The Comparison of DNA Quantity between Full and Half Volume Single Cell Whole Genome Amplification by Linker-Adapter PCR Technique

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Background: *Whole genome amplification (WGA) is a very important step in providing sufficient DNA templates for many high-throughput genetic analyzes. WGA approaches can be subdivided into PCR- or non-PCR-based methods. The PCR amplification category includes PEP-PCR, DOP-PCR and linker-adapter PCR, but only the linker-adapter PCR is suitable for application in preimplantation genetic diagnostic screening because it provides the necessary rapid turnaround time.*
Objective: *Evaluate the ability of linker-adapter WGA commercial kits by using half volume compare with full volume of the reagent amplified DNA extracted from single cell fibroblast.*
Material and Method: *Single cell fibroblast was used based on known genetic profiles. The authors reduced the volume of the reagent and compared the DNA yields and fragmented DNA products with yields and products using the original protocol.*
Results: *Our result did not show a significant difference between the amount of DNA products between full and half volume method (4.72 vs. 4.89 μg, p-value = 0.56). We achieve a slightly different of fragmented length of WGA products, full volume of reagent received slightly longer length than half volume (502.83 vs. 478.30 bp, p-value = 0.19).*
Conclusion: *In this study, we have shown that the half volume of the reagent of linker-adapter WGA method amplified DNA extracted from single cell fibroblast was comparable DNA yield and DNA fragmented length with the original method. We need further study extrapolate to evaluate the outcome.*

Keywords: *Whole genome amplification, Linker-adapter PCR, DNA yields*

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Preimplantation genetic diagnosis (PGD) is the aim to select normal oocytes or embryos for eliminating the chance of abnormal pregnancy by chromosomal aberrations. PGD can be offered to patients with a high risk of chromosome abnormality and monogenic diseases. A variety of methods may be chosen for testing, such as fluorescence in situ hybridization (FISH), polymerase chain reaction (PCR), and comparative genomic hybridization (CGH). There have been dramatic changes in the method of diagnosing small numbers of cells for PGD. In the PGD field, they are often limited amount of DNA sample available. Samples contain only one cell or 5 to 10 cells depending on the stage of embryo biopsy. Now, array-CGH and SNP arrays have been introduced as a new innovation for detecting chromosome aberrations, both will result in an increase in delivery rates, is a more accurate, reliable and faster method than the older one. As the number of high-throughput genetic analysis was developed, it became even more important to have adequate quantity and quality of DNA template. Standard PCR methods were difficult to obtain this goal. Therefore, whole genome amplification (WGA) was developed to overcome this limitation. The ability of WGA to increase the amount of starting material, and/or the quality of the template would be invaluable for PGD applications, provided the product is representative of the original template[1].

WGA amplify small amount of whole DNA template into an ample amount, thus providing

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sufficient DNA templates for many high-throughput genetic analysis. The technology enables amplification of picogram amounts of DNA into microgram quantities. WGA is different from the conventional PCR technique. Conventional PCR is performed for one specific genomic sequence of interest, while WGA technique aim to non-selectively amplify the genome DNA for further genetic analysis. WGA methods has been successfully utilized in several clinical applications, such as PGD, PND, microbial studies, and forensics. In addition, several WGA method has also been integrated with other genotyping technologies such as DNA microarrays and SNP GeneChip platforms[2-4].

Over the last years different types of WGA methods, utilizing a variety of mechanisms that are PCR or non-PCR-based, have been developed and established for genetic investigations from a limited number of cells or small quantities of DNA[5]. Most WGA methods used random primers and low stringency annealing conditions. Currently, there are several WGA methods, although some of the early methods of WGA, such as Primer Extension Preamplification PCR (PEP-PCR), Degenerate Oligonucleotide-primed PCR (DOP-PCR) generate nonspecific amplification artifacts, incomplete genome coverage, due to amplification bias of PCR reactions over certain loci and generate short fragmented DNA (less than 1 kb) that cannot be used in many applications[6-8].

There are several commercial WGA kits, and the authors investigated one of the commercial WGA kits to evaluate its ability to amplify small amounts of DNA sample from single cell fibroblast by using only half the volume of reagent compared with the standard full volume. GenomePlex WGA (Sigma, St. Louis, MO, USA) is an adapter ligation-based PCR method, introduced to markets in 2002, involving randomly digesting the DNA template into a fragmented library of DNA molecules, followed by the ligation of adapters to each fragment and PCR with universal primers. The fragment library can then be amplified several 1,000-fold to generate milligram quantities of DNA starting with as little as 10-100 ng[9,10]. GenomePlex is able to generate amplified DNA products sufficient in yield (4-8 μg) to perform several molecular procedures.

The focus of this study was to evaluate the ability of linker-adapter WGA commercial kits by using half the volume compared with the full volume of reagent amplified DNA extracted from single cell fibroblast. The purpose for reducing the volume of reagent is to observe if the result will be compatible to the regular volume in order to save expenses.

Material and Method

This study was experimental designed to evaluate DNA quantity from different volume of linker-adapter commercially WGA methods on single cell fibroblast.

DNA samples and single cell isolation

Fibroblast cell samples were used based on known genetic profiles. The karyotype of cell line was 47,XX,+21 cell line (GM02767) from Coriell Cell Repository (Camden, NJ, USA). Cells were cultured in 1x Minimum Essential Medium (Gibco, Carlsbad, CA, USA) with 15% Fetal Bovine Serum (Invitrogen Corp., Carlsbad, CA, USA), 1% Non-Essential Amino Acid and 1% Penicillin-Streptomycin-Glutamine (Gibco, Carlsbad, CA, USA) at 37°C and 5% CO_2. Single cells were isolated following treatment with 0.05% trypsin/EDTA (Gibco, Carlsbad, CA, USA) to detach the adherent fibroblast cultures as recommended. Single cell fibroblast was picked up in 2.5 μl 1x PBS using clean tip pipette under a dissecting microscope and place in the bottom of a 0.2 ml PCR tube (Eppendorf, Hamburg, Germany). Sixty cells were picked up and divided into 30 single cells per group. Individual cells were collected in a separate tube. One μl of PBS was removed to serve as negative controls for each WGA method.

Whole genome amplification: GenomePlex Single cell lysis and fragmentation

GenomePlex (Rubicon Genomics, Inc., Ann Arbor, MI) WGA was performed according to the manufacturer's instructions. Single cells in 1 μl of culture media were loaded into 0.2 ml PCR tubes containing 2.5 μl PBS buffer followed by the freshly prepared lysis then fragmentation buffer/proteinase K solution 0.5 μl were added, mix thoroughly and then incubated at 50°C for 60 minutes, and then heated to 99°C for four minutes, it is time sensitive. The sample cooled on ice and spin down before proceeding to library preparation.

Library preparation

GenomePlex libraries were created by adding 1 μl of library preparation buffer and 0.5 μl of library stabilization solution. Tubes were mixed thoroughly for 30 seconds and centrifuged briefly, then incubated in GeneAmp PCR System 9700 (Life Technologies

corporation, NY, USA) at 95°C for two minutes. When incubation time was done, samples were removed from PCR system and placed instantly on ice. Then 0.5 µl library preparation enzyme was added. The tubes were reinserted in PCR system and incubated at 16°C for 20 minutes, 24°C for 20 minutes, 37°C for 20 minutes, and 75°C for 5 minutes, and finally 4°C hold.

PCR amplification

Amplification reactions were performed as follows: 3.75 µl of 10x amplification master mix, 24.25 µl of nuclease free water and 2.5 µl WGA DNA polymerase are added to 7 µl library mix. Samples were amplified using an initial denaturation of 95°C for three minutes followed by 25 cycles each consisting of a denaturation step at 94°C for 30 seconds and an annealing/extension step at 65°C for five minutes and final hold step at 4°C. Positive controls with 100 pg of standard human genomic DNA and 1 µl of media serve as negative controls, both of them were performed with each set of reactions. PCR products were run in 2% agarose gel. The amplified DNA product was stored at -20°C until ready for analysis or purification.

Purification of amplified products

Amplified DNA product from each WGA methods described above was purified with the PCR Cleanup Kit (QIAGEN, Hilden, Germany) as described in the manufacturer's instructions.

Quantitation of WGA products

The initial assessment of WGA amplified DNA was done by 5 µl of WGA DNA products, positive and negative controls were mixed with 1 µl of loading dye and loaded to 2% agarose gel in 1x TBE buffer and stained with ethidium bromide. A 50-bp ladder is loaded as a reference for DNA smear size. Ideal amplified DNA product shows as a smear that ranged in size between 100 and 1,000 bp (Fig. 1).

The concentration of purified WGA DNA was quantitated using 2 methods: spectrophotometry and high sensitivity DNA analyzer. Spectrophotometric quantitation was performed with a Nanodrop 2000 spectrophotometer (ThermoScientific, Wilmington, DE) at 260, 280, and 230 nm wavelengths. High 260/280 and 260/230 ratios obtained in the range of 1.8 to 2 indicate high purity of the sample. High sensitivity DNA analyzer was performed with Agilent 2100 Bioanalyzer (Agilent Technologies, Inc., Santa Clara, CA), using 2 µl of purified WGA products.

Successful WGA was defined as a single cell sample that yielded greater than the required input WGA DNA amount for microarray based analysis (200 ng). For each method, the reliability was defined as the percentage of sample that met this definition.

The mean and standard deviation (SD) or median and range were used to describe continuous data. Results were analyzed using computer program STATA version 12. Data with normal distribution were analyzed by paired Student's t-test. Data without normal distribution were analyzed by sign test. A p-value of less than 0.05 was considered as statistical significant.

Results

A commercially available WGA kits, GenomePlex was investigated for use on single cell in the different volume of reagent. Single cell fibroblast was sampled and divided into 30 cells for each group.

DNA yield and quality

The extracted DNA products from 60 single cells fibroblast was subjected to amplify by the same methods (GenomePlex) but at different volumes: full and half volume. Thirty cells were provided for each method. The WGA products were analyzed on a 2% agarose gel. In the aspect of amplification success rate: for the full volume, we detected bands for 29 of the 30 samples (96.67%) when electrophoresed on an ethidium bromide stained agarose gel, while 28 of the 30 samples (93.33%) for the half volume. Interesting, half volume GenomePlex WGA from single cell

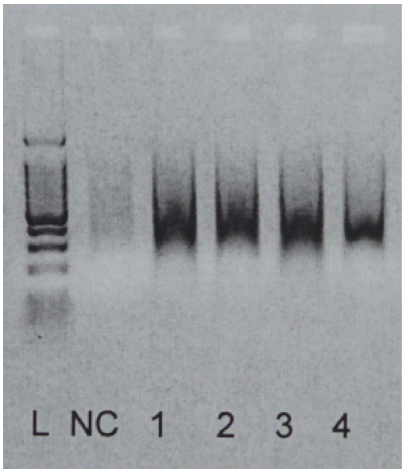

Fig. 1 Electrophoresis gel shown average of amplified DNA products from GenomePlex in size between 100-1,000 bp (L = ladder; NC = negative control).

fibroblast failed more than the full volume, but it was a slightly difference. All of the positive controls were amplified successfully and none of the negative controls were amplified. In addition, the authors were unable to detect any PCR product larger than 800 bp using either method. However, the band intensities varied considerably between the two methods, and among samples. Fig. 2 shows electrophoresis band of GenomePlex full volume and Fig. 3 shows band of the half volume. Half volume PCR bands were weaker than full volume. The most intensive PCR bands were generated with full volume GenomePlex products.

WGA products yields varied from 3.2 to 8.3 µg. The average results of full and half volume GenomePlex were 4.72 µg, 4.89 µg respectively. It was not significantly different between both methods (p-value = 0.56). In addition, the amplification yield is in agreement with the manufacturer's declarations (Table 1).

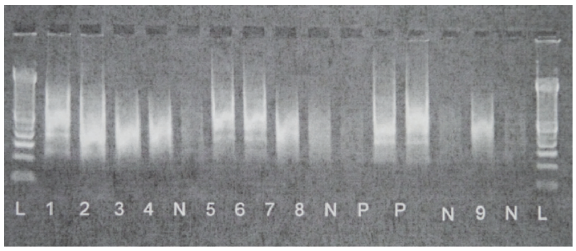

Fig. 2 Electrophoresis band of full volume GenomePlex (L = ladder; N = negative control; P = positive control).

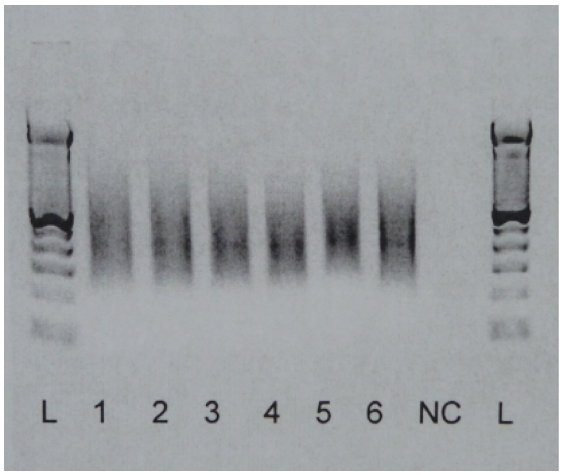

Fig. 3 Electrophoresis band of half volume GenomePlex (L = ladder; NC = negative control).

DNA product length

The average lengths of half volume products were 478.30 bp, whereas the full volume were on average 502.83 bp. Full volume GenomePlex produced WGA DNA that was equivalent in DNA length to that of the half volume GenomePlex (p-value = 0.19). GenomePlex WGA DNA product size ranged from 100 to 1,000 bp (Table 2).

Discussion

Following the development of high-through put instrumentation, it became even more important to have sufficient template DNA. The source of DNA is often limited, especially in PGD studies, which require good quality samples as well as enough DNA to perform numerous downstream procedures. Therefore, WGA method developed to solve the problem of stock DNA limitation. WGA is capable of increasing both the quality and quantity of DNA, and has the potential to improve profiling success from difficult samples in forensic casework[11]. A variety of WGA amplification kits were launched in the market, but GenomePlex have the advantage for handling single cell in PGD field

Table 1. Comparison of DNA yields between full and half volume of GenomePlex

GenomePlex	Average yield (SD) (µg)*	Yield range (µg)*	p-value
Full volume (29 cells)	4.72 (1.12)	3.20-8.30	0.56
Half volume (28 cells)	4.89 (1.20)	3.00-7.10	

* Quantified using high sensitivity DNA analyzer (agilent 2100) as per the manufacturer's recommendations

Table 2. Comparison of fragmented DNA products between full and half volume of GenomePlex

GenomePlex	Average DNA concentration (SD) (bp)	Fragmented DNA size (min-max) (bp)	p-value
Full volume	502.83 (93.31)	358-615	0.19
Half volume	478.30 (73.76)	366-741	

because it is designed to work with small quantity of samples and has a fast turnaround time (<5 hours). In addition, it is a significantly better genome coverage, lower amplification bias and drop-out rate when compared with the other methods of WGA. The important point was the primary amplification products were stable and stored at 4°C (up to a minimum of 6 months) without change.

Here, the authors evaluated the ability of linker-adapter PCR WGA method (GenomePlex Single Cell Whole Genome Amplification kit, Sigma-Aldrich, UK) amplified single cell fibroblasts by using half volume of reagent compared with full volume. Based on the study the authors found the protocol for the GenomePlex system is adjustable in reagent volumes, and we observed that it is possible to increase product yield using this system by increasing the concentration of polymerase[11,12]. This protocol will not only adjust the concentration of polymerase but also reduce all of the reagent volume. Product quality and quantity were determined by an agarose gel electrophoresis and high sensitivity DNA analyzer. Negative and positive controls should always be included. Since this is a method that is highly sensitive to DNA contamination, inclusion of a negative control is a must to ensure contaminant free WGA amplified products. Otherwise, if there was any trace of DNA in the negative control apparent from gel electrophoresis imaging, the experiment is considered unsuccessful, even though target samples were amplified and displayed a white smear on gel. However, the negative control sample in this present study did not show any background amplification.

Each of the GenomePlex WGA systems tested on DNA extracted from single cell fibroblasts successfully amplified DNA from picograms of starting material to micrograms of product. The percentage of success rate of amplification was slightly higher in full volume than half volume of reagent but it is not significantly different. Results from half volume of GenomePlex reagent used were satisfying because of the high rate of successfully amplification for enough products that will be process for downstream analysis. We found that the protocol for the GenomePlex system is adjustable in reagent volumes, and the authors observed that it is possible to increase product yield using this system by increasing the concentration of polymerase[11,12]. Fiegler et al (2007) applied the GenomePlex single cell kit which is based on linker adapter PCR to single cells from tumor cell lines as well as lymphocytes and were able to detect copy number changes as small as 8.3 Mb[2]. The average DNA yield varied from 3.0 to 8.3 µg. Our results did not show a significant difference between the amount of DNA products between full and half volume method (p-value = 0.56). It is explained by the basic of PCR technique when we used a very small volume of reagent it will make the reaction more precise. This study reduced all of the reagent in the kit proportionally that made better results. In addition, DNA yield achieved following manufacturer's suggestion.

In the aspect of the fragmented length of WGA products, we achieved a slightly different result, full volume of reagent received slightly longer length of products than half volume. GenomePlex fragments tended to be less than 800 bp. The average fragmented size of full volume reagent was 502.83 bp while half volume of reagent was 478.30 bp. Increased amounts of starting DNA template did not appear to result in longer length fragments after amplification[12].

The merit was following this aspect, it is a first study compared between half and full volume of reagent in linker-adapter PCR technique leads to reduced costs.

The limitation was the intermediate outcomes. This study measured only DNA yield and fragmented DNA size, we still need further studies for outcomes.

The application of this study was the half volume of reagent was enough in small amounts of DNA template in PGD or forensic field, it can achieve adequate amount of DNA product and reduce the cost of the reagent. In addition, the most typical PGD application requires the completion of single cell analysis within 24 hours of initiating the procedure in order to avoid embryo cryopreservation. Therefore, GenomePlex is suitable for application to PGD screening because it provides a rapid turnaround time (5 hours).

Conclusion

The present study reported the half volume reagent of GenomePlex method amplified DNA extracted from single cell fibroblast was comparable DNA yield and DNA fragmented length with full volume method. We need further studies to extrapolate and evaluate the outcome.

What is already known on this topic?

From the previous publications, it has been known that the DNA quantity from the single cell amplification with the full volume reagent are from GenomePlex.

What this study adds?

Base on the basic understanding of PCR method and knowledge from the previous study of full volume single cell whole genome amplification, the authors tried to reduce the volume of the reagent proportionally and expected the same outcome.

Potential conflicts of interest

None.

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การศึกษาเปรียบเทียบปริมาณ DNA ระหว่างการทำ whole genome amplification ในเซลล์เดี่ยวด้วยวิธี linker-adapter PCR technique โดยใช้ปริมาตรปกติและปริมาตรครึ่งหนึ่ง

มัธชุพร สุขประเสริฐ, ศศิวิมล รัตนศิริ, Kungpu Xu

ภูมิหลัง: *Whole genome amplification เป็นกระบวนการสำคัญในการเพิ่มปริมาณ DNA ให้เพียงพอสำหรับนำมาใช้ในการวิเคราะห์ทางพันธุกรรมขั้นสูง WGA สามารถแบ่งออกเป็นวิธีที่ใช้ PCR และไม่ใช้ PCR เข้ามาเกี่ยวข้อง โดยวิธีที่ใช้ PCR ในการเพิ่มจำนวนประกอบด้วย PEP-PCR, DOP-PCR และ linker-adapter PCR แต่พบว่ามีเพียงเทคนิค linker-adapter ที่เหมาะสมในการนำมาใช้เพื่อตรวจวิเคราะห์พันธุกรรมของตัวอ่อนก่อนการฝังตัว เนื่องจากเป็นวิธีที่ได้ผลรวดเร็วที่สุด*

วัตถุประสงค์: *เพื่อศึกษาความสามารถของ linker-adapter WGA ในการเพิ่มปริมาณ DNA ของเซลล์เดี่ยว fibroblast โดยเปรียบเทียบระหว่างการลดปริมาตรสารที่ใช้เป็นตัวทำปฏิกิริยาลงครึ่งหนึ่งกับปริมาตรปกติ*

วัสดุและวิธีการ: *ผู้นิพนธ์ลดปริมาตรสารที่ใช้เป็นตัวทำปฏิกิริยาลงครึ่งหนึ่งในกระบวนการ WGA โดยเปรียบเทียบปริมาณ DNA และความยาวของชิ้นส่วน DNA กับวิธีที่ใช้ตัวทำปฏิกิริยาที่ปริมาตรปกติ โดยทำในเซลล์เดี่ยว fibroblast ที่ทราบความผิดปกติทางพันธุกรรม*

ผลการศึกษา: *ไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติของปริมาณ DNA ระหว่างการใช้สารทำปฏิกิริยาลดปริมาตรลงครึ่งหนึ่งเทียบกับปริมาตรปกติ (4.72 vs. 4.89 μg, p-value = 0.56) และพบว่าเมื่อใช้สารทำปฏิกิริยาปริมาตรปกติได้ชิ้นส่วนของ DNA ที่ยาวกว่าการใช้สารทำปฏิกิริยาครึ่งหนึ่งเล็กน้อยไม่มีนัยสำคัญทางสถิติ (502.83 vs. 478.30 bp, p-value = 0.19)*

สรุป: *การศึกษานี้แสดงให้เห็นว่าการลดปริมาตรสารที่ใช้ทำปฏิกิริยาลงครึ่งหนึ่งในกระบวนการ linker-adapter WGA ได้ปริมาณ DNA และความยาวของชิ้นส่วน DNA เท่ากับการใช้สารทำปฏิกิริยาปริมาตรปกติ แต่ทั้งนี้ยังต้องการการศึกษาเพิ่มเติมเพื่อดูถึงผลลัพธ์สุดท้าย*