Isolation of DNA from 0.1 – 3 million HeLa Cells using Norgen's Cell and Tissue Genomic DNA Isolation Kit

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INTRODUCTION

Isolation of genomic DNA from mammalian cells is essential in many research laboratories. Certain cell samples may contain a lower cell number than others, and therefore require an isolation method that is able to obtain genomic DNA from a wide range of cell concentrations. It is important that the isolation kit being used have the ability to extract DNA of the same caliber from both low and high concentrations of cells.

The purpose of this study is to isolate cellular genomic DNA from mammalian cell suspensions with inputs of 0.1, 0.25, 0.5, 1, 1.5, 2 and 3 million cells using Norgen's Cells and Tissue Genomic DNA Isolation Kit (Cat# 53100).

MATERIALS AND METHODS

Cellular DNA extraction

DNA was extracted from cell growth media suspensions using Norgen's Cells and Tissue Genomic DNA Isolation Kit (Cat# 53100), as per the manufacturer's instruction. Cell inputs of 0.1, 0.25, 0.5, 1, 1.5, 2 and 3 million HeLa cells were pelleted by centrifuging at 2000 rpm for 10 minutes. One hundred µL of PBS was added to the pellet and vortexed thoroughly in order to resuspend the pellet. Next, Proteinase K was added to the PBS suspension, followed by the addition of 300 µL Lysis Solution. The samples were then vortexed and incubated at 55ºC for 15 minutes. Following this, 10 µL RNAse A was added to each sample and vortexed. The samples were then incubated at 37ºC for 15 minutes. Next, 250 µL ethanol was added to each sample and samples were bound, washed and eluted as per the manufacturer's protocol.

Spectrophotometry

DNA quantity was measured using the UltraSpec 2100 Pro (Fisher Scientific). Twenty-five µL of each DNA elution was diluted with 475 µL of nuclease-free water, and OD measurements were taken using the cuvette-based spectrophotometry method.

Real-Time PCR

The purified DNA was then used as the template in a real-time TaqMan® PCR reaction. Briefly, 2 µL of isolated DNA was added to 20 µL of real-time PCR reaction mixture containing 10 µL of Norgen's 2X PCR Mastermix (Cat# 28007), 0.4 µL of 25 µM GAPDH primer pair mix and 0.2 µL of the TaqMan® probe. The volume was brought up to 20 µL using nuclease-free water. The PCR samples were amplified under the real-time program; 95°C for 3 minutes for an initial denaturation, 40 cycles of 95°C for 15 seconds for denaturation, 60°C for annealing and extension. The reaction was run on an iCycler iQ Realtime System (Bio-Rad).

RESULTS AND DISCUSSION

In this study, DNA was isolated from cell growth media suspensions using Norgen's Cells and Tissue Genomic DNA Isolation Kit. Fifteen microliters of each 200 µL elution was then run on a 1X TAE 1.0% agarose gel to visually inspect the isolated genomic DNA **(Figure 1)**. Isolated DNA shows high integrity of greater than 24kbp with increasing yield correlating with increasing cell number.

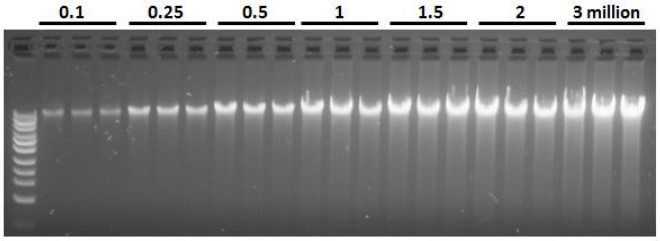

Figure 1. Resolution of DNA isolated from 0.1-3 million HeLa cells. DNA was isolated using Norgen's Cells and Tissue Genomic DNA Isolation Kit**.** Fifteen microliters of 200 µL elutions were run on 1X TAE 1.0% agarose gel. The used marker is Norgen's UltraRanger DNA Ladder.

In order to determine the yield of the isolated DNA, samples were measured using a cuvette-based spectrophotometry method (UltraSpec 2100 Pro; Fisher Scientific) **(Figure 2).** The DNA yield correlates with the input number of cells; a higher number of cells results in a higher DNA yield.

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DNA quality was determined through the use of a TaqMan® Real-Time PCR method. Two µL of each sample was used in the reaction, and the Ct values were then graphed **(Figure 3)**. The number of cells and the Ct value were found to display an inverse relationship, indicating the increased concentration with increasing cell input. The linearity in Ct decrease indicates that the eluted DNA from all cell inputs is free of PCR inhibitors.

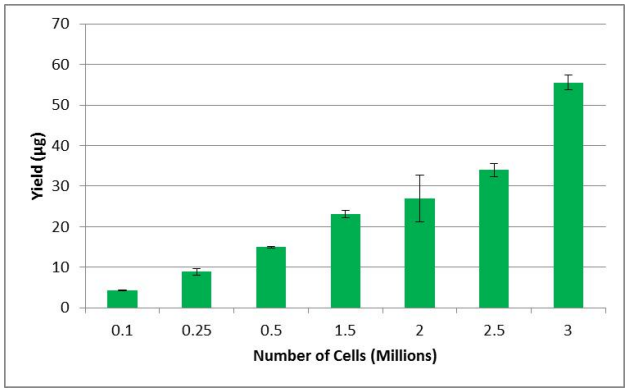

Figure 2. Yield of DNA isolated from 0.1-3 million HeLa cells. DNA was isolated using Norgen's Cells and Tissue Genomic DNA Isolation Kit**.** Twenty-five microliters of the 200 µL samples were diluted in 475 µL of nuclease-free water, and DNA concentrations were measured using the UltraSpec 2100 Pro (Fisher Scientific).

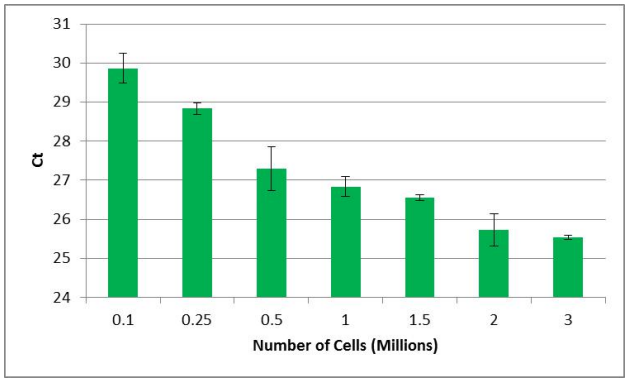

Figure 3. The difference in Ct values obtained from a Taqman® qPCR reaction performed on DNA isolated from 0.1-3 million HeLa cells. DNA was isolated using Norgen's Cells and Tissue Genomic DNA Isolation Kit**.** Two microliters of each elution were used in a 20 µl qPCR reaction involving GAPDH primers.

CONCLUSIONS

From the data presented in this report, the following can be concluded:

- Norgen's Cells and Tissue Genomic DNA Isolation Kit isolated high integrity DNA, free of PCR inhibition and suitable for sensitive downstream applications.
- The kit has a dynamic range of 0.1 million to 3 million mammalian cells.

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