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DSBCapture: *in situ* single-nucleotide resolution DNA double-strand break mapping

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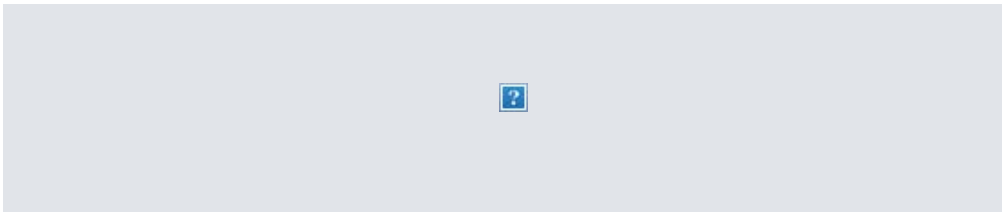
Protocol Exchange (2016) | doi:10.1038/protex.2016.52
Published online 23 August 2016

Abstract

DSBCapture is a double-strand DNA break (DSB) mapping methodology that maps DSBs *in situ* to single-nucleotide resolution. DSBs are captured through the ligation of a modified Illumina adapter to broken DNA ends inside isolated, fixed nuclei. Break sites are subsequently decoded to single-nucleotide resolution by Illumina sequencing.

Figures at a glance



Subject terms: Cell biology ▪ Genomics ▪ Nucleic acid based molecular biology ▪ Epigenomics

Keywords: double-strand DNA break ▪ double-strand DNA break mapping ▪ next generation sequencing ▪ molecular biology ▪ DNA damage ▪

G-quadruplex ▪ Transcription

Introduction

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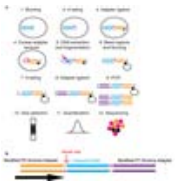
DSBCapture is a substantially improved DSB mapping methodology derived from BLESS[1] that maps DSBs *in situ* to single-nucleotide resolution. The procedure involves the ligation of a modified Illumina adapter to DSBs in fixed nuclei. The subsequent lysis of the nuclei and the fragmentation of the DNA generates a second free end, to which a second modified Illumina adapter is ligated. PCR amplification of the ligated DNA fragments generates a library that can be directly sequenced on the Illumina platform. DSBs are decoded to single-nucleotide resolution in single-end sequencing, where the first base sequenced identifies the site of *in situ* DSB formation. The DSBCapture workflow is depicted in Fig.1. DSBCapture can be universally applied for the study of DSBs; these can for example be endogenous, drug-induced or nuclease cleavage sites. The complete experimental procedure takes approximately 8 days. We suggest using 20 million cells per sample: 10 million for the full procedure and 10 million for a control performed without T4 DNA ligase in the first ligation reaction.



Reagents

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• Cells
• Cell culture medium
• Fetal Bovine Serum
• Formaldehyde (Pierce, cat. no. 28908)
• 2 M Glycine
• PBS (- $CaCl_2$ / – $MgCl_2$)
• Lysis buffer:
- 10 mM Tris-HCl pH 8
- 10 mM NaCl
- 1 mM EDTA
- 1 mM EGTA
- 0.2 % NP40 substitute (Sigma, 74385)
- freshly added: cOmplete Roche protease inhibitors (Roche, REF: 11873580001)
- freshly added: 1 mM DTT
• Nucleus Break buffer:
- 10 mM Tris-HCl pH 8
- 150 mM NaCl
- 1 mM EDTA
- 1 mM EGTA
- 0.3 % SDS
- freshly added: 1 mM DTT
• 1 × NEBuffer 2 (pH 7.9 at 25 °C):
- 10 mM Tris-HCl
- 50 mM NaCl
- 10 mM $MgCl_2$
- freshly added: 1 mM DTT
• 1 × W&B buffer:
- 5 mM Tris-HCl pH 7.5

- 1 mM EDTA
- 1 M NaCl
• Proteinase K (Ambion, cat. no. AM2546)
• PMSF (Sigma, cat. no. 93482)
• Quick Blunting Kit (NEB, cat. no. E1201L)
• Blunting Buffer (NEB, cat. no. E1201L)
• BSA (NEB, cat. no. B9000S)
• Klenow Fragment 3’-5’ exo- (NEB, cat. no. M0212L)
• dATP (Promega, cat. no. U120D)
• T4 Ligase Reaction Buffer (NEB, cat. no. B0202S)
• T4 DNA Ligase (NEB, cat. no. M0202M)
• Lambda Exonuclease (NEB, cat. no. M0262L)
• Lambda Exonuclease Reaction Buffer (NEB, cat. no. M0262L)
• Nuclease free water (e.g. Ambion cat. no. AM9937)
• Dynabeads MyOne Strepdavidin C1 (Invitrogen, cat. no. 65001)
• Modified P5 Illumina adapter (for sequence see end of protocol)
• Modified P7 Illumina adapter (for sequence see end of protocol)
• PCR F and PCR R primers (for sequence see end of protocol)
• NEBNext PCR High Fidelity 2X PCR Master Mix (NEB, cat. no. M0541L)
• MinElute PCR Purification Kit (Qiagen, cat. no. 28004) or MinElute Gel Extraction Kit (Qiagen, cat. no. 28604) depending on method of DNA extraction (pippin versus manual, respectively)
• KAPA Library Quantification Kit (Kapa Biosystems, cat. no. kk4824)
• Propoan-2-ol (molecular biology grade)
• Ethanol (molecular biology grade)
• GlycoBlue (Invitrogen, cat. no. AM9515)
• 1 M Potassium acetate
• 3 M Sodium acetate pH 5.2 (for pH adjustment during PCR clean up using Qiagen kit; e.g. Thermo Fisher Scientific, cat. no. R1181)
• Triton X-100
• Trypan blue

Equipment

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• Manual Pipettes
• Pipette tips
• Tissue culture facilities and equipment
• Light microscope
• Cell counting equipment
• Haemocytometer or Ibidi slides (Ibidi, cat. no. 80621)
• Sonicator e.g. Biorupter
• Rotator
• 4 °C room or incubator
• 37 °C incubator
• Thermocycler
• Vortex
• Centrifuge with refrigeration capability (e.g. Eppendorf 5810R)
• Microcentrifuge with refrigeration capability (e.g. Eppendorf 5415R)
• Water bath
• Heat block
• ThermoMixer e.g. Eppendorf comfort
• Gel electrophoresis equipment
• Magnetic rack (1.5 or 2 mL)
• Bioanalyser (Agilent) or TapeStation (Agilent) or similar (with high sensitivity reagents)
• DNA quantification equipment (e.g. Qubit fluorimeter, Thermo Fisher Scientific)
• Quantitative PCR machine (if performing library quantification)
• Falcon tubes
• 2 mL Eppendorf tubes

- Eppendorf LoBind tubes (1.5 or 2 mL, depending on type of magnetic rack used)
- Sonication tubes (Diagenode, cat. no. C30010010)
- PCR tubes
- 0.2 µM filter (e.g. Millipore, cat. no. SLGP033RS) and syringe
- Access to an Illumina sequencer
- Optional: BluePippin (Sage Science)

Procedure

Day 1: Cell fixation

Note: centrifugation steps were performed in a bench top centrifuge e.g. Eppendorf 5810R. Once cells have been harvested, work with cells in Falcon tubes.

1. Grow cells to 70 % confluency
Note: We suggest using 20 million cells per sample: 10 million for the full procedure and 10 million for a control performed without T4 DNA ligase in the first ligation reaction.
2. Wash cells with PBS (-/-)
3. Detach cells using trypsin (e.g. HeLa/U2OS) or accutase (e.g. NHEKs)
4. Add an equal amount of complete growth medium to the detached cells
5. Transfer cells to a Falcon tube and centrifuge at 1300 rpm for 5 min at room temperature to pellet the cells
6. Remove the supernatant, re-suspend cells in complete medium and count
7. Make a single cell suspension (by pipetting up and down 10 times) of 1 million cells/1.5 mL in DMEM 10 % FBS (maximum of 25 million cells / 50 mL Falcon tube)
8. To the single cell suspension add formaldehyde to a final concentration of 2 %
9. Rotate (7 rpm) at room temperate for 30 min
10. Add 2 M glycine to achieve a final concentration of 125 mM
11. Rotate (7 rpm) for a further 5 min at room temperature
12. Chill samples on ice for 10 min
From this point forward work on ice for the rest of the day unless stated otherwise.
13. Centrifuge cells at 1200 rpm for 5 min at 4 °C with the break switched off
14. Remove the supernatant, tap tube against hand to loosen the pellet and add ice cold PBS (same volume as was removed)
Note: we suggest loosening pellet by tapping, rather than re-suspending using pipettes, to avoid loss of material.
15. Invert tube 5 times
16. Centrifuge the cells at 1200 rpm for 5 min at 4 °C with the break switched off
17. Remove the supernatant, tap tube against hand to loosen the pellet and add ice cold PBS (same volume as was removed)
18. Store fixed cells in PBS at 4 °C over night or alternatively proceed immediately to day 2

Day 2: Isolation of nuclei

Note: centrifugation steps were performed in a bench top centrifuge e.g Eppendorf 5810R. Work with cells in Falcon tubes. Work on ice unless stated otherwise.

19. Centrifuge cells at 1200 rpm for 5 min at 4 °C with the break switched off
20. Remove supernatant, tap tube against hand to loosen the pellet
21. Add chilled Lysis buffer (see reagents; 200 µL/10^6 cells)
22. Invert tube gently until the pellet is re-suspended
23. Incubate for 90 min at 4 °C rotating at 7 rpm
24. Centrifuge cells at 1200 rpm for 5 min at 4 °C with the break switched off
25. Remove supernatant and tap tube against hand to loosen the pellet (image cells, see Fig.2b for representative images of cells at this point)
26. Add room temperature Nucleus Break buffer (see reagents; 200 µl/10^6 cells)

27. Invert tube gently until the pellet is re-suspended
28. Incubate at 37 °C for 45 min whilst gently rotating
29. Centrifuge cells at 1200 rpm for 5 minutes at room temperature with the break switched off. Note: room temperature is required to avoid the precipitation of SDS.
30. Remove the supernatant. Pellet may be loose, remove as much supernatant as possible without disturbing the pellet
31. Add chilled 1 × NEBuffer 2 (see reagents) + 0.1 % Triton X-100 (freshly added) so that the final volume in the tube is equivalent to 10 million cells/mL
32. Re-suspend by pipetting up and down and transfer nuclei to 2 ml Eppendorf tubes (10 million nuclei/tube i.e. 1 mL/tube)
Note: familiarise yourself with steps 33-35 before proceeding.
33. Place samples on ice and add Proteinase K (20 mg/ml) to a final concentration of 100 µg/ml
34. Vortex samples briefly at minimum power and then incubate for 8 minutes at 37 °C in a water bath
35. Work quickly: Place samples on ice and immediately add an equal volume of NEBuffer 2 + 0.1 % Triton X-100 (freshly added) + 1:50 PMSF (freshly added; filter buffer through 0.2 µm filter before adding)
36. Invert tubes to mix
Note: now that cells are in 2 ml Eppendorf tubes centrifuge samples in a bench top microcentrifuge (e.g. Eppendorf 5415R).
Critical steps applying to all centrifugation steps until the nuclei are lysed: the pellet may not be visible in the tube by eye. Centrifuge with the hinge of the Eppendorf tube facing outwards so that the pellet will accumulate against the wall of the tube at the side with the hinge. Knowing the position of the pellet carefully remove as much of the supernatant as possible (approximately 50 µL can be left behind). If the pellet is accidentally removed it may be visible in the pipette tip. Do not discard the supernatant, but transfer it to another Eppendorf tube. Check the supernatant for nuclei (see below) – if the supernatant contains nuclei, centrifuge the supernatant again with a spin speed 1000 g greater than the indicated spin speed. Remove the supernatant and add the recovered nuclei back to the original tube (this is most easily done by adding the wash buffer or reaction buffer for the subsequent step to the pelleted nuclei and then transferring the nuclei/buffer solution back the original tube. Also make sure that the supernatant from this repeated centrifugation step is free from nuclei. Different cell types behave differently during the centrifugation steps and thus require slightly different spin-speeds. It is important not to loose material during the wash steps so we recommend checking the supernatant for nuclei after each centrifugation step and to re-centrifuge if necessary. Checking for nuclei: mix 10 µL of supernatant with 10 µL trypan blue and visualise nuclei using a light microscope. We use Ibidi slides to aid this process.
37. Centrifuge the nuclei at 1200 g for 10 min at 4 °C and remove the supernatant
38. Add chilled 1 × NEBuffer 2 + 0.1 % Triton X-100 (freshly added) (100 µL /10^6 cells). Close tube and invert rapidly to re-suspend the pellet
39. Centrifuge the nuclei at 1200 g for 10 min at 4 °C and remove the supernatant
40. Add chilled 1 × NEBuffer 2 + 0.1% Triton X-100 (freshly added) (100 µL/10^6 cells). Close tube and invert rapidly to re-suspend the pellet
Image nuclei: see Fig.2c for representative images of nuclei at this point.
41. Store nuclei at 4 °C overnight or alternatively proceed immediately to day 3

Day 3: *In situ* adapter ligation (modified P5 Illumina adapter)

Work on ice unless specified otherwise.
Note: anneal the modified P5 Illumina adapter with its complement before use (see methods in manuscript).

42. Centrifuge the nuclei at 1200 g for 10 min at 4 °C and remove the supernatant
43. Add chilled 1 × Blunting Buffer + 100 µg/ml BSA (200 µl/10^6 nuclei). Close tube and invert rapidly to re-suspend the pellet
44. Centrifuge the nuclei at 1200 g for 10 min at 4 °C and remove the supernatant.
45. Add 1 × Blunting Buffer + 100 µg/ml BSA to the pellet to create a final volume of roughly 86 µl/tube, add:

- 10 µl 1 mM dNTPs
- 4 µl Blunting Enzyme Mix

46. Mix gently by pipetting up and down 10 times
47. Incubate the nuclei for 45 min at 25 °C, shaking at 800 rpm for 10 sec every 5 min

48. Wash the nuclei 3 × in chilled 1 × NEBuffer 2 + 0.1 % Triton X-100 (freshly added) (100 µl/10^6 nuclei)
49. Centrifuge the nuclei at 2200 g for 10 min at 4 °C, between each wash and remove the supernatant. After the addition of wash buffer close the tube and invert rapidly to re-suspend the pellet
50. Add chilled 1 × NEBuffer 2 without Triton X-100 (100 µl/10^6 nuclei). Close tube and invert rapidly to re-suspend the pellet
51. Centrifuge the nuclei at 2200 g, remove the supernatant and leave behind approximately 50 µl (if less than 50 µl was left behind add more 1 × NEBuffer 2 to make volume up to 50 µl, add:

- 0.5 µl 10 mM dATP
- 3 µl Klenow Fragment exo-

52. Mix gently by pipetting up and down 10 times
53. Incubate the nuclei at 37 °C for 45 min, shaking at 800 rpm for 10 sec every 10 min
54. Wash the nuclei 3 × in chilled 1 × NEBuffer 2 + 0.1% Triton X-100 (freshly added) (100 µl/10^6 nuclei)
55. Centrifuge the nuclei at 2200 g for 10 min at 4 °C between each wash and remove the supernatant. After the addition of wash buffer close the tube and invert rapidly to re-suspend the pellet
56. Add chilled 1 × T4 Ligase Reaction Buffer + 0.1 % Triton X-100 (freshly added) (100 µl/10^6 nuclei), close the tube and invert rapidly to re-suspend the pellet
57. Centrifuge nuclei at 2200 g for 10 min at 4 °C
58. Remove the supernatant
59. Add chilled 1 × T4 Ligase Reaction Buffer without Triton X-100 (100 µl /10^6 nuclei), close the tube and invert rapidly to re-suspend the pellet
60. Centrifuge the nuclei at 2200 g for 10 min at 4 °C. Remove the supernatant, leaving behind approximately 40 µl, add:

- 10 µl 10 µM modified P5 Illumina adapter
- 0.6 µl T4 DNA Ligase (2,000,000 units/mL)

61. Prepare one control without ligase (10 million nuclei)
62. Mix gently by pipetting up and down 10 times
63. Incubate at 16 °C for 15-20 h, shaking at 350 rpm, every 45 min for 15 sec.

Day 4: Excess adapter removal and DNA extraction

Work at room temperature unless specified otherwise.

64. Wash nuclei twice in 1 × W&B buffer + 0.1 % Triton X-100 (freshly added) (100 µl/10^6 nuclei)
65. Centrifuge nuclei at 3500 g for 10 min at 25 °C between each wash and remove the supernatant. After the addition of wash buffer close the tube and invert rapidly to re-suspend the pellet.
Note: when checking supernatant for nuclei, first dilute the supernatant in water, otherwise aggregates form upon the addition of trypan blue).
66. Add 1 × Lambda Exonuclease reaction buffer (50 µl/10^6 nuclei), close the tube and invert rapidly to re-suspend the pellet
67. Centrifuge nuclei at 3500 g for 10 min at 25 °C
68. Remove the supernatant
69. Make volume up to 50 µl/10 million cells using 1 × Lambda Exonuclease reaction buffer
70. Add 50 units of Lambda Exonuclease
71. Mix gently by pipetting up and down 10 times
72. Incubate at 37 °C for 30 min
From here on in work at 4 °C unless specified otherwise.
73. Add chilled 1 × NEBuffer 2 + 0.1 % Triton X-100 (freshly added) (100 µl/10^6 nuclei), close the tube and invert rapidly to re-suspend the pellet
74. Centrifuge at 4500 g for 10 min at 4 °C
75. Remove the supernatant
76. Add chilled 1 × NEBuffer 2 + 0.5 % Triton X-100 (freshly added) (100 µl/10^6 nuclei), close the tube and invert rapidly to re-suspend the pellet
Note: set aside 5 µl.
77. Add 20 mg/ml Proteinase K to a final concentration of 200 µg/ml
78. Incubate for 30 min at 55 °C, shaking at 800 rpm

79. Incubate for 30 min at 65 °C, shaking at 800 rpm
Note: whilst the nuclei are digesting image the nuclei using the 5 µl that were set aside (see Fig.2d for representative images of nuclei at this point).
80. Centrifuge the sample at maximum speed for 1 min
81. Place the samples on ice and divide each tube into 2 (500 µl per tube)
82. Add 214 µl 1 M potassium acetate per tube
83. Vortex for 5 sec
84. Add 2 µl GlycoBlue per tube
85. Vortex for 5 sec
86. Add 500 µl ice cold propan-2-ol per tube
87. Vortex at max speed until the aqueous and organic phases have mixed
88. Chill samples on ice for 20 min, store at -80 °C overnight
Note: samples are stable at this point and can be stored for longer periods at -80 °C.

Day 5: DNA fragmentation

Work on ice unless stated otherwise.

89. Centrifuge samples for 30 min at max speed, 4 °C
90. Remove the supernatant
91. Add 500 µl ice cold 70 % ethanol per tube
92. Vortex for 5 sec
93. Centrifuge for 5 min at max speed, 4 °C
94. Remove the supernatant
95. Add 500 µl ice cold 70 % ethanol per tube
96. Vortex for 5 sec
97. Centrifuge for 5 min at max speed, 4 °C
98. Remove any remaining ethanol
99. Air dry pellets
100. Dissolve each pellet in 90 µl of nuclease-free water
101. Incubate samples at 55 °C, shaking at 800 rpm for 1 h
102. Centrifuge for 1 min at maximum speed, pool two tubes of one kind together (180 µl total)
Note: material may be extremely gloopy, this is normal and expected.
103. Transfer material to a sonication tube and sonicate: we use the Biorupter plus on HIGH setting with parameters: 30 seconds ON; 30 seconds OFF for a total of approx. 25 cycles, with the water bath chilled to 4 °C
Note: aim for an average fragment length of 200-500 bp. After 15 cycles, image 1 µl DNA on an agarose gel to determine the average fragment length and assess whether further cycles of sonication are necessary.
104. Once the desired fragment length has been obtained transfer DNA to LoBind Eppendorf tube
Note: work with DNA in LoBind Eppendorf tubes wherever possible.
105. Quantify DNA (e.g. Qubit fluorimeter)
106. Store samples at -20 °C or alternatively proceed directly to day 6

Day 6: Second adapter ligation (modified P7 Illumina adapter)

Work at room temperature unless specified otherwise.
Note: anneal the modified P7 Illumina adapter with its complement before use (see methods in manuscript).

107. Use 5 µl Dynabeads MyOne Strepdavidin C1 per 50 µg of DNA
Note: 10 million cells should yield over 50 µg of DNA but if less DNA is available use as much as possible; we have also performed experiments using 20 µg and observed a similar number of peaks (see: Supplementary Fig. 4c in the manuscript).
108. Wash beads twice with 1 mL 1 × W&B buffer + 0.1 % Triton X-100 (freshly added)
109. Add an equal volume of 2 × W&B buffer to the DNA to create a 1 × final concentration
110. Add the DNA to the beads (50 µg of DNA to 5 µl beads/tube, make volume up to 0.6 mL using 1 × W&B buffer) and rotate at 7 rpm for 45 min at 4 °C
111. Place tubes onto magnetic rack to capture the beads and remove the supernatant

112. Wash beads 3 × in 1 mL 1 × W&B buffer + 0.1 % Triton X-100 (freshly added)
Critical step: when washing the beads, replace buffer immediately after removing it. Do not let the beads dry.
113. Re-suspend the beads in 42 µl 1 × Blunting Buffer, add:

- 0.8 µl 10 × Blunting Buffer
- 0.25 µl BSA (20 mg/mL)
- 5 µl 1 mM dNTPs
- 2 µl Blunting Enzyme Mix

114. Incubate for 45 min at 25 °C, shaking at 800 rpm every 5 min for 10 sec
115. Wash beads 3 × in 1 ml 1 × W&B buffer + 0.1 % Triton X-100 (freshly added)
Critical step: when washing the beads, replace buffer immediately after removing it. Do not let the beads dry.
116. Re-suspend sample in 23 µl 1 × NEBuffer 2 without Triton X-100, add:

- 0.5 µl 5 mM dATP
- 1.5 µl Klenow Fragment exo-

117. Incubate at 37 °C for 45 min, every 10 min shake for 10 sec at 800 rpm
118. Wash beads 3 × in 1 ml 1 × W&B buffer + 0.1 % Triton X-100 (freshly added)
Critical step: when washing the beads, replace buffer immediately after removing it. Do not let the beads dry.
119. Re-suspend sample in 39.4 µl 1 × T4 Ligase Reaction Buffer, add:

- 10 µl 10 µM modified P7 Illumina adapter
- 0.6 µl T4 DNA Ligase (2,000,000 units/mL)

120. Ligate for 15-20 h at 16 °C, every 45 min shake samples for 1 min at 1200 rpm

Day 7: Library amplification

121. Wash beads 3 × in 1 ml W&B buffer + 0.1 % Triton X-100 at room temperature
Critical step: when washing the beads, replace buffer immediately after removing it. Do not let the beads dry.
122. Re-suspend beads in 25 µl nuclease free water
123. Place samples on ice
From this point forward work on ice for the rest of the day unless stated otherwise.
124. Perform PCR: each 25 µl sample will make up 5 reactions
Note: Multiple PCR R (reverse) primers are available (see end of protocol). Each has a different barcode sequence to enable multiplexing of samples on the Illumina platform during sequencing. If multiple samples will be sequenced together in one sequencing reaction ensure that each sample is amplified with a different PCR R primer. Ensure to record the AD number of the primer used to amplify each sample. The AD numbers reflect Illumina TruSeq adapter sequences and thus DSBCapture libraries can be sequenced like Illumina TruSeq libraries (i.e. single index, 6 bp).
125. PCR reagents per reaction:

- 1.25 µl 20 µM F primer
- 1.25 µl 20 µM R primer
- 5 µl Beads
- 17.5 µl nuclease free water
- 25 µl NEBNext High-Fidelity 2 × Master Mix

Note: after the addition of all reagents pipette sample up down, do not centrifuge as this will cause the beads to pellet.
126. Cycling parameters (total of 15 cycles):

1. 98 °C for 30 sec

2. 98 °C for 10 sec
3. 65 °C for 30 sec
4. 72 °C for 30 sec
Loop back to 2. 14 times, for a total of 15 cycles
5. 72 °C for 5 min

127. Centrifuge to pellet the beads and recover PCR product (supernatant). Store the PCR product at -80 °C or alternatively proceed directly to day 8. Beads can now be discarded.

Day 8: Size selection, quantification and sequencing

128. Either pass the PCR sample through a Qiagen MinElute PCR clean up column (100 µl PCR product / column) and then load sample into BluePippin gel extraction system (1.5 % agarose cassette) and extract 250-1200 bp OR omit PCR clean up step and directly load PCR product into 2 % hand cast agarose gel; manually extract band at 250-1200 bp and then clean up the DNA using Qiagen MinElute gel extraction kit.
Note: if performing manual gel extraction: the DNA concentration can sometimes be low and therefore the DNA may not always be visible, in such cases cut the gel using the reference ladder as a guide and continue. The DNA will be visible on the subsequent Bioanalyser profile.
129. Run sample on Bioanalyser or TapeStation (high sensitivity chip) to determine the library size. All negative controls should not contain any DNA. See anticipated results (Fig.3)
130. Quantify DNA using the KAPA Library Quantification Kit
131. Proceed to sequencing and store excess DNA at -20 °C

Oligonucleotide sequences

Modifications: P = phosphorylated, * = phosphorothioate linkage, Btn = biotin
Oligonucleotides are shown in the 5'-3' orientation
Oligonucleotides have HPLC purity

• Modified P5 Illumina adapter:

BtnA*ATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATC*T

• Control modified P5 Illumina adapter:

A*ATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATC*T

• Modified P5 Illumina adapter complement / Control modified P5 Illumina adapter complement:

PGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGTAGATCTCGGTGGTCGCCGTATCATT

• Modified P7 Illumina adapter:

PGATCGGAAGAGCACACGTCTGAACTCCAGTCAC

• Modified P7 Illumina adapter complement:

GTGACTGGAGTTCAGACGTGTGCTCTTCCGATC*T

• PCR Forward primer:

AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGA

Reverse primers contain Illumina barcodes (AD); the number refers to the sequence of the barcode.
Note: PCR amplify each sample with a different reverse primer to enable multiplexing of samples during sequencing. If only one sample is made choose any PCR R Primer. If two samples are to be sequenced together choose either the combination of AD06 and AD12 or AD05 and AD19. If 3 or more samples are to be sequenced together choose AD06 and AD12 plus any others or AD05 and AD19 plus any others (see:

TruSeq Library Prep Pooling Guide[2] for more information on multiplexing).
• PCR R reverse primer AD01:
CAAGCAGAAGACGGCATACGAGATCGTGATGTGACTGGAGTTCAGACGTGT
• PCR R reverse primer AD02:
CAAGCAGAAGACGGCATACGAGATACATCGGTGACTGGAGTTCAGACGTGT
• PCR R reverse primer AD03:
CAAGCAGAAGACGGCATACGAGATGCCTAAGTGACTGGAGTTCAGACGTGT
• PCR R reverse primer AD04:
CAAGCAGAAGACGGCATACGAGATTGGTCAGTGACTGGAGTTCAGACGTGT
• PCR R reverse primer AD05:
CAAGCAGAAGACGGCATACGAGATCACTGTGTGACTGGAGTTCAGACGTGT
• PCR R reverse primer AD06:
CAAGCAGAAGACGGCATACGAGATATTGGCGTGACTGGAGTTCAGACGTGT
• PCR R reverse primer AD12:
CAAGCAGAAGACGGCATACGAGATTACAAGGTGACTGGAGTTCAGACGTGT
• PCR R reverse primer AD19:
CAAGCAGAAGACGGCATACGAGATCGTTTCACGTGACTGGAGTTCAGACGTGT

Anticipated Results

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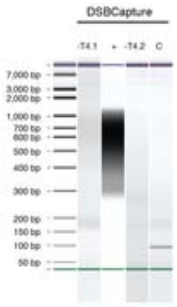
Fig.3 shows a completed DSBCapture library that is ready for sequencing.



References

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Figures

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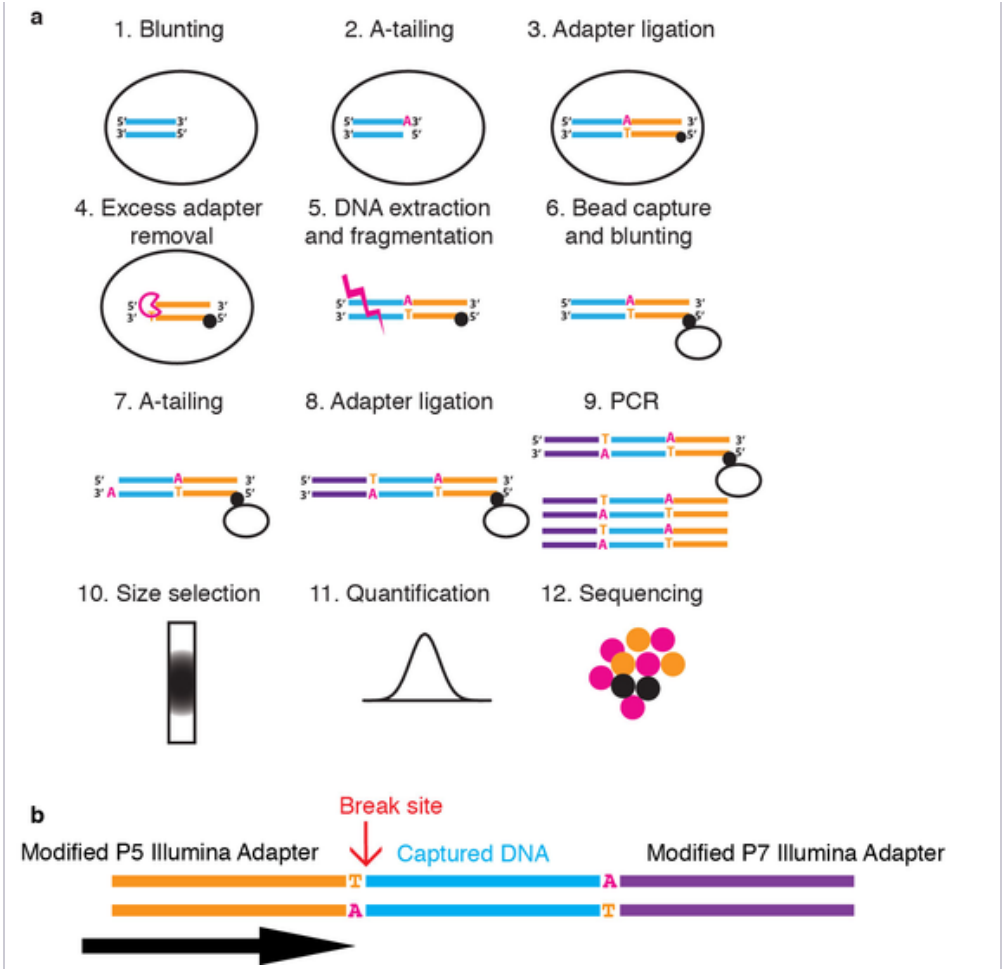
Figure 1: DSBCapture workflow



(a) DNA processing (1) DSBs in fixed nuclei are blunt-end repaired, (2) A-tailed and (3) ligated to a biotinylated (black ball) modified P5 Illumina adapter (orange lines). (4) Excess adapters are removed by Lambda Exonuclease digestion; (5) DNA is extracted from lysed nuclei and fragmented by sonication, (6) bead-captured (hollow ball) and blunted-end repaired, (7) A-tailed, and (8) ligated to a modified P7 Illumina adapter (purple lines). (9) Captured break sites are PCR amplified, (10) size-selected, (11) quantified and (12) sequenced. (b) Sequencing of the completed DSBCapture library. Large black arrow indicates the site at which sequencing is initiated; the first base sequenced is the site of *in situ* DSB formation.

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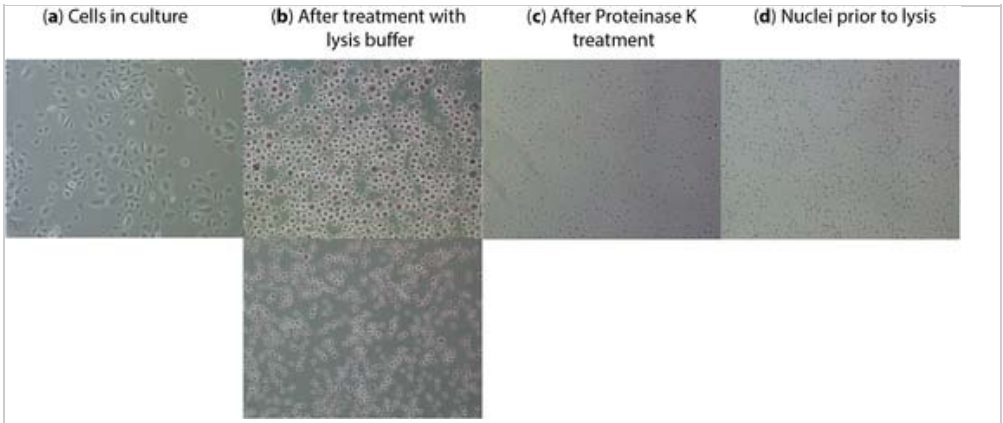
Figure 2: Representative images of cells and nuclei at various stages



(a) cells in culture, (b) cells after treatment with Lysis buffer (two different cell lines are shown), (c) nuclei after an 8 min treatment with Proteinase K and (d) nuclei prior to lysis with Proteinase K.

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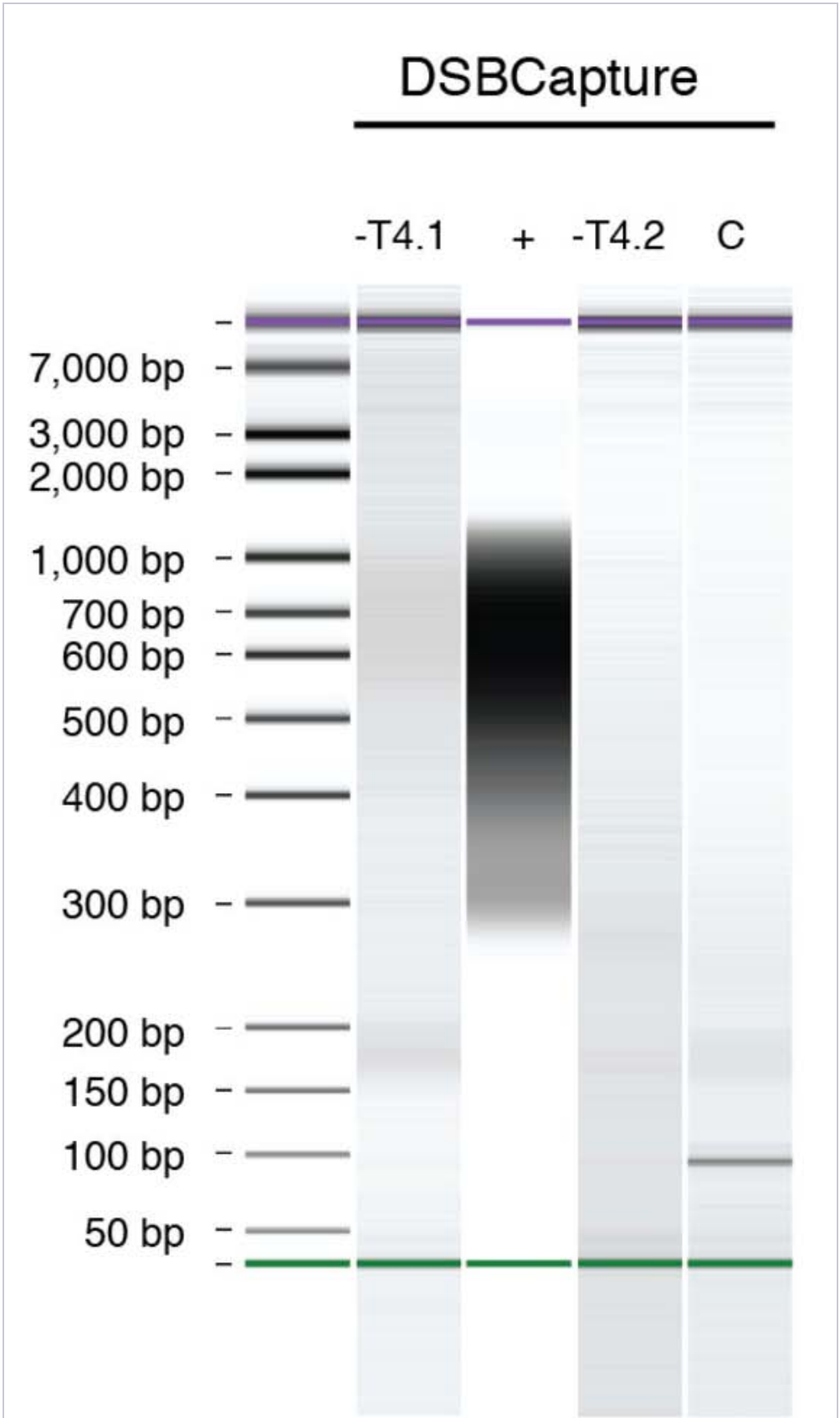
Figure 3: Expected results: DNA library



Bioanalyser profiles of the DNA products from DSBCapture libraries. The anticipated result of a DSBCapture library is shown (+). No product should be present in the negative controls performed. Shown are controls performed without T4 DNA ligase during the first (-T4.1) or second (-T4.2) ligation reactions

and for a control performed with the non-biotinylated control modified P5 Illumina adapter (C).

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Associated Publications

Main ▪ Abstract ▪ Introduction ▪ Reagents ▪ Equipment ▪ Procedure ▪ Anticipated Results ▪ References ▪ Figures ▪ Associated Publications ▪ Author Information

This protocol is related to the following articles:

DSBCapture: in situ capture and sequencing of DNA breaks

Stefanie V Lensing, Giovanni Marsico, Robert Hänsel-Hertsch, Enid Y Lam, David Tannahill, and Shankar Balasubramanian

Nature Methods | doi:10.1038/nmeth.3960

Author information

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Competing financial interests

The authors declare no competing financial interests.

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