An Integrated Nanoliter DNA Analysis Device

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A device was developed that uses microfabricated fluidic channels, heaters, temperature sensors, and fluorescence detectors to analyze nanoliter-size DNA samples. The device is capable of measuring aqueous reagent and DNA-containing solutions, mixing the solutions together, amplifying or digesting the DNA to form discrete products, and separating and detecting those products. No external lenses, heaters, or mechanical pumps are necessary for complete sample processing and analysis. Because all of the components are made using conventional photolithographic production techniques, they operate as a single closed system. The components have the potential for assembly into complex, low-power, integrated analysis systems at low unit cost. The availability of portable, reliable instruments may facilitate the use of DNA analysis in applications such as rapid medical diagnostics and point-of-use agricultural testing.

Conventional biochemical DNA analyses—such as Sanger sequencing, polymerase-assisted amplification, and restriction endonuclease digestion—typically require several linked steps to proceed from an unknown sample to base pair information (*1*). The analyses have several technical steps in common, including (i) precise volume measurement of reagent, enzyme, and DNA-template solutions; (ii) mixing of solutions; (iii) controlled thermal reaction of the mixture; (iv) loading of the reaction products onto an electrophoresis device; and (v) detection of size-fractionated reaction products. In a standard laboratory, the complete process relies on intervention at several stages to manipulate or observe samples and record results. Although DNA analysis typically involves small liquid volumes (10 to 50 μl) and linked process steps, the reagents, labor, and supporting equipment required for high throughput processing remain costly (*2*).

We have developed an integrated analysis system in which DNA and reagent solutions are placed on the device and electronic signals corresponding to genetic information are the primary output. The device (Fig. 1) includes a nanoliter liquid injector, a sample mixing and positioning system, a temperature-controlled reaction chamber, an electrophoretic separation system, and a fluorescence detector. All the components of the system, with the exception of the excitation source, pressure source, and control circuitry, are microfabricated by means of photolithographic techniques and are contained on a single glass and silicon substrate.

Photolithographic construction begins with diode photodetector formation within a crystalline silicon substrate. The diodes were constructed by implanting the entire surface of a silicon wafer (200 ohm·cm, ⟨100⟩, float zone, boron-doped, p-type) with boron (50 keV, 2×10^{12} cm^{-2}). Diffused layers of phosphorus (50 keV, 5×10^{14} cm^{-2}) and boron (30 keV, 1×10^{15} cm^{-2}) were then ion-implanted onto the sample in lithographically defined regions to form the substrate contact and the main lead for the diode, respectively. Thermal silicon oxide was grown (0.2 μm at 900°C) over the wafer to provide an insulating barrier and to activate the diodes. A quarter-wavelength interference filter consisting of alternating layers of titanium oxide and silicon oxide was deposited above the diodes to allow the transmission of fluorescent light (515 nm in the existing design; ZC&R Coatings for Optics Inc., Carson, CA) while rejecting the excitation light (<500 nm). The metal heaters and temperature sensors required for temperature control were fabricated between two barrier layers of vapor-deposited plastic (*p*-xylylene) (*3*). Platinum metal was placed on the surface at defined locations for use as electrophoresis electrodes (*4*). Finally, a glass substrate containing etched microfluidic channels (500 μm wide and 50 μm deep) was bonded to the silicon substrate to complete the device (*5*). The interior surface of the glass channel had defined hydrophobic regions for fluid control. The finished microfabricated assembly (47 mm long, 5 mm wide, and 1 mm high) was wire-bonded to a printed circuit board, linked to air-pressure lines through a manifold, and fitted with electrophoresis buffer wells. Polyacrylamide gels were formed in situ in the electrophoresis stage immediately before use (*6*).

An important aspect of the device is the ability to meter a small, accurate volume of fluid by means of a hydrophobic patch and injected air. A pipette is used to place a sample of DNA-containing solution on one fluid-entry port and a reagent-containing solution on the other port. Capillary action draws each solution into the device, but hydrophobic patches positioned just beyond the vent line in each injection channel stop the samples (Fig. 2A). Pressure through an air vent is used to split off precise nanoliter drops whose volumes equal the distance between the vent line and the hydrophobic patch times the channel cross section (drop volumes formed in the device shown are 120 nl). The drops mix together and move forward to the thermal reaction region. Once the merged sample passes the third vent, the driving force for motion is diverted and the sample is automatically positioned in the reaction region (Fig. 2B). Microfabricated heaters and temperature sensors are then used to control the temperature in the reaction chamber for a specified time (*7*).

Of comparable importance is the ability of the device to detect size-fractionated products with microfabricated fluorescence detectors. After the reaction is complete, the sample is moved forward by pressure to the intersection at the start of the gel electrophoresis channel. The presence of the electrically conducting sample drop in this loading zone is confirmed by electrical connectivity across the gel system. The DNA is electrokinetically loaded onto the gel and size-fractionated under applied fields of <10 V/cm. As the fluorescently labeled DNA migrates though the gel, an external blue light–emitting diode (LED) excites emission while an integrated photodiode beneath the microfabricated filter captures the fluorescent signal. The output from the detector indicates the migration time of the DNA reaction products.

The raw electronic output from an integrated analysis run is shown in Fig. 3. In this strand displacement amplification (SDA) experiment, one channel of the device was loaded with a mixture of Bso B1 restriction and Bst DNA polymerase enzymes. The second channel was loaded with a reaction solution containing template DNA and amplification site–specific oligonucleotides (*8*). Discrete 120-nl drops were injected from each channel, mixed together, and positioned in the reaction area. The temperature was slowly raised to 50°C (10 to 20 s) and controlled at 50°C for 17 min with the resistive heaters and integral temperature sensors. The sample was then moved forward and, after merging with a drop (~25 nl) of intercalating dye, stopped in the gel electrophoresis intersec-

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tion (*9*). The DNA products were loaded onto the gel for ~30 s at 8 V/cm and migrated at 8 V/cm for 30 min (*10*). The active diode was 2 mm from the running gel's interface, and the single large peak obtained from continuous reading of that diode indicates that the specific target DNA was successfully amplified and detected (*11*).

In addition to the potential impact of the multicomponent integrated device, several of the individual components are useful in themselves. For example, the fluorescence detection system we have constructed to detect DNA band migration data is very sensitive. The low-wavelength rejection of this filter, combined with the near 100% transmission in the fluorophore's emission range, allows the diode to detect DNA at concentrations down to ~10 ng/μl (*12*). Although a similar diode without the filter could detect radioactively labeled DNA (*7*), the sensitivity of a properly constructed fluorescence detector will be several orders of magnitude better. The close positioning of the detector to the sample (distance of ~6 μm) results in efficient collection of the fluorescent signal. We have used this detection system in combination with gel electrophoresis for a variety of separations, including analysis of standard DNA base-pair ladders and restriction digest products (*13*).

Another feature of the device is the spatial DNA resolution obtained in the polyacrylamide gel after only a few millimeters of electrophoretic migration. Figure 4 shows the results of a 50–base pair (bp) DNA ladder run in the gel electrophoresis section. The crossed intersection used to electrophoretically load sample onto the gel is robust and allows stacking of the aqueous DNA at the gel boundary. Sample loads typically require 10 to 30 s, and the subsequent runs have durations on the order of minutes. In each case, DNA separation was achieved in a distance of 0.5 to 3 mm. Other non–cross-linked separation media can also be used in the device and have the advantage of being replaceable (*14*). Although we have achieved similar resolution with those media (*15*), cross-linked acrylamide allows simpler loading of discrete samples on the gel.

The liquid injection system provides a solution to the challenge of introducing and metering precise nanoliter-size liquid samples. The key to the system is the ability to place hydrophobic regions or patches in the injection channels with micrometer precision. The patches are constructed by reacting a silane on a patterned aluminum mask (*5*, *16*). After removing the mask, the hydrophobic surface treatment remains on areas where the substrate was exposed. By using these precise hydrophobic patches and an air-pressure source, drops of <1 nl can be isolated and moved within the channels. Although the device shown can only meter one size of drop, a design incorporating multiple vent lines could be used to vary the injection volume. Air pressure can be supplied by an external source or by on-chip expansion chambers (*16*); the extent of the pressure increase has

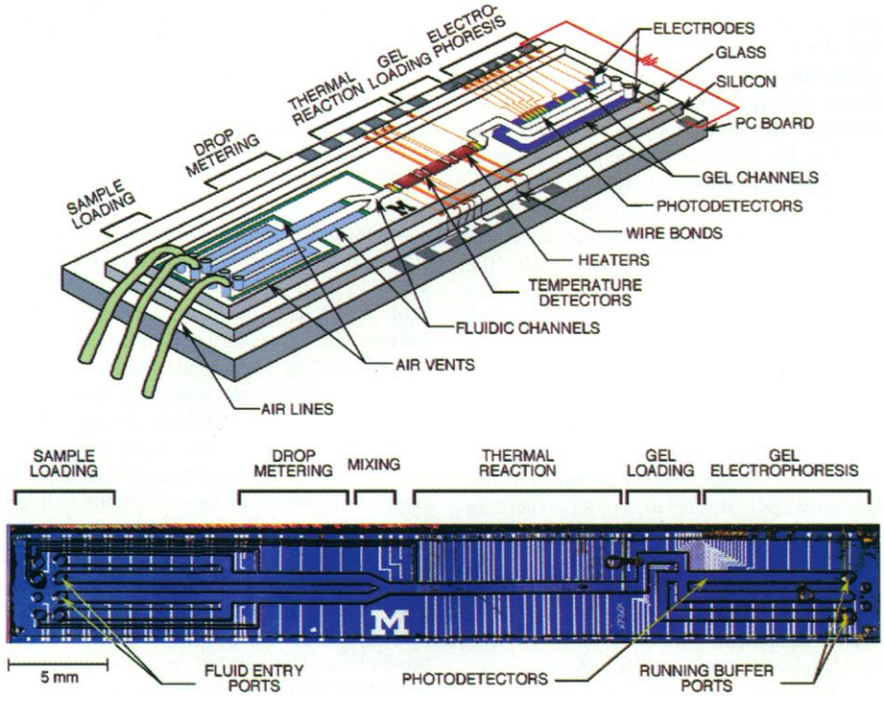

Fig. 1. (Top) Schematic of integrated device with two liquid samples and electrophoresis gel present. The only electronic component not fabricated on the silicon substrate, except for control and data-processing electronics, is an excitation light source placed above the electrophoresis channel. Color code: blue, liquid sample (ready for metering); green, hydrophobic surfaces; purple, polyacrylamide gel. (Bottom) Optical micrograph of the device from above. Wire bonds to the printed circuit board can be seen along the top edge of the device. The blue tint is due to the interference filter reflecting the short-wavelength light. The pressure manifold and buffer wells that fit over the entry holes at each end of the device are not shown.

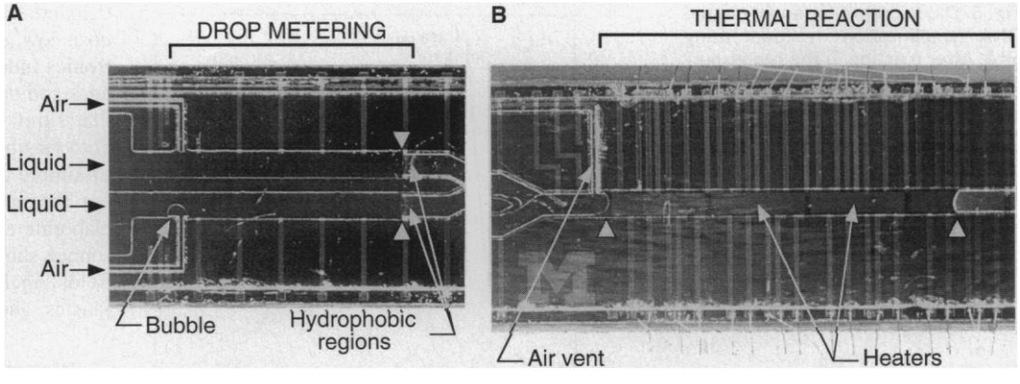

Fig. 2. Optical micrographs of the injection system, showing the movement of two 120-nl drops into the 500-μm-wide reaction chamber. White arrowheads indicate drop menisci. (**A**) An excess of solution is placed at the openings of the two injection channels. Both solutions are drawn in by capillary action and stop at the hydrophobic patches. The bubble at the end of the bottom air vent indicates that a slight increase in air pressure has occurred and the metering is about to occur. (**B**) After air pressure on both air lines has split off and moved the two drops down the channels, they merge at the intersection and flow into the reaction zone. The air vent at the trailing end of the reaction zone relieves the pressure and prevents the drop from moving beyond this position. When the reaction is completed, that air vent is pressurized to move the drop from the reaction zone to the sample loading intersection.

no effect on the volume of the drop. The location of a drop in the device can be readily determined with diode detectors located beneath the channel; the presence of a solution with a refractive index different from that of air results in a step change in the illuminated diode current (*17*). Once metered, these individual samples can also be moved by differential heating in place of air pressure (*7*).

The reaction region of the system has the same channel cross section as the injection and electrophoresis components. Resistive metal heaters are embedded beneath a plastic coating immediately below the reaction area. The coating is necessary to electrically and chemically isolate the electronic components; however, we have successfully used other passivating layers such as silicon oxide and nitride to perform this function (*7*). One of the heaters is used as a temperature sensor because the resistance of the metal is a fairly linear function of temperature. The temperature of the reaction chamber is controlled to ±0.1°C using these sensors and a proportional-integral controller, and controlled transitions of 1° to 10°C per second are easily obtainable. Even though only one side of the liquid is being heated, we have found the vertical variation in liquid temperature at this size scale, both experimentally and theoretically, to be <1°C (*18*). Our current chamber is compatible with a variety of biochemical reactions with DNA, including restriction digestion and DNA polymerase extension. In general, the results are similar to conventional macroscale reactions (*19*).

The described nanoliter device, although still at an early stage in its development, constitutes an integrated fluidic and electronic system for multistep DNA analysis. The inclusion of sample injection, movement, mixing, reaction, separation, and detection in one device suggests that increasingly complicated systems can be constructed at the nanoliter scale. Because the only external connections needed for this device are input electronic control lines, data output lines, and an air pressure manifold, the device approaches a completely "hands-free" analysis technology. The closed fluidic system allows processing steps to occur on liquid volumes far smaller than conventional human or robotic handling methods.

The removal of human interaction with analytical samples will become increasingly attractive as the demand for genetic information increases (*20*). Although the methods and chemistries for the analysis of DNA have improved enormously over the past decade, the costs for reagents and labor remain high (*21*). Incremental improvements in efficiency and cost will continue within the existing experimental technology; however, fully integrated microscale methods may provide order-of-magnitude advances. The goal of constructing the nanoliter analysis device was to replace conventional hardware and trained personnel as an initial step toward more complex systems.

The strategy presented here relies on developing compatible components that are essentially a complete set of analytical tools. The components are compatible both in their operation and their construction; hence, there are few constraints on assembling more complex systems. We have also focused on discrete drops and standard biochemistries (*7, 20*) to take advantage of the many existing protocols. Working with discrete drops of known concentrations and volumes should limit the problems associated with the adaptation of microliter-size reactions to nanoliter-size systems. In addition, essentially all necessary components, including the detector, have been fabricated on the substrates to eliminate the need for additional, expensive equipment.

Although this strategy has advantages for complex chemical analysis systems, many other microfabricated devices currently exist. Most published microseparation systems use electroosmotic flow to deliver the sample and use a non–cross-linked separation medium (*14*). In these systems, samples can be electronically manipulated. Integration of these systems with reaction chambers and detectors has also been accomplished (*22*). The high voltages (100 to 200 V/cm) used in these systems may limit the ability to integrate sensitive electronic components in the same device. Hybridization array devices with external imaging have the advantage of detecting components without a separation stage (*23*). The vast scope of problems that can be solved by these devices, from determining the presence of a single region of DNA to sequencing several noncontiguous regions, dictates that a variety of these solutions will be successful.

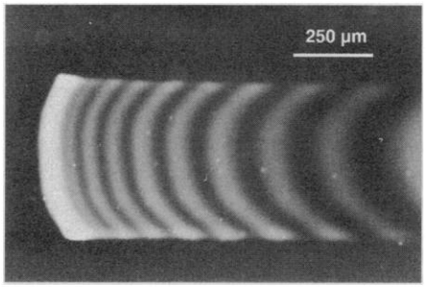

Fig. 4. Optical micrograph of a 50-bp DNA ladder in a 500-μm-wide polyacrylamide gel. The DNA (0.12 μg/μl) was loaded onto the gel for 15 s at 8 V/cm. After flushing the intersection with buffer, the gel was run for 15 min. The bright band at the far right is the 350-bp band; the six smallest bands in the sample have already passed out of the frame of view. The remaining bands are between 400 bp and 800 bp and are all resolved in ~1 mm.

The standardized interconnections between each component in our device, coupled with the inexpensive photolithographic techniques, should be ideal for the low-cost production of integrated devices. However, important technical challenges remain, including maintaining minimal liquid evaporation and solute adsorption, increasing detector sensitivity, and controlling complex fluidic and electronic operations. Although the construction and testing of micrometer-size integrated systems is difficult, the power of these systems compensates for the additional effort. The availability of robust microfabrication procedures and high unit demand should keep future production costs low, as has occurred in the microelectronics industry (*24*). Finally, the low voltages and power used in this device suggest that simple hand-held battery operation is both feasible and practical. The availability of simple devices that analyze DNA without the need for specialized laboratories, elaborate equipment, or highly skilled personnel should yield benefits across many fields, including medical diagnostics, forensics, and agricultural testing.

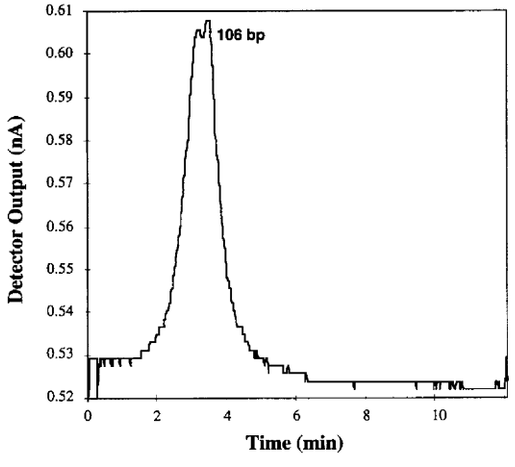

Fig. 3. Diode output for an integrated DNA amplification reaction using SDA. After reaction in the center portion of the device, the amplification products were mixed with SYBR Green, moved onto the electrophoresis gel, and run at 8 V/cm for 25 min. The single peak recorded by the integral diode indicates successful amplification of the target DNA.

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3. For formation of the heaters, a 2-μm layer of *p*-xylylene was deposited as a moisture barrier and a reactive ion–hydrofluoric acid etch was used to form connections with the diodes. A 3.3-μm layer of Microposit 1400-37 photoresist was patterned to define the dual–meandering-line heaters (area 500 μm by 500 μm, line thickness 5 μm), and 50 nm of chromium was evaporated over the resist, followed by 400 nm of gold. Liftoff of the resist left the heaters on the surface. The heaters were then covered with a second, insulating layer of *p*-xylylene (2 μm thick).
4. For the electrodes, 20 nm of titanium and 30 nm of platinum were deposited on top of the second *p*-xylylene layer, using the same liftoff procedure as for the heaters.
5. Channels were prepared on 500-μm-thick glass wafers (Dow Corning 7740) using standard aqueous-based etch procedures, as described (*7*). Al (1000 nm) was then evaporated and patterned using photoresist AZP4620 (Hoechst Celanese). The wafer was dipped in a solution of heptadecafluro-1,1,2,2-hydrodecyl dimethylchlorosilane to form hydrophobic regions on the surface, and the aluminum was then removed. Holes through the glass substrate at the ends of the fluid channels were drilled by applying 37 V to a metal point touching the glass surface in a 50 weight % sodium hydroxide solution. For assembly, the glass channel was placed on top of the silicon substrate, and optical adhesive (SK-9 Lens Bond; Sumers Laboratories, Fort Washington, PA) was applied to the edge of the channel and allowed to wick between the glass and silicon substrate. The adhesive did not enter the channel area and was cured under an ultraviolet lamp for 24 hours.
6. Raised polymer walls were fashioned around the buffer ports to prevent excess buffer from contacting the surface electronics. Monomer acrylamide electrophoresis gel material [10% acrylamide, 0.3% bis(acrylamide), 89 mM tris-HCl, 89 mM borate, 10 mM EDTA, 0.001% *N,N,N′,N′*-tetramethylethylenediamine (TEMED), and 0.01% ammonium persulfate] was allowed to wick into the two channels and polymerize for 30 min. In some cases, a hydrophobic patch at the crossed channels was used to aid in the formation of flat gel interfaces. The gel present in two of the four channels of the intersection serves to restrict the motion of the sample and to ensure that the DNA remains at the running gel interface.
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9. SDA does not work in the presence of intercalating dyes such as SYBR Green. For our integrated restriction digest runs, a separate dye injection step was not necessary; the dye was included in the DNA sample solution. Buffer was kept on the gel during reaction to prevent drying. The buffer was removed before the sample was loaded onto the gel.
10. Sample was removed after injection and replaced with buffer; runs were also performed without this step, but removing the sample led to more uniform and sharp peaks. Platinum electrodes placed in the buffer wells were used for both the injection and running of the sample.
11. A lock-in amplifier and data acquisition program were used to pulse a blue LED with accompanying low-pass filter at 288 Hz and to record the output of the microfabricated diode detector.
12. Diode detection limits for this system were obtained using dilute solutions of 4.0-kb plasmid DNA with intercalating dye. In a 50 μm by 500 μm channel over a 10 μm by 500 μm detector, DNA solutions at concentrations of 10 ng/μl were readily detected.
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18. When the reaction chamber is at 52°C, a thermocouple at the top surface of the glass (500 μm above) measures ~48°C. Therefore, the vertical temperature difference in the 50-μm-high channel is on the order of 0.5°C. A finite-difference solution of the heat-transfer equations for the system, using a constant-temperature boundary condition at the heater and a natural convection heat-transfer coefficient at the top, gives a similar result (T. S. Sammarco and M. A. Burns, data not shown).
19. Nanoliter reactions may be affected by evaporation and surface adsorption. Evaporation was reduced in our design by having only a few small openings from the device to the outside. Surface adsorption was reduced by pretreatment of the microfluidic channels with a dilute protein solution (BSA); other researchers have solved the adsorption problem in different ways [for example, M. A. Shoffner, J. Cheng, G. E. Hvichia, L. J. Kricka, P. Wilding, *Nucleic Acids Res.* **24**, 375 (1996)].
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24. As an example of the cost of these devices, a slightly more compact version of the current design would yield about 30 devices per wafer. The costs for 25 silicon and glass substrates are about $600 and $200, respectively. The lithography ($500), ion implants and oxidation ($100), high-pass optical filter ($800), passivation layer ($200), and depositions ($500), along with laboratory fees ($1700) to process 25 wafers, would therefore be $3800, yielding a total device cost of just over $6 per device. An optimized design combined with a larger batch run could easily bring the cost down by at least an order of magnitude.
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Phosphorylation and Activation of 13*S* Condensin by Cdc2 in Vitro

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13*S* condensin is a multisubunit protein complex essential for mitotic chromosome condensation in *Xenopus* egg extracts. Purified 13*S* condensin introduces positive supercoils into DNA in the presence of topoisomerase I and adenosine triphosphate in vitro. The supercoiling activity of 13*S* condensin was regulated by mitosis-specific phosphorylation. Immunodepletion, in vitro phosphorylation, and peptide-mapping experiments indicated that Cdc2 is likely to be the kinase that phosphorylates and activates 13*S* condensin. Multiple Cdc2 phosphorylation sites are clustered in the carboxyl-terminal domain of the XCAP-D2 (*Xenopus* chromosome-associated polypeptide D2) subunit. These results suggest that phosphorylation of 13*S* condensin by Cdc2 may trigger mitotic chromosome condensation in vitro.

Chromosome condensation is a fundamental cellular process that ensures the faithful segregation of genetic information during mitosis and meiosis. Activation of the protein kinase Cdc2 triggers a series of downstream mitotic events including chromosome condensation, but the underlying molecular mechanisms are poorly understood (*1, 2*). 13*S* condensin, a five-subunit protein complex purified from *Xenopus* egg extracts, is an essential regulator of mitotic chromosome condensation (*3, 4*). The two core subunits of 13*S* condensin, XCAP-C and XCAP-E, belong to the SMC (structural maintenance of

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