A

SPO11 oligos (RPM)

60

0

500 bp

DSB sites

5′
3′

3′
5′

Normalized SSDS coverage

Watson

Crick

1

0

1

ssDNA coverage

−2000   0   2000

Position relative to hotspot center (bp)

B

Possible resection at a certain position (Crick strand):

	Position of DSBs	**Length of resection**
i	On site	>0 bp
	1 bp to the left	>1 bp
	2 bp to the left	>2 bp
	3 bp to the left	>3 bp
	...	...
	x bp to the left	>x bp

$$\text{ssDNA coverage at position } i = \sum_{x=0}^{d} \left(\begin{array}{l}\text{Frequency of DSBs at } x \text{ bp} \\ \text{to the left of position } i\end{array} \cdot \begin{array}{l}\text{Frequency of resection} \\ \geq x \text{ bp in length}\end{array} \right) + \text{Error at position } i$$
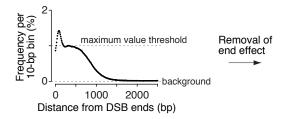
d = distance from position i to left end of chromosome

Possible resection at a certain position (Watson strand):

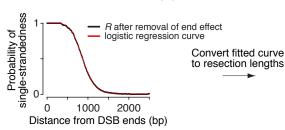|	**Position of DSBs**	**Length of resection**
i	On site	>0 bp
	1 bp to the right	>1 bp
	2 bp to the right	>2 bp
	3 bp to the right	>3 bp
	...	...
	x bp to the right	>x bp

$$\text{ssDNA coverage at position } i = \sum_{x=0}^{d} \left(\begin{array}{l}\text{Frequency of DSBs at } x \text{ bp} \\ \text{to the right of position } i\end{array} \cdot \begin{array}{l}\text{Frequency of resection} \\ \geq x \text{ bp in length}\end{array} \right) + \text{Error at position } i$$

d = distance from position i to right end of chromosome
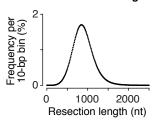
C

Initial resection model (R')

Frequency per 10-bp bin (%)

2

0

maximum value threshold

background

0   1000   2000

Distance from DSB ends (bp)

Removal of end effect →
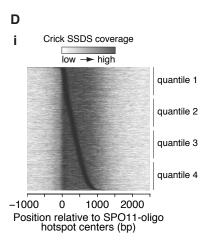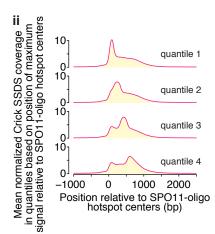
Corrected resection model (R)

Probability of single-strandedness

1

0

— R after removal of end effect
— logistic regression curve

0   1000   2000

Distance from DSB ends (bp)

Convert fitted curve to resection lengths →
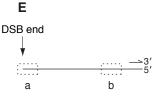
Estimated distribution of resection tract lengths

Frequency per 10-bp bin (%)

2

0

0   1000   2000

Resection length (nt)

D

i

Crick SSDS coverage

low → high

quantile 1
quantile 2
quantile 3
quantile 4

−1000   0   1000   2000

Position relative to SPO11-oligo hotspot centers (bp)

ii

Mean normalized Crick SSDS coverage in quantiles based on position of maximum signal relative to SPO11-oligo hotspot centers

10
0   quantile 1
10
0   quantile 2
10
0   quantile 3
10
0   quantile 4

−1000   0   1000   2000

Position relative to SPO11-oligo hotspot centers (bp)

E

DSB end

3′
5′

a   b

a Short fragments that include the DSB end need to be sheared only **once** by sonication.

b Short fragments far from the DSB end need to be sheared **twice** by sonication.

F

Frequency per bp (%)

0.1

0

0   1000   2000

Distance from DSB ends (bp)