tubes in the hot air oven at 70 °C for 30 minutes. After drying put the tubes at 4 °C. Next day morning add 100 ul of tris EDTA to get the DNA solution. Put the tubes in hot water bath for to dissolve the DNA pallet in the solution. After this the PCR was performed to quantify the DNA. Confirmation of DNA was done by running the sample on pre-stained agarose gel with ethidium bromide in a technique called horizontal agarose gel electrophoresis and subsequently image was visualized by using Gel Doc System.

Results

The desired goal of every molecular genetic analysis laboratory is successful extraction of DNA from the tissue sample without compromising the quality and quantity of DNA. The quantity of the chemical added and time allotted for each process which every laboratory has to standardize will definitely affect the overall yield of the DNA. We successfully isolated the HPV DNA and the quality of the DNA was also very good. The bands of HPV DNA were confirmed with horizontal electrophoresis. We have observed clear DNA bands which indicated that very precisely we standardized the protocol for HPV DNA extraction from cervical exfoliated cells. So this cost effective phenol-chloroform method of DNA extraction is very good for projects with low funds in rural settings.



Discussion

The causative role of different types of Human Papilloma Viruses in the development cancer of cervix of uterus (CaCx) is well established. Worldwide,

Carcinoma cervix is one of the most common malignancy affecting women, in terms of both incidence and mortality rates (Das BC et al 2000, Sankaranarayanan et al 2003, Kahesa et al 2008). Eighty percent of cases occur in developing countries where it is the leading cause of Cancer-related deaths among women (Winkler et al 2008). In India, about 100,000 women develop this cancer every year constituting about 16% of the world's annual incidence (Das BC et al 2000). It accounts for about 70000 deaths annually in India. Thus the screening programmes for the detection and diagnosis of premalignant and malignant lesions of the cervix is effective in decreasing the incidence and mortality from Cervical Cancer (Miller D 2003). To confirm these screened HPV positive cases, it requires PCR analysis, for which HPV DNA has to be extracted with high quality and quantity yield. So we can conclude that for the laboratory with fewer funds, the phenol chloroform method of HPV DNA extraction from cervical samples is an ideal method.

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