

A simple filtration technique for obtaining purified human chromosomes in suspension

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Here we present a simple method for cleaning polyamine human mitotic chromosomes in solution. This was achieved by filtering intact (unburst) nuclei along with both large and small cytoplasmic debris through a series of different pore sized filters. Pure human chromosomes were recovered using a simple reverse filtration step. Fluorescence microscopy was used to validate the chromosome suspension after each filtration step. This reverse filtration technique is an improvement in both procedure time and chromosome recovery compared to currently used post-purification methods. Chromosomes purified by our method could be used for many applications, such as structural studies using microfluidics and high resolution imaging or generation of chromosome paints and sequencing after flow cytometry.

Human polyamine chromosomes retain both their proteins and nucleic acid intact when purified by standard procedures (1,2). These isolated chromosomes in solution are widely used—after sorting by flow cytometry—for a number of important applications, including physical mapping, generating libraries, developing DNA markers, and chromosome painting (3). They have also been used in chromosome isolation studies where streptavidin magnetic beads capture

selected biotin-labeled chromosomes in solution (4). Chromosomes isolated in this manner are not suitable for investigating chromosome structure because the procedure involves a protein denaturation step; however, they could be used for DNA-based studies. Polyamine chromosomes have been used in a microfluidic device from which individual DNA fibers were isolated and sequenced (5). As the polyamine suspension preserves the structure of chromosomes (1), imaging

studies using microfluidics (6), scanning electron microscopy (SEM) (7), and 3-D X-ray CDI diffraction (8) have been used to investigate the structure of human chromosomes. Hundreds of chromosomal proteins have also been isolated from polyamine chromosome preparations and studied using gel electrophoresis (9).

The success of the above applications depends on the quality of the isolated chromosomes used as starting material. The polyamine method involves bursting chromosomes out of the nucleus by vigorous vortexing (2) of nuclei or by passing nuclei through a syringe needle. The final in-solution chromosome preparation includes chromosomes, nuclei that failed to burst, and bits of cytoplasmic debris that are both larger and smaller than the chromosomes. This can lead to various problems, as the unwanted nuclei and debris have the tendency to stick to one another and even to the chromosomes, making the preparation unsuitable for the required application.

Several post-purification methods for cleaning polyamine-prepared chromosomes have been published. These include centrifugation to remove unwanted nuclei and allow recovery of a chromosome rich fraction (1), but this method does not remove smaller debris and nuclei are present in the final suspension. Other such methods include gradient centrifugation using sucrose (10) and Percoll (Hayashihara et al., 2008; <http://dx.doi.org/10.1038/nprot.2008.166>), and separation chambers subjected to a gravitational field (11). These methods, although useful in some situations, are time-consuming, involve specialized equipment such as chambers and high-speed centrifuges, require a large amount of starting material, are costly in terms of reagents, and the recovery of chromosomes is limited.

Here, we present a simple technique for obtaining purified chromosomes. Our aim was to overcome the problem

METHOD SUMMARY

We used filters of different pore sizes to remove intact nuclei and cytoplasmic debris left over in human polyamine chromosome preparations. After reversing the final filter and eluting the chromosomes with polyamine buffer, a rich pool of chromosomes was recovered. The quality and yield of the filtered chromosome suspension was verified by fluorescence microscopy. This new, simple, and robust post purification method allows recovery of native human chromosomes in less than 20 minutes.

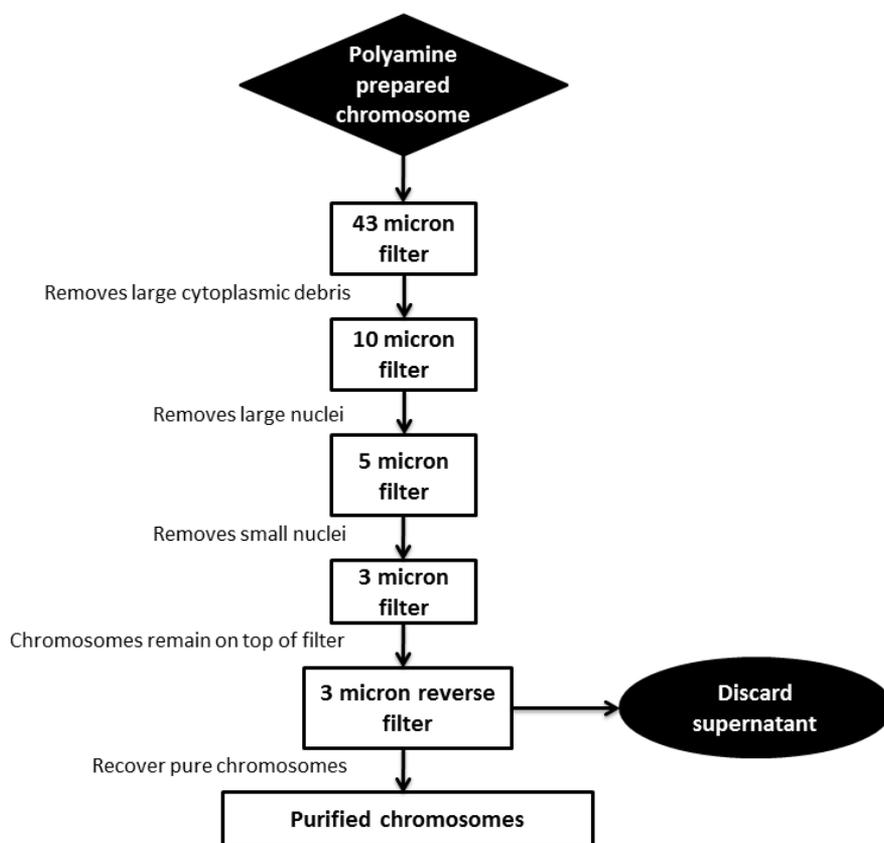


Figure 1. Procedure for chromosome cleanup using the reverse filtration method.

of having unwanted debris and intact (unburst) nuclei in the final chromosome suspension by using a simple and fast filtration technique (Supplementary Protocol). Polyamine chromosomes were prepared according to published protocols (1,2). Figure 1 shows a flowchart of the entire filtration procedure. Dropping the suspension onto glass slides, staining with SYBR Gold, and imaging using a fluorescence microscope allowed the presence of chromosomes, nuclei, and debris to be assessed (Figure 2A). SYBR Gold stain does not stain proteins or lipids but instead binds to the single- and double-stranded RNA/DNA contained in chromosomes and nuclei (12) (Supplementary Figure S1, A and B). These chromosomes were filtered through several filters of different pore sizes according to the manufacturer's (Catalog # NY4102500, NY41002500, SVLP01300, and TSTP01300; Millipore, Watford, UK) instructions. The first step involved using a syringe to pass the suspension through a 41-micron filter

into a tube. The recovered suspension was further filtered through a 10-micron filter. This step removed any nuclei that were larger than 10 microns (Figure 2B). Nuclei larger than 5 microns but less than 10 microns were removed by filtering the recovered suspension through a 5-micron filter (Figure 2C). Even though larger unwanted debris and nuclei were removed, the suspension still contained smaller debris. The smaller debris in the suspension is likely to be nuclear matter that remained in the sample after bursting of the nuclei during the preparation procedure. The suspension recovered from the 5-micron filtration step was passed through a 3-micron filter. This allowed any material above 3 microns to be retained on the filter, in this case the chromosomes. Unwanted small debris that passed through the filter was discarded. Reversing the 3-micron filter and washing it with fresh polyamine buffer allowed a rich suspension of chromosomes to be recovered. The recovered solution was imaged by fluorescence microscopy

(Figure 2D) and was greatly enriched in chromosomes. These results have been reproduced many times and the method has proven to be quick and reliable.

The morphology of chromosomes in the starting preparation of polyamine chromosomes (Figure 3A) was compared with chromosomes recovered after further purification by centrifugation (Figure 3B), 40% sucrose gradient centrifugation (Figure 3C), and our 3-micron reverse filtration method (Figure 3D). All of preparations showed the typical chromosome morphology. Characterization of chromosome morphology was made difficult due to the chromosomes being compact in polyamine buffer and imaged while wet. This made it difficult to image the smaller chromosomes, which were recovered mainly from the sucrose gradient and 3-micron reverse-filtered methods.

Chromosomes recovered by centrifugation were of various sizes. The sucrose gradient centrifugation method had a rich pool of chromosomes recovered from the 40% gradient that on average were ~2 microns in length. The 3-micron reverse-filtered chromosomes were of similar size, >3 microns and <5 microns. In some cases, we observed chromosomes less than 3 microns as these were most likely stuck on the filter before the reverse filtration step. This method was performed on human chromosomes that are larger in size compared with chromosomes from other species (e.g., chicken microchromosomes), which would be lost during the final step of filtration. Microchromosomes would also be difficult to distinguish from small debris.

The percentage yield of chromosomes recovered was 8.7% after centrifugation and 0.5% after the sucrose gradient centrifugation (in the 40% fraction). A much higher percentage yield of 36.1% was obtained after 3-micron reverse filtration (Supplementary Table S1).

This reverse filtration technique is an improvement in terms of procedure time and yield compared with the methods currently used for recovering clean chromosomes. Centrifugation removes a large number of nuclei, but nuclei and cytoplasmic debris are still present in the final suspension containing the

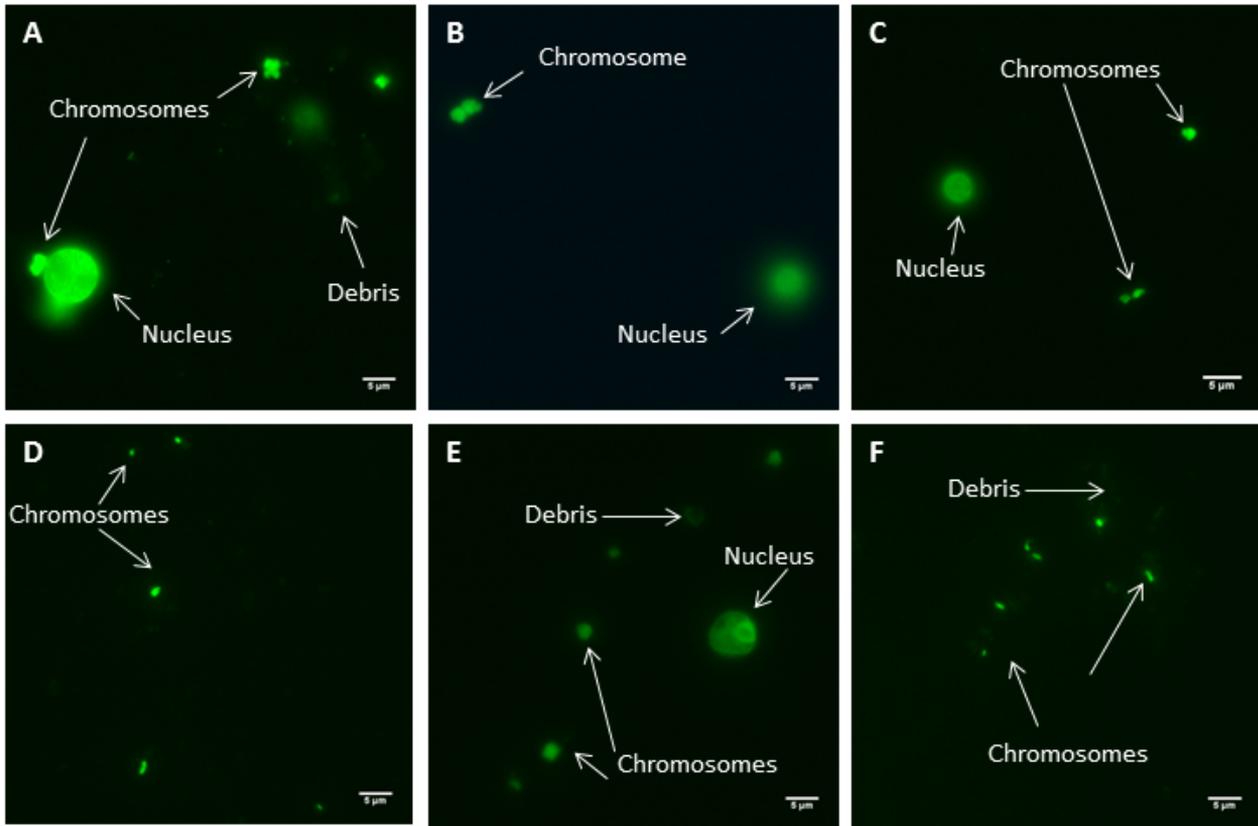


Figure 2. Chromosomes recovered after different post purification methods. (A) Polyamine chromosome starting sample showing nuclei, chromosomes, and debris. (B) After the 10-micron filter step of the filtration method, nuclei and debris remain in the suspension. (C) The 5-micron filter step of the filtration method allows chromosomes through but retains smaller nuclei. (D) After passing the sample through a 3-micron filter and a reverse filtration step, only chromosomes are seen. (E) Chromosomes recovered after the centrifugation method show nuclei and debris. (F) Chromosomes recovered after the sucrose gradient (40%) method show debris in the sample. All samples were stained with 150 μM SYBR Gold and imaged with a 63 \times objective.

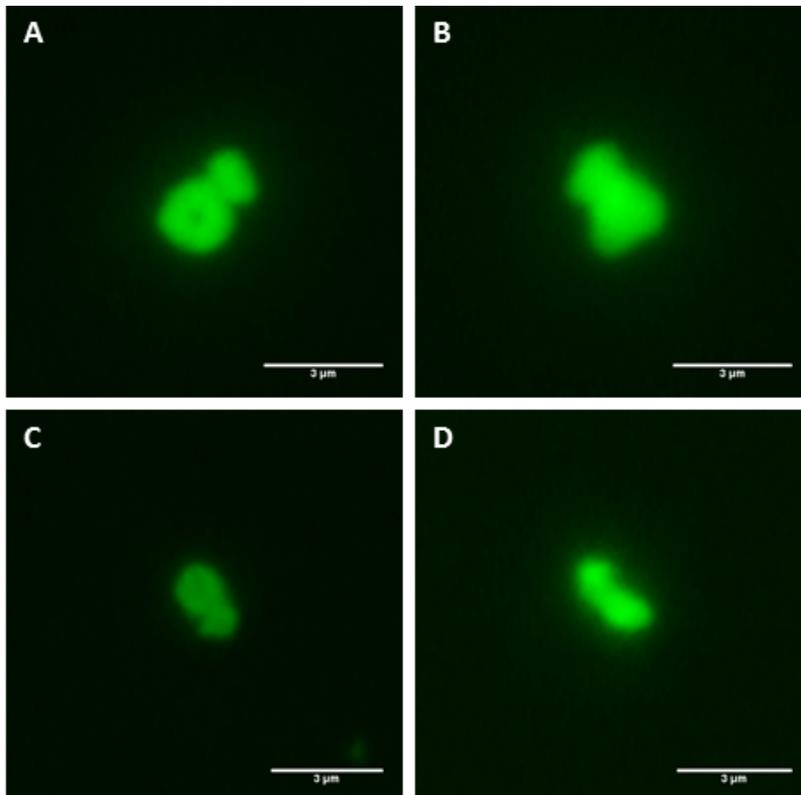


Figure 3. Chromosome morphology after different post purification procedures. Typical chromosome morphology can be seen from (A) the starting sample, (B) centrifugation method, (C) sucrose gradient (40%) centrifugation method, and (D) 3-micron reverse filtration method. All samples were stained with 150 μM SYBR Gold and imaged with a 63 \times objective. Photos are 5 \times magnifications.

chromosomes (Figure 2E). Sucrose gradient centrifugation takes much longer, has a lower yield, and retains debris (Figure 2F). By comparison, the reverse filtration method showed a significant improvement in (i) yield of chromosomes; (ii) length of the procedure, as it takes only about 20 min; and (iii) cost, as only commercially available mesh filters are used, and no specialized equipment is required.

The procedure described here provides a rapid and efficient way to isolate highly purified human chromosomes as starting material for many applications, and this rich pool of chromosomes offers exciting new possibilities. Getting rid of cellular debris would be useful for downstream flow cytometry applications, in particular those that involve subsequent manipulation of chromosomes in suspension, such as FISH or immunofluorescence. Furthermore, chromosomes isolated by this method would be beneficial for flow within microfluidic devices as larger debris and unwanted nuclei can clog the channels of the device. They would also be useful for high resolution microscopy such as X-ray imaging, as any object in the membrane window should be identified as a chromosome. Finally, they could also be used for extracting proteins from chromosomes without contamination by unwanted nuclear proteins.

Author contributions

M.Y. designed the experiment and wrote the manuscript. M.Y., N.P., and G.K.B. wrote the Supplementary Material for the manuscript and finalized figures. M.Y., N.P., G.K.B., and I.K.R. amended the manuscript. M.Y., N.P., and G.K.B. performed the experiments. M.Y. and I.K.R. supervised the study.

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Competing interests

The authors declare no competing interests.

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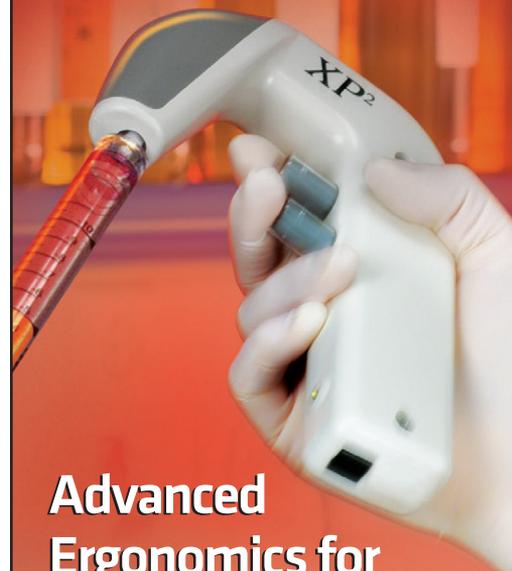
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