A device for extraction, manipulation and stretching of DNA from single human chromosomes

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  University of Valladolid, Spain

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We describe the structure and operation of a micro/nanofluidic device in which individual metaphase chromosomes can be isolated and processed without being displaced during exchange of reagents. The change in chromosome morphology as a result of introducing protease into the device was observed by time-lapse imaging; pressure-driven flow was then used to shunt the chromosomal DNA package into a nanoslit. A long linear DNA strand (>1.3 Mbp) was seen to stretch out from the DNA package and along the length of the nanoslit. Delivery of DNA in its native metaphase chromosome package as well as the microfluidic environment prevented DNA from shearing and will be important for preparing ultra-long lengths of DNA for nanofluidic analysis.

The ability to extract, handle and visualize DNA from each individual chromosome (from the human set of 46, for example) would enable the native long-range organization of diploid genomes and the variation between chromosomes within (i.e. homologs) and between individuals to be investigated. Nanofluidic devices have emerged as powerful tools for handling and analyzing single DNA molecules. DNA stretching in nanofluidics has been applied to repressor binding, restriction mapping and recently, denaturation mapping and barcoding of viral DNA or BACs of limited length (100–400 kbp). However, if the size of the DNA that can be handled could extend beyond the megabase scale then individual haplotypes and much of the structural variation in genomes would become accessible. However, extracting mega base-pair long DNA from real-world samples is a major challenge as single molecules of such lengths are known to be sheared during micropipetting. Given that a microfluidic device has been used for manipulating single chromosomes, we hypothesized that the metaphase chromosome could act as a robust packaging for delivering DNA that would mitigate against the shearing effects of pipetting (Fig. 1B). In this paper we report the design, fabrication and use of a device (Fig. 1A) for handling and extracting DNA from metaphase chromosomes. The device design aims to immobilize a single metaphase chromosome in an isolation zone (Fig. 1C) through which reagents can be exchanged by diffusion (Fig. 1D) enabling proteins to be digested. The DNA thus extracted can then be shunted out of the isolation zone into a nanoslit for stretching (Fig. 1E).

The device was designed, with the aid of finite element simulations (COMSOL, USA; see Fig. S1, ESI†), to have a series of isolation zones to slow down the chromosomes in the trap area while maintaining a high flow rate through the device. The parallel isolation zones increased in area with increasing distance (3000 \( \mu \text{m}^2 \), 6000 \( \mu \text{m}^2 \), 9000 \( \mu \text{m}^2 \), etc.) from the sample entry point, in order to obtain a homogeneous flow rate into each of the zones during the introduction of the sample. This was to ensure that all chromosomes were slowed down in the trap area while being able to maintain a high flow rate throughout the device. This ensures a homogeneous flow rate to each of the zones.

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marker into the isolation zones (Fig. 2) validated the device design

Observation of the introduction and spread of the Cy3 fluorescent

chromosome isolation and protease digestion was conducted.

the reagent into the isolation zones to verify device operation before

stagnant volume inside the zones occurred by diffusion only.

there was no flow into the isolation zones; reagent exchange with the

occurring perpendicular to the isolation zones. In this configuration

protease reagent was introduced from the top of the device with flow

sealed by fusion bonding to a 500

powder blasting from the backside of the device which was finally

in order to later allow fusion bonding. Inlet holes were made by

device enabled a high flow rate of 0.6 nL min

and indicated that the reagent is able to spread quite well throughout

the isolation zones by time, 300s.

The device and reagent exchange process was then applied to a sample containing metaphase chromosomes. The chromosomes were isolated from Jurkat cells (DSMZ, Germany: ACC282) in a polyamine buffer as described by Cram et al. Fluorescence imaging was performed using an inverted microscope (Nikon Eclipse TE2000, Japan) equipped with a 60

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incubated for 45 minutes at 37 °C. The swollen cells were collected at

200g for 10 minutes and re-suspended in an ice-cold isolation buffer‡

at approximately 8 × 10⁶ cells per mL. The cells were lysed by vigorous vortex for 30 s. The chromosome content was estimated to be in the order of 10⁷ cells per mL. The device was mounted on a holder interfacing the inlet holes of the device with pressured air allowing movement of the solution inside the device as described elsewhere. Fluorescence imaging was performed using an inverted microscope (Nikon Eclipse TE2000, Japan) equipped with a 60×/1.00 water immersion objective and an EMCCD camera (Photometrics Cascade II512, USA). The temperature inside the device was controlled by a cartridge heater held in contact with the backside of the silicon device. Inlet holes were loaded with 30 μL of solution unless otherwise mentioned. Prior to receiving the chromosomes, the device was flushed by 1% sodium dodecyl sulfate, buffer solution§ and BSA at 1 mg mL

for 10 minutes. 1000–2000 chromosomes were added to the diagonal inlet port (Fig. 1B); the depth of the microfluidic structure allowed the cell extract to be flushed quickly through the isolation zone while watching for the appearance of chromosomes that could be isolated (Fig. 1C). A single chromosome was trapped in an isolation zone of the device (as illustrated in Fig. 1C). Simultaneously the temperature was adjusted to 37 °C and a 100 μg mL⁻¹ solution of protease K

was introduced (Fig. 1D). The device enabled a high flow rate of 0.6 nL min⁻¹ allowing the protease to diffuse quickly into the stagnant volume within the isolation zone. Moreover, a continuous flow through the device ensured that after 4 minutes the protease concentration around the isolated chromosome was maintained above 50 μg mL⁻¹ (Fig. S1† and Fig. 2) and that the digestion products were washed away from the isolation zone

entering isolation zones were moving at the same horizontal speed in order to facilitate selection of individual chromosomes from the parade of chromosomes and cell debris flowing through the device.

The device was fabricated using UV lithography and reactive ion etching of a silicon substrate. Briefly, a 500 nm dry thermal oxide was grown on a silicon wafer. The protease inlet slit and the slit for DNA stretching were defined by UV masking and deep reactive ion etching in the oxide at the depth of 500 nm and 100 nm respectively. The 50 μm wide microfluidic channels connecting the inlet ports and the 400 × 400 μm trap area were defined using a third UV lithography step and were etched in silicon at a depth of 10 μm. A thermal oxide was grown in order to later allow fusion bonding. Inlet holes were made by powder blasting from the backside of the device which was finally sealed by fusion bonding to a 500 μm thick borofloat glass wafer. The protease reagent was introduced from the top of the device with flow occurring perpendicular to the isolation zones. In this configuration there was no flow into the isolation zones; reagent exchange with the stagnant volume inside the zones occurred by diffusion only.

We used streptavidin labelled with Cy3 to visualize the diffusion of the reagent into the isolation zones to verify device operation before chromosome isolation and protease digestion was conducted. Observation of the introduction and spread of the Cy3 fluorescent marker into the isolation zones (Fig. 2) validated the device design

and indicated that the reagent is able to spread quite well throughout the isolation zones by time, 300s.

The device and reagent exchange process was then applied to a sample containing metaphase chromosomes. The chromosomes were isolated from Jurkat cells (DSMZ, Germany: ACC282) in a polyamine buffer as described by Cram et al. with some modifications. Briefly, the Jurkat cells were grown at 37 °C in a 5% CO₂ atmosphere. At exponential growth, they were arrested in metaphase with colcemide at 0.06 μg mL⁻¹ for 12–16 hours. The cells were collected at 200g for 10 minutes and re-suspended in a swelling buffer (55 mM NaNO₃, 55 mM CH₃COONa, 55 mM KCl, 0.5 mM spermidine, 0.2 mM spermine) at approximately 10⁶ cells per mL and incubated for 45 minutes at 37 °C. The swollen cells were collected at 200g for 10 minutes and re-suspended in an ice-cold isolation buffer‡ at approximately 8 × 10⁶ cells per mL. The cells were lysed by vigorous vortex for 30 s. The chromosome content was estimated to be in the order of 10⁷ cells per mL. The device was mounted on a holder interfacing the inlet holes of the device with pressured air allowing movement of the solution inside the device as described elsewhere. Fluorescence imaging was performed using an inverted microscope (Nikon Eclipse TE2000, Japan) equipped with a 60×/1.00 water immersion objective and an EMCCD camera (Photometrics Cascade II512, USA). The temperature inside the device was controlled by a cartridge heater held in contact with the backside of the silicon device. Inlet holes were loaded with 30 μL of solution unless otherwise mentioned. Prior to receiving the chromosomes, the device was flushed by 1% sodium dodecyl sulfate, buffer solution§ and BSA at 1 mg mL⁻¹ for 10 minutes. 1000–2000 chromosomes were added to the diagonal inlet port (Fig. 1B); the depth of the microfluidic structure allowed the cell extract to be flushed quickly through the isolation zone while watching for the appearance of chromosomes that could be isolated (Fig. 1C). A single chromosome was trapped in an isolation zone of the device (as illustrated in Fig. 1C). Simultaneously the temperature was adjusted to 37 °C and a 100 μg mL⁻¹ solution of protease K

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through diffusion. As proteolysis took place, the chromosome swelled (Fig. 3A) and self-aligned in the plane of the device allowing reliable and reproducible fluorescence time-lapse imaging. Although no visible change of the chromosome was observed after $t = 25$ minutes, digestion was allowed to proceed for one hour as recommended by protocols for digestion in bulk solution.\textsuperscript{9} It is striking that even after a digestion treatment that should be sufficient to remove all proteins, sister chromatids could still be clearly identified and chromosomes of different sizes and with different centromere positions could be seen (Fig. 3B). Moreover heterogeneity in the chromatin topography of chromosomes will be important for mapping the linear organisation of sequence along the molecule which is essential for obtaining ultra-long range haplotype and genome structural information. It is expected that the ability to visualize the unravelling of chromatin and extraction of DNA will enable studies into the organisation and topology of chromatin in mitotic chromosomes. The device architecture, which contains isolation zones of various sizes, will provide flexibility for analysing other biological samples that may be of interest such as large macromolecular complexes and individual cells.

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**Notes and references**