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Detection of DNA Using Bioactive Paper Strips

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General:
All DNA oligonucleotides were purchased from Integrated DNA Technologies (IDT) and purified in house by 10% denaturing PAGE. Straptavidin-coupled microgels were synthesized as previously described.[1] The fluorescently labeled DNA oligonucleotide was obtained from IDT and purified by 10% denaturing PAGE. T4 DNA ligase (T4L), Phi29 DNA polymerase (ϕ29DNAP) and T4 polynucleotide kinase (T4 PNK) were purchased from MBI Fermentas (supplied with relevant reaction buffers). [γ-^{32}P]ATP was purchased from Perkin Elmer. Water used in this work was double-distilled. The autoradiogram and fluorescent images of paper strips were obtained using Typhoon and analyzed using ImageQuant software (Molecular Dynamics).

Preparation of DNA1-MG conjugates:
Biotinylated DNA1 (200 pmol) and streptavidin-conjugated microgel (SAV-MG, 20 pmol SAV, prepared and characterized as previously reported[1]) were allowed to incubate for 1 h in 100 μL of the binding buffer (50 mM Tris-HCl, 100 mM NaCl, 0.1% Tween 20, pH 7.5 at room temperature). Thus coupled DNA1-MGs were precipitated by centrifugation at room temperature for 30 min at 22,000 g. Following the removal of the supernatant, the pellet was washed twice with 200 μL of the same binding buffer to remove any uncoupled DNA1, re-suspended in 50 μL of the binding buffer and stored at 4 °C.

Preparation of 5′-phosphorylated DNA2:
Phosphorylation with [γ-32p]ATP
A 10-μL reaction mixture was prepared using10 pmol of DNA2, 1.0 μL 10× PNK buffer, 1.0 μL [γ-^{32}p]ATP and ddH_2O. The reaction was started by adding 1 μL of PNK and continued at 37 °C for 20 min. Following this, 1 μL of non-radioactive ATP (10 mM) was added to the reaction mixture and incubated at 37 °C for 10 min. The reaction was quenched by adding 10 μL of 2× gel loading buffer (80 mM EDTA, pH 8.0 at 25 °C, 16 M urea, 180 mM Tris, 180 mM boric acid, 20% sucrose (w/v), 0.05% xylene cyanol and 0.05% bromophenol blue), followed by heating at 90 °C for 5 min. The DNA was subjected to 10% denaturing PAGE. The DNA was isolated from the gel by crash and soak method, followed by ethanol precipitation. Finally, the DNA pellet was dried by speed vac and dissolved in 20 μL of ddH_2O.

Phosphorylation with non-radioactive ATP
DNA2 (500 pmol) was phosphorylated in the same way as described above with the omission of [γ-^{32}p]ATP. The DNA pellet was dried by speed vac and dissolved in 50 μL of ddH_2O. Following isolation, the DNA was quantified by UV-vis spectrometer and the concentration was adjusted to 5 μM.

DNA ligation with paper strips:
A series of six paper strips with the size of ~3 × 25 mm were made from Whatman filter paper #1 (VWR). The paper strips in each series were marked as a-f (see Figure 2B in the main text) for tracking purpose. An aliquot of 5 μL of the DNA1-MGs (as prepared above) was taken out from the stock by a pipette and carefully spread even onto the bottom 10 mm of each paper strip using the pipette tip. After air-drying for ~20 min at room temperature, each paper strip was dipped into a 1.5-mL microcentrifuge tube containing 500 μL of the binding buffer. After rocking gently by hand for 30 sec, the binding buffer was removed from the tube by pipetting. Another 500 μL of the binding buffer was added to the tube and rocked by hand and then removed (to remove the loosely bound DNA-MG on the paper strip). This was followed by the addition of 100 μL of a mixture containing 0.1 μM of nonradioactive 5′-phosphorylated DNA2, trace amount of radioactive 5′-phosphorylated DNA2, 0.1 μM of DNA3, T4L reaction buffer (40 mM Tris-HCl, 10 mM $MgCl_2$, 10 mM DTT, 0.5 mM ATP, pH 7.8 at 23°C) and T4L (0.1 U/μL) to the tubes hosting strips c and f. To the tubes hosting strips b and e, the same amount of the master mixture lacking only T4L was added. A similar mixture omitting DNA3 was added to the tubes with a and d. The immersed paper strips were incubated for the DNA ligation reaction for 60 min at room temperature. Following the removal of the reaction solution, each paper strip was carefully washed 4 times each with 500 μL of the binding buffer. Strips d-f were further washed once with 500 μL of 0.1 M NaOH and twice with 500 μL of the binding buffer. Finally, all the paper strips were scanned for radioactivity using a phosphorImaging scanner (Storm from GE Health). This experiment was repeated three times and one set of results is given in Figure 2B of the main text.

RCA with paper strips:
A series of three paper strips of ~3 × 25 mm in size were made once again from Whatman filter paper #1 and marked as a-c. Each paper strip was absorbed with DNA1-MGs in the same way as described above, placed into a 1.5-mL microcentrifuge tube. A master mixture (100 μL) containing 0.1 μM of non-radioactive 5'-phosphorylated DNA2, T4L reaction buffer, and T4 DNA ligase (0.1U/μL) was added to each tube. An aliquot (1 μL) of H_2O, DNA4 (10 pmol), or DNA3 (10 pmol) was added to the tubes with strips a, b and c, respectively. The portion of the paper strips immobilized with DNA1-MGs was submerged into the reaction mixture and incubated for DNA ligation at room temperature for 1 h. Following the removal of the reaction mixture by pipetting, each paper strip was washed twice with 500 μL of the binding buffer. Thereafter, 100 μL of a master mixture containing the circular template (RCAT, 0.1 μM), ϕ29DNAP reaction buffer (33 mM Tris-acetate, pH 7.9 at 37 °C, 10 mM Mg-acetate, 66 mM K-acetate, 0.1% Tween 20, 1 mM DTT), dNTPs (0.5 mM each of dATP, dCTP, dGTP and dTTP) and ϕ29DNAP (0.1 U/μL) was added into each tube. The paper strips and the RCA mixture were incubated at 30 °C for 1 h. At this point, 1 μL (50 pmol) of the fluorescent probe (DNA5) was added into the reaction mixture, which was further incubated for 2 h at room temperature. Following the removal of the solution by pipetting, each paper strip was washed three times each with 500 μL of the binding buffer. The paper strips were placed between two glass plates and scanned for fluorescence using a fluorescence scanner (Typhoon 9200 from GE Health). This experiment was repeated three times and one set of results is given in Figure 3B of the main text.

Quantitative detection with paper strips:
A series of six paper strips were made and immobilized with DNA1-MGs in the same way as described above. These paper strips were used in the ligation/RCA reactions conducted in the same way as described above except that DNA3 was used at 0, 10, 100, 500, 1,000 and 10,000 pM. This experiment was repeated five times and one set of images obtained is given in Figure 4 of the main text. The fluorescence intensities of all examined paper strips were quantified with ImageQuant software. The

relative fluorescence intensity is calculated as the ratio of the fluorescence intensity of a test strip over that of a blank paper. The detection limited is taken as the concentration that produces a fluorescence intensity that is at least equal to the sum of the intensity at no DNA target and 3× standard deviation.

References

1. Su S, Ali MM, Filipe CD, Li Y, Pelton R, *Biomacromolecules*. **2008**, 9, 935-941