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

Rasmussen, Kristian Hagsted; Marie, Rodolphe; Moresco, Jacob Lange; Svendsen, Winnie Edith; Kristensen, Anders; Mir, Kalim U.

Published in:
Lab on a Chip

Link to article, DOI:
10.1039/c0lc00603c

Publication date:
2011

Document Version
Publisher's PDF, also known as Version of record

Citation (APA):

DTU Library
Technical Information Center of Denmark

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.
COMMUNICATION
Marie et al.
A device for extraction, manipulation and stretching of DNA from single human chromosomes

Analytical Research Forum 2011
25 - 27 July 2011
University of Manchester, UK

Registration now open!

The annual Analytical Research Forum (ARF) is a major event for researchers in Analytical Science and is run by the RSC at a location in the United Kingdom or Republic of Ireland. The meeting is aimed primarily toward early career stage analytical science students (PhD students and postdoctoral researchers). The invited lectures have been selected to reflect the broad range of research interests giving attendees exposure to a number of analytical science applications.

Keynote Speakers
Invited lectures have been selected to present research in areas of topical importance:

A Random Walk Through Analytical Chemistry
Professor Neil W Barnett
Deakin University, Australia

Micro Total Analytical Systems - Are we there yet
Professor Stephen Haswell
University of Hull, UK

From Hand Waving to Hardware: The Path to Fielded Instrumentation
Dr Raymond P. Mariella Jr
University of Valladolid, Spain

Scanning Electrochemical Microscopy and Possible Applications in Bioelectrochemistry and Materials Research
Professor Dr Wolfgang Schuhmann
Ruhr University, Bochum, Germany

Workshop: Analytical Science in the Manchester Interdisciplinary Biocentre (MIB)
Monday 25th July 2011, prior to the commencement of ARF

Ever wondered what Analytical Science can do for research in the life sciences?
Then this is the workshop for you!
Fee £25 - book online when registering for ARF

Key Deadlines
Oral abstracts: 25 February 2011
Poster abstracts and early bird registration: 27 May 2011
Standard registration: 24 June 2011

Submit your abstract NOW and register early to take advantage of discounts
A device for extraction, manipulation and stretching of DNA from single human chromosomes†

Kristian H. Rasmussen,*a Rodolphe Marie,*a Jacob M. Lange,a Winnie E. Svendsen,a Anders Kristensena and Kalim U. Mirb

Received 15th November 2010, Accepted 1st February 2011
DOI: 10.1039/c0lc00603c

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 µm2, 6000 µm2, 9000 µm2, 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 etched in silicon at a depth of 10 nm. Marker into the isolation zones (Fig. 2) validated the device design.

Observation of the introduction and spread of the Cy3 fluorescent marker into the isolation zones to verify device operation before stagnation. In this configuration occurred 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.7 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 50 × 10^6 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 × 10^6 cells per mL. The cells were lysed by vigorous vortex for 30 s. The chromosome content was estimated to be in the order of 10^7 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 into the device as described elsewhere.9 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¶ 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.

![Fig. 2](image1.png)  
Fig. 2 Experimental time-lapse imaging of the increasing fluorescence in the trap area due to the diffusion of streptavidin-Cy3 as it is injected at 0.6 nL min−1. The diffusion constant is 60 × 10^{−12} m^2 s^{−1}.

![Fig. 3](image2.png)  
Fig. 3 (A) Time-lapse image series of a single metaphase chromosome during digestion with protease at 37 °C. (B) A panel of different individually isolated chromosomes after 40 minutes digestion.
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.\(^9\) 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 could be seen (Fig. 3B). Moreover heterogeneity in the chromatin of different sizes and with different centromere positions could be seen (Fig. 3B).

The chromosomal DNA could be easily manipulated by using the sample inlet/outlet microchannels and the reagent inlet/outlet slits as a bi-directional flow system inside the trap area. This enabled the chromosomal DNA to be moved in front of 100 nm high slit and then forced in (Fig. S2, ESI†). Although, the bi-directional flow in the trap area would enable DNA extracted from chromosomes trapped in different isolation zones to each be individually manipulated and moved toward the slit, the present study has worked with a dilute solution of chromosomes and so only one chromosome at a time was processed. The post-digestion chromosomal DNA was observed as a densely packed core composed of separated loops (Fig. 4). The chromosomal DNA was highly pliable: the DNA stretched by increasing the flow through the nanoslit and recoiled when the flow was stopped as in Fig. 4A. Loops of DNA were seen to escape from the main core of the chromosomal DNA (Fig. 4B) and a longer separate strand stretched across the whole length of the 450 \( \mu \)m long nanoslit and out into a microchannel (Fig. 4C). This corresponded to a minimal length of \( \sim 1.3 \) Mbp (1.3 million bases) of fully elongated DNA. Such separated DNA strands were also visible around the chromosomal DNA before the introduction to the nanoslit (see last frames of Fig. S2†).

In summary, we designed a silicon device able to trap a particle and to exchange reagent over the particle without dislodging it from its site of isolation. We applied the device to the trapping of single metaphase chromosomes and were able to digest chromosomal proteins to obtain a DNA package that retained a loose two-chromatid structure. The DNA package could be fluidically manipulated and was forced into a nanoslit where a single linear strand was elongated out. The stretching out of DNA from individual 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.

### Acknowledgements

The research leading to these results has received funding from the European Union’s Seventh Framework Programme (FP7/2007-2013) under grant agreement no 201418 (READNA) and from the Danish research council for technology and production under grant number (274-06-0237). We thank David Bauer for comments on the manuscript.

### Notes and references

\(^\dagger\) 15 mM Tris–HCl, 2 mM EDTA, 80 mM KCl, 20 mM NaCl, 0.5 mM EGTA, 0.5 mM spermidine, 0.2 mM spermine, 0.12% digitonin, and 7 mM mercaptoethanol.

\(^\ddagger\) 0.5x TBE, 3% β-mercaptoethanol (BME) and 0.5% Triton X-100.

\(^\bullet\) 1 \( \mu \)M of YOYO-1 is added to the protease K solution for staining the DNA strands while cut free from the chromatin in the vicinity of the bright chromosome body.