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Institut Pasteur

Meiotic silencing by unpaired DNA (MSUD) in *Neurosopora crassa*: a new approach to studying recombination-independent homologous pairing

Tinh-Suong NGUYEN

How are homologous chromosomes recognized in meiosis ?

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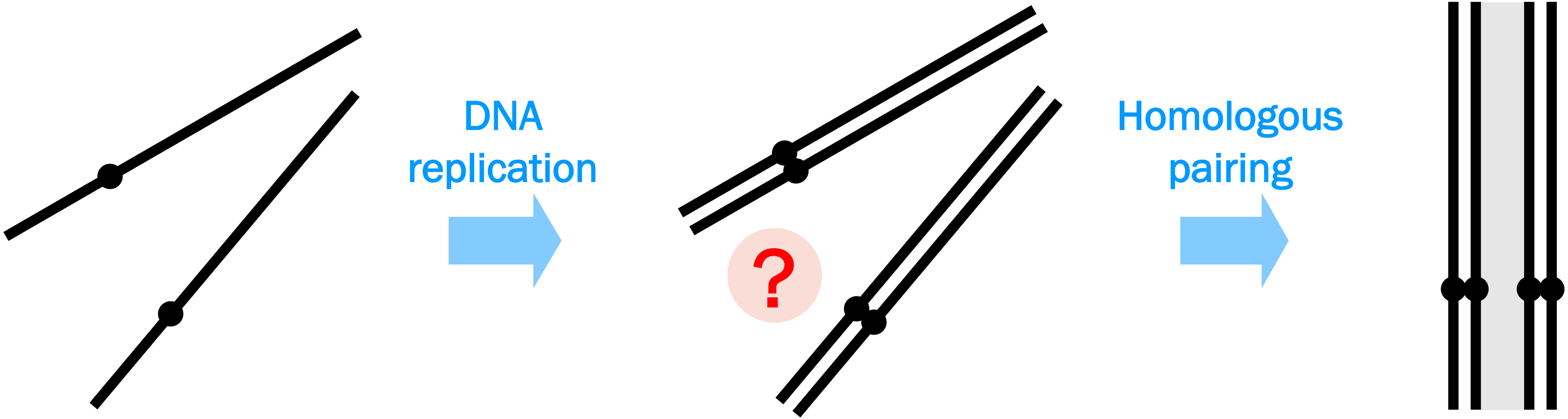

Recombination-mediated homologous pairing

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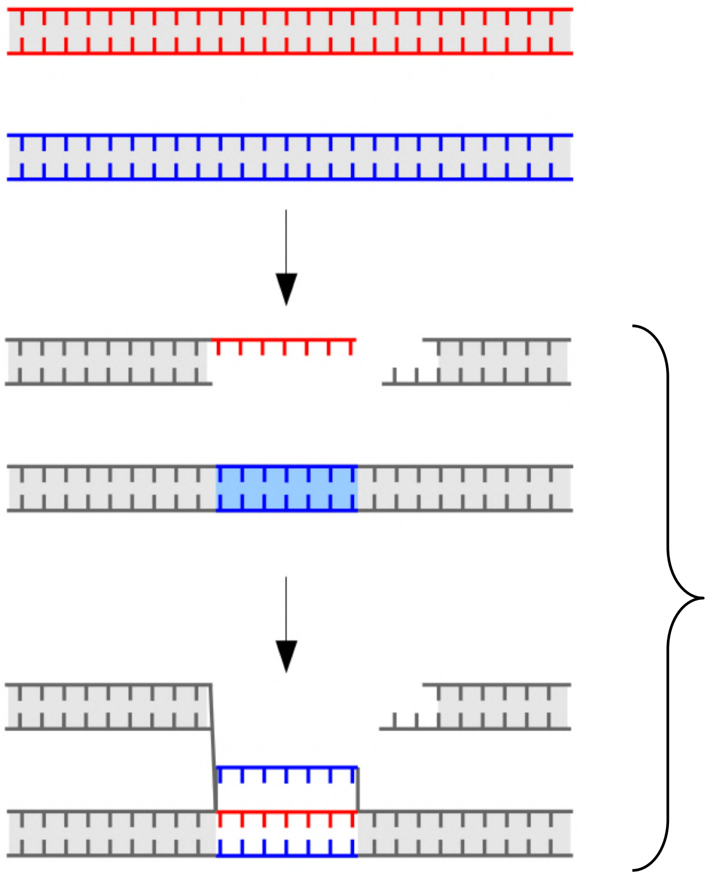


Double-strand break
(normally by **Spo11)**

Homology search
by **Rad51/Dmc1**

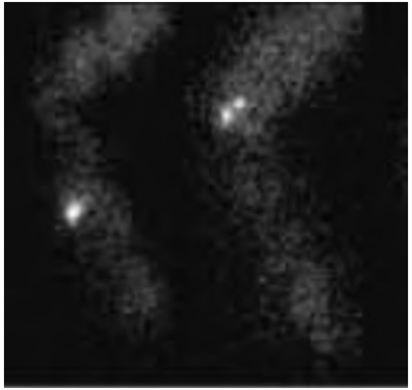
many steps

Widespread recombination-independent homologous pairing

In meiosis:

Fungi

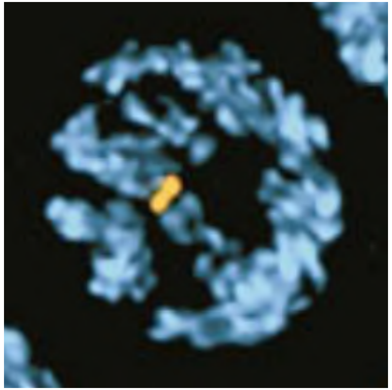
S. pombe



Dev Cell (2004) 6: 329-41

Worms

C. elegans



Cell (1998) 94: 387-98

Mammals

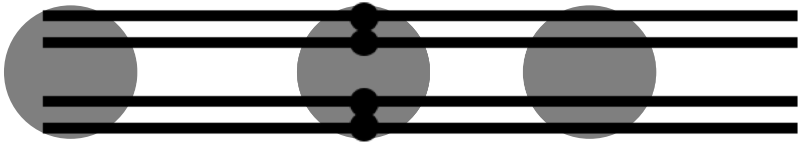
M. musculus

Dev Cell (2013) 24: 196-205

How are homologous chromosomes recognized in these situations ?

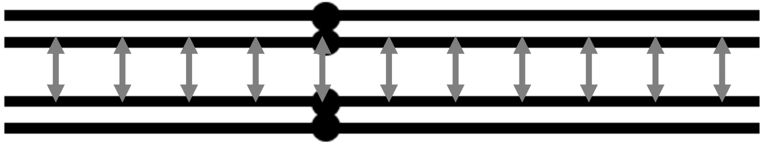
Recombination-independent homologous pairing: how?

- Landmark chromosomal features (centromeres, telomeres)
- Transcriptional factories
- Other forms of bar-coding by RNAs or proteins

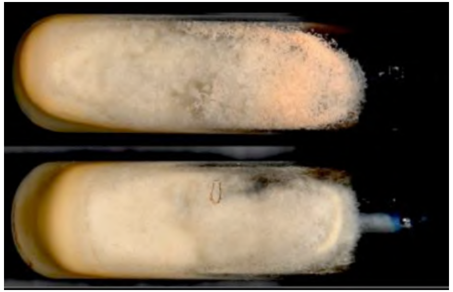


versus

- Direct pairing



Neurospora crassa, a model organism



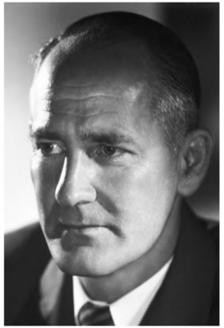


Photo from the Nobel Foundation archive.

George Wells Beadle

Prize share: 1/4

Photo from the Nobel Foundation archive.

Edward Lawrie Tatum

Prize share: 1/4

Photo from the Nobel Foundation archive.

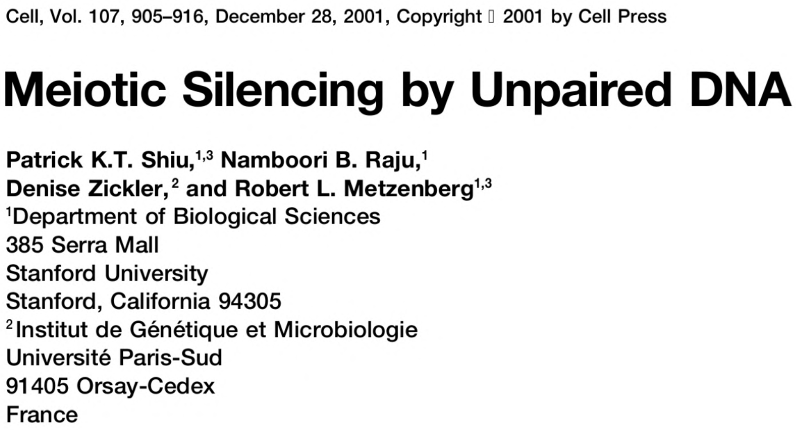
Joshua Lederberg

Prize share: 1/2

The Nobel Prize in Physiology or Medicine 1958

- *Ascomycota, Sordariomycetes, Sordariales*
- Genome sequenced: haploid, 43 Mb, 7 chromosomes ~10,000 genes
- Growth rate: 5 mm/h at 30°C
- Ease of culture, non-pathogenic
- Vegetative cycle (conidia)
  Sexual cycle (ascospores)
- Two sexual types : A and a
- 10-15 days for a complete sexual cycle

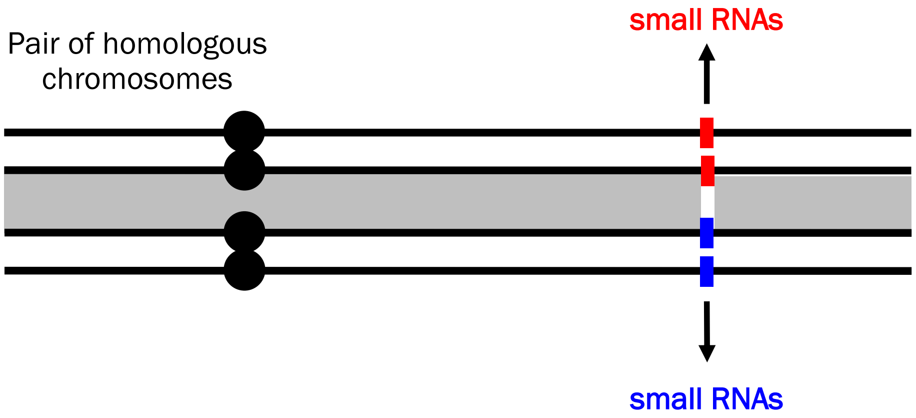
Meiotic Silencing by Unpaired DNA (MSUD)



Cell, Vol. 107, 905–916, December 28, 2001, Copyright © 2001 by Cell Press

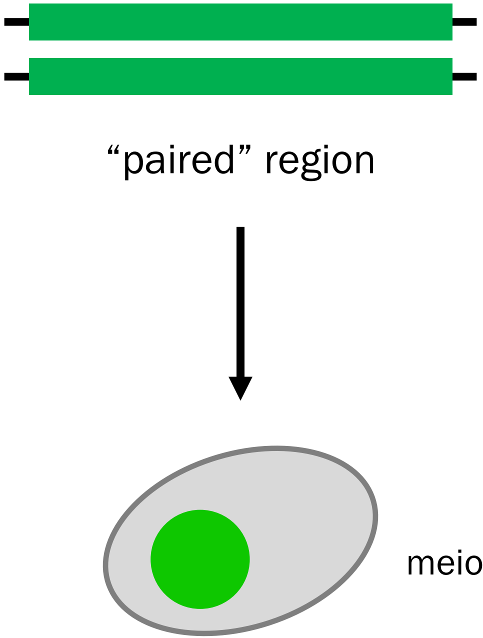
Meiotic Silencing by Unpaired DNA

Patrick K.T. Shiu,[1,3] Namboori B. Raju,[1]
Denise Zickler,[2] and Robert L. Metzenberg[1,3]
[1]Department of Biological Sciences
385 Serra Mall
Stanford University
Stanford, California 94305
[2]Institut de Génétique et Microbiologie
Université Paris-Sud
91405 Orsay-Cedex
France



- **General:** any DNA sequence can be recognized as unpaired
- **Sensitive:** such unpaired sequences can be as short as ~1 kbp
- **Efficient:** occurs in a few hours

Does MSUD require Rad51/Dmc1 and Spo11?



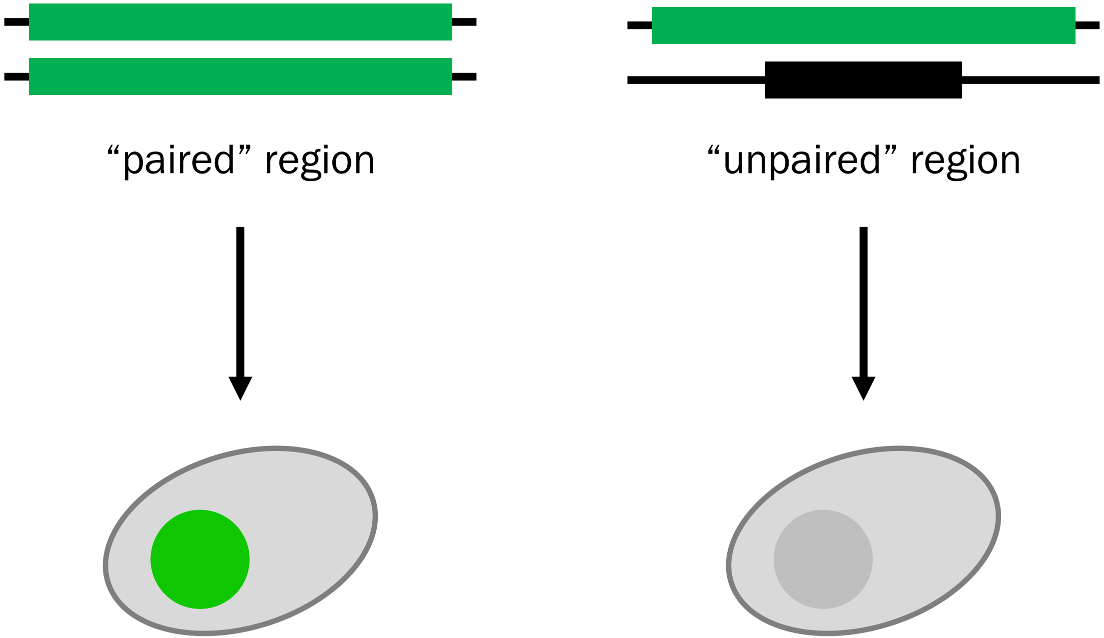
Histone H1 fused to GFP

“paired” region

meiotic cell (ascus)

hH1-GFP is expressed

Does MSUD require Rad51/Dmc1 and Spo11?



Histone H1 fused to GFP

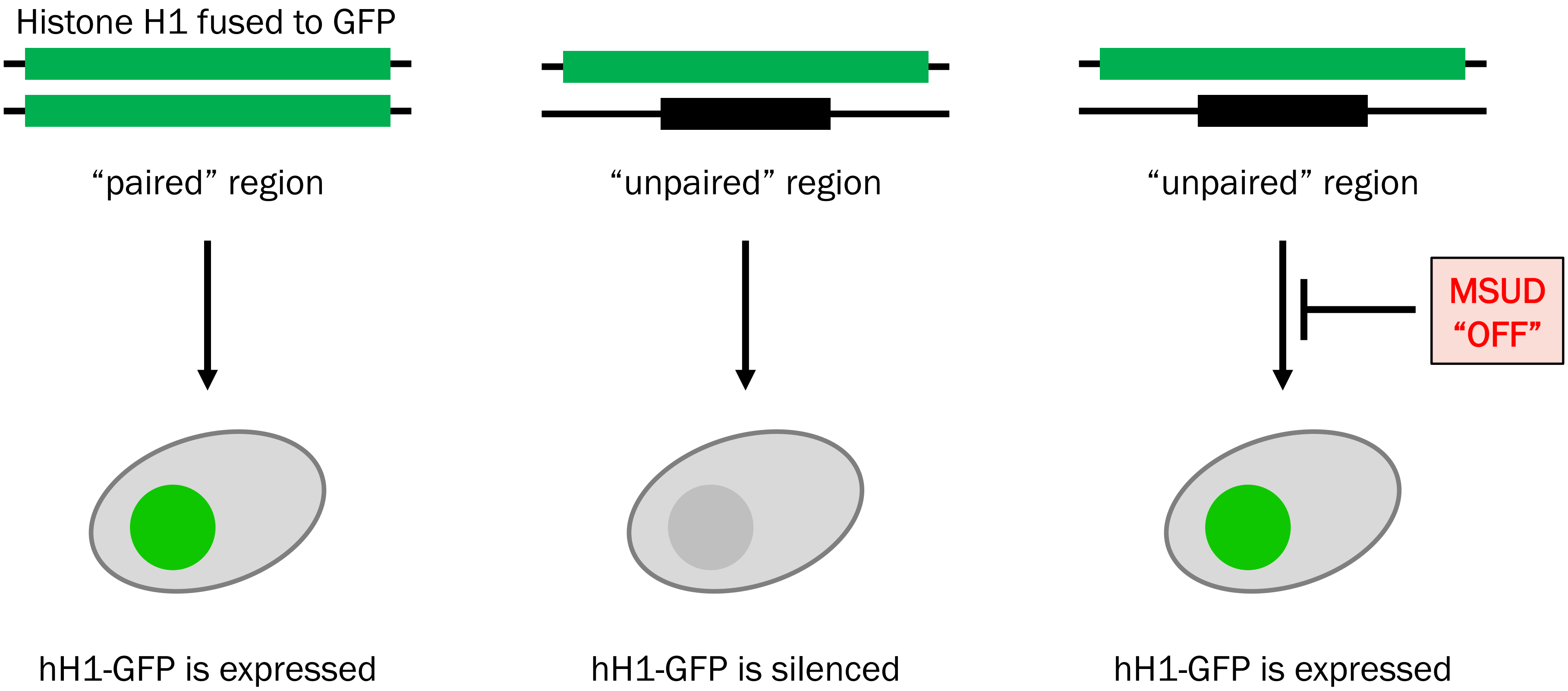
“paired” region

“unpaired” region

hH1-GFP is expressed

hH1-GFP is silenced

Does MSUD require Rad51/Dmc1 and Spo11?



Histone H1 fused to GFP

“paired” region

“unpaired” region

“unpaired” region

MSUD
“OFF”

hH1-GFP is expressed

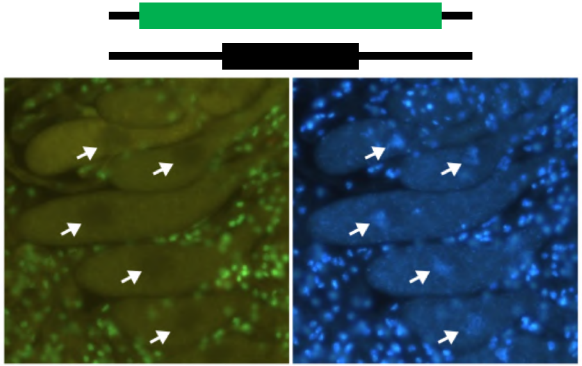
hH1-GFP is silenced

hH1-GFP is expressed

MSUD does not require Rad51/Dmc1 and Spo11

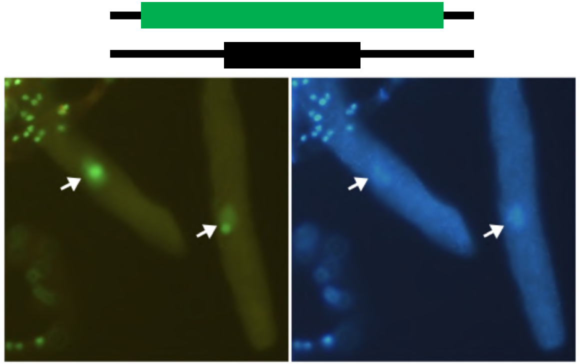
MEI-3 is the only Rad51/Dmc1-like recombinase in *N. crassa*

MEI-3 ✓ Spo11 ✓ MSUD ✓



silenced

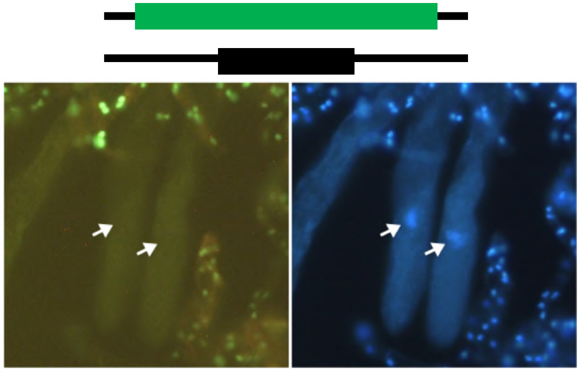
MEI-3 ✓ Spo11 ✓ MSUD ~



expressed

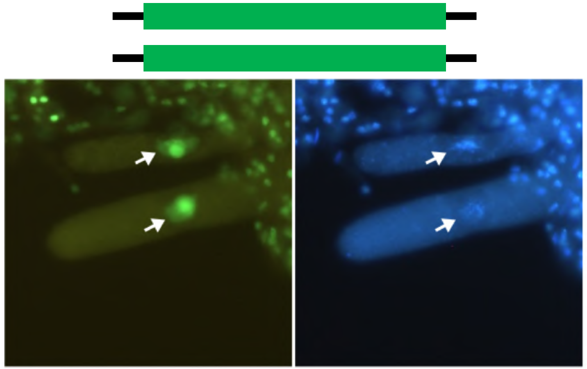
MEI-3 ✗: *mei-3* Δ/Δ
Spo11 ✗: *spo11* Δ/Δ
MSUD ~: *sad2+*/Δ

MEI-3 ✗ Spo11 ✗ MSUD ✓



silenced

MEI-3 ✗ Spo11 ✗ MSUD ✓



expressed

N. Rhoades *et al.*, preprint 2019

Another mechanism based on DNA homology: the Repeat Induced Point mutation (RIP) in *N. crassa*

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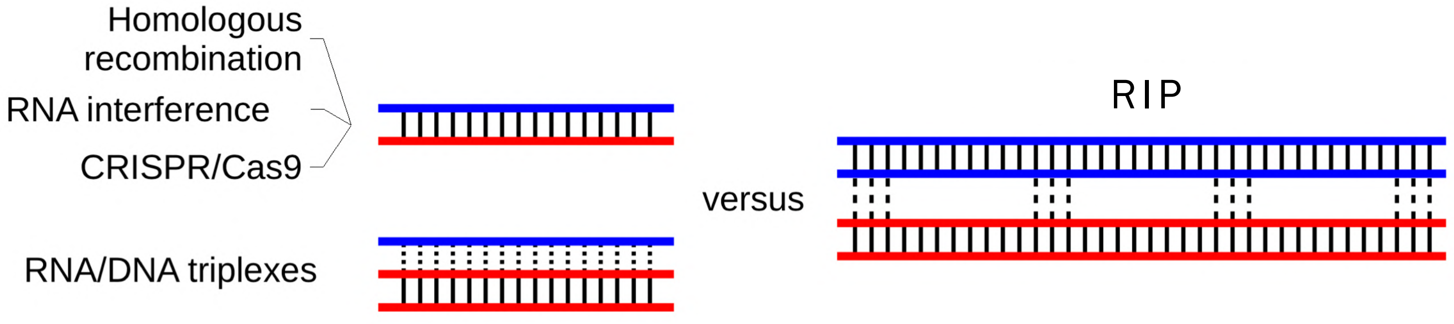
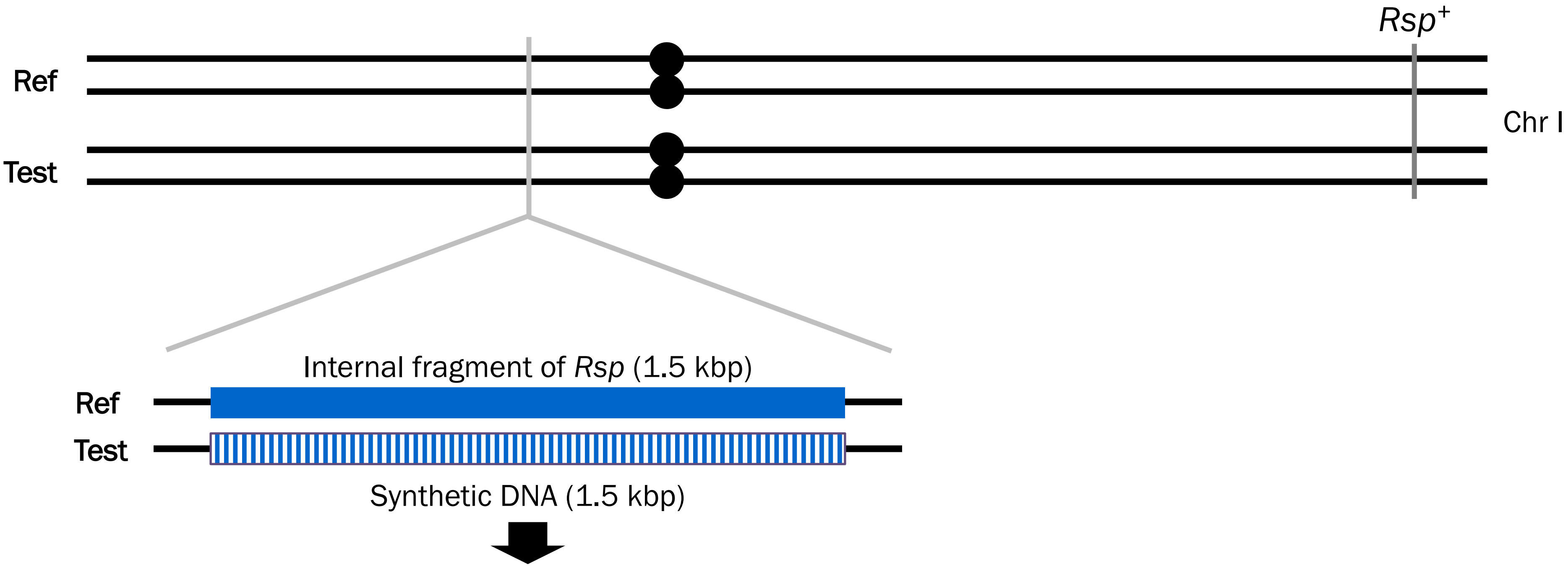


(Mazur *et al*., 2019)

- Does not require MEI-3 and Spo11
- DNA molecules are likely double-stranded and co-aligned
- Homology information is integrated over several hundred base-pairs
- Recognizes patterns of weak interspersed homology

Does MSUD recognize weak interspersed homology?



Rsp^+

Ref

Test

Chr I

Internal fragment of *Rsp* (1.5 kbp)

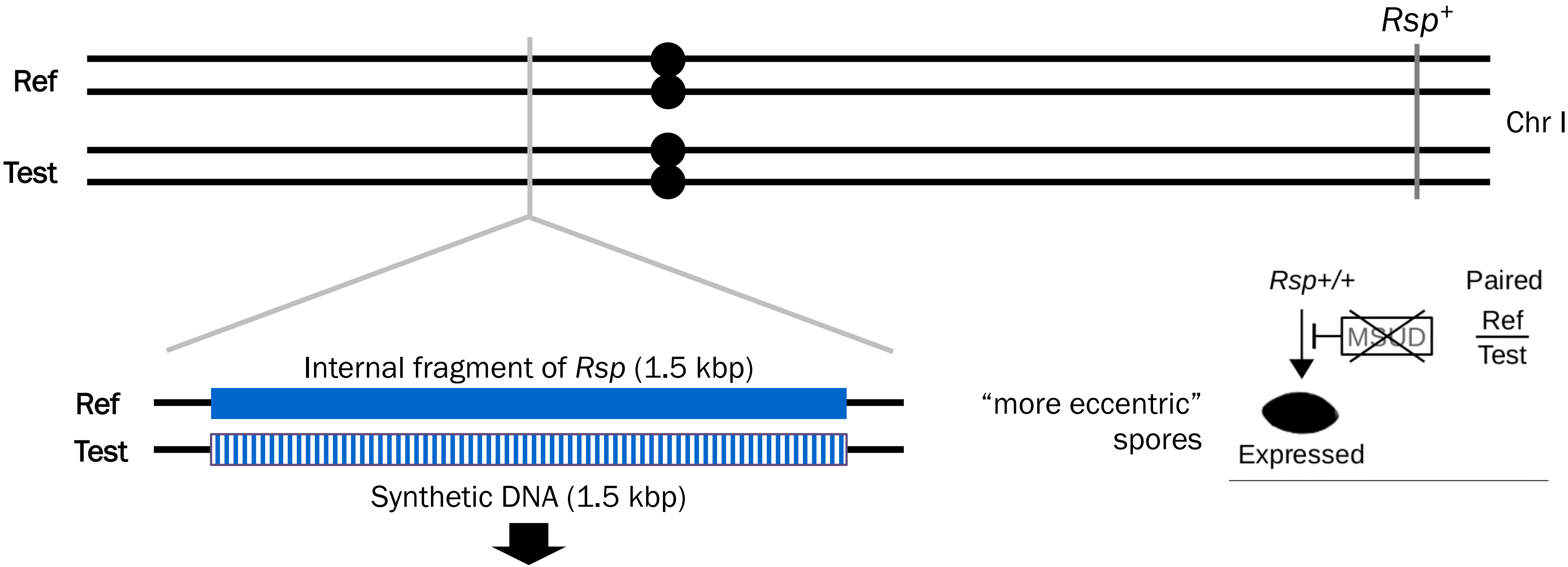
Ref

Test

Synthetic DNA (1.5 kbp)

pattern of homology is manipulated experimentally

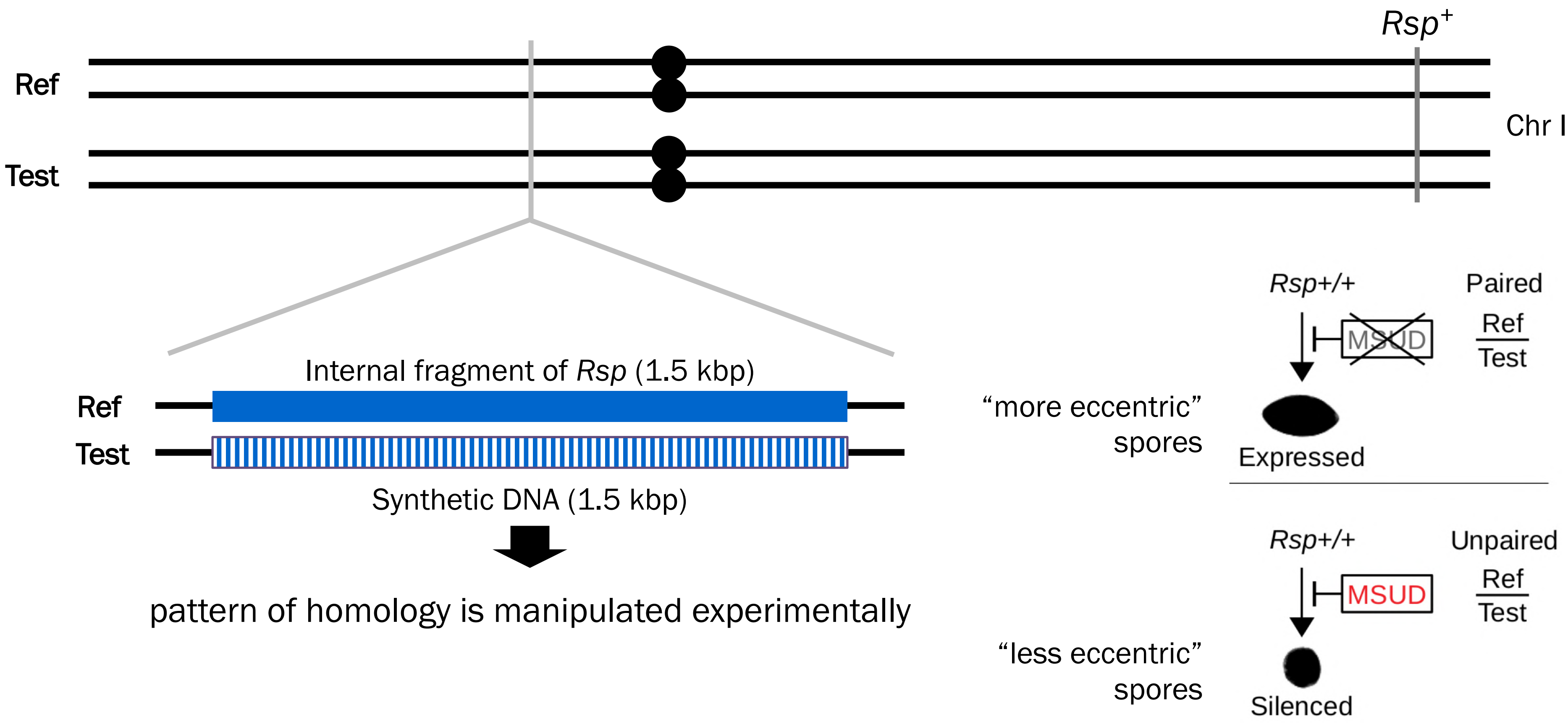
Does MSUD recognize weak interspersed homology?



"more eccentric" spores

pattern of homology is manipulated experimentally

Does MSUD recognize weak interspersed homology?



Rsp$^+$

Ref

Test

Chr I

Internal fragment of *Rsp* (1.5 kbp)

Ref

Test

Synthetic DNA (1.5 kbp)

pattern of homology is manipulated experimentally

Rsp+/+

Paired

~~MSUD~~

Ref
Test

“more eccentric” spores

Expressed

Rsp+/+

Unpaired

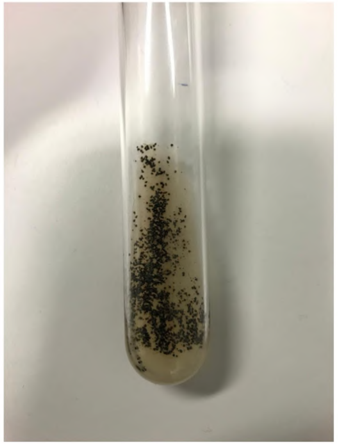
MSUD

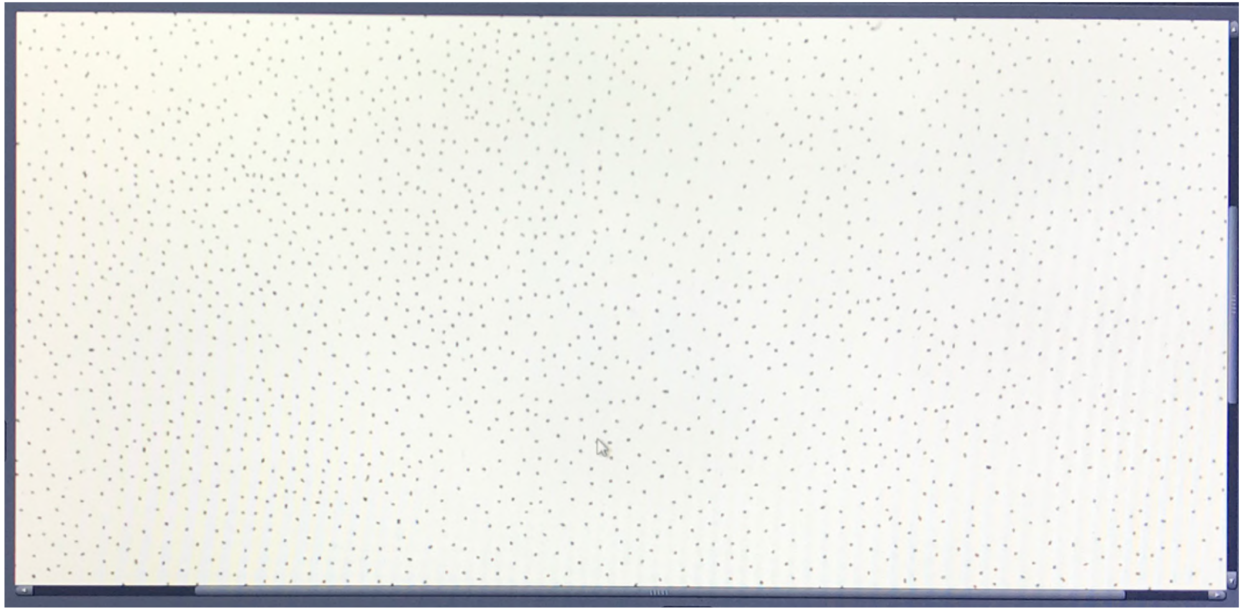
Ref
Test

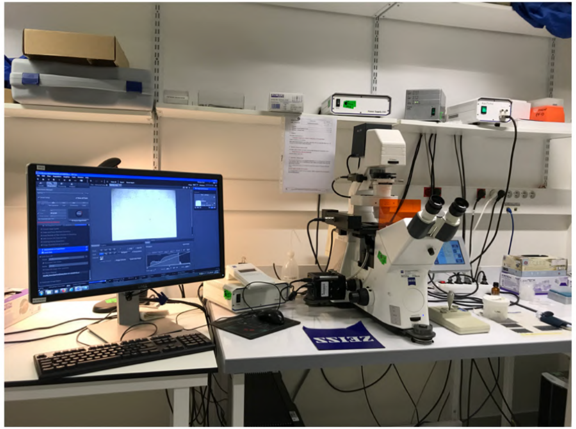
“less eccentric” spores

Silenced

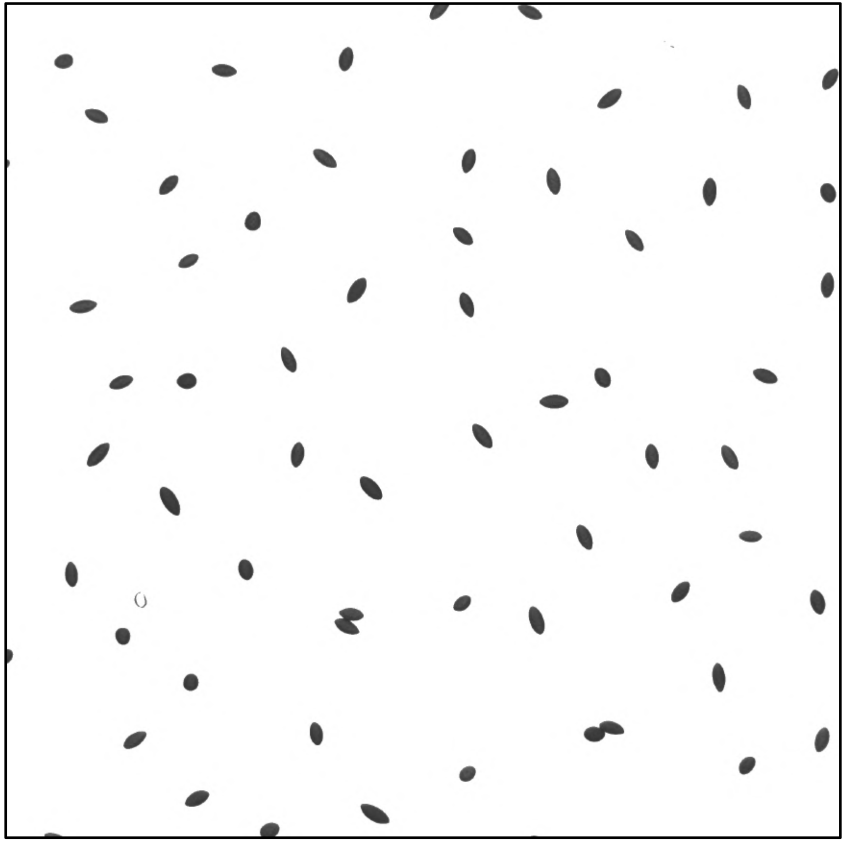
Roundspore assay: quantifying eccentricity





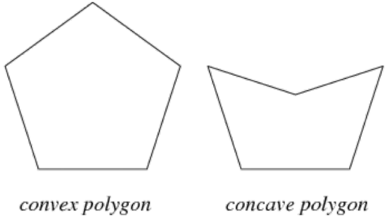


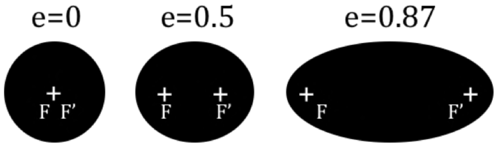
Roundspore assay: quantifying eccentricity



Code in python
- scikit-image
- scikit-learn

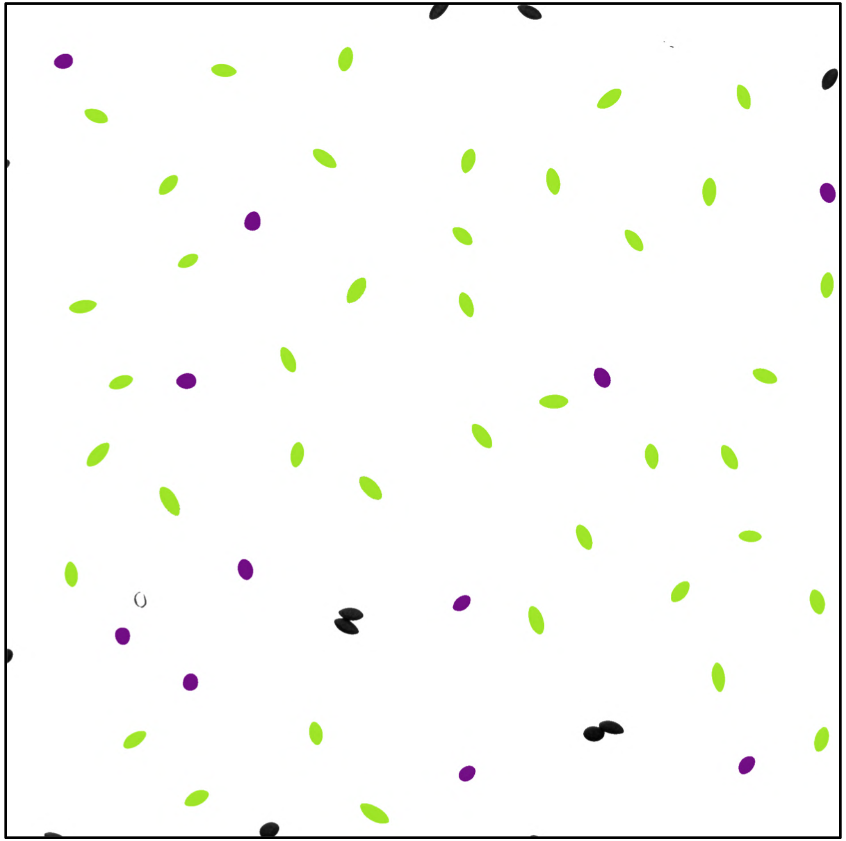
Spores are selected by
- area (min, max)
- convexity





Guillaume Witz
(Universität Bern)

Roundspore assay: quantifying eccentricity



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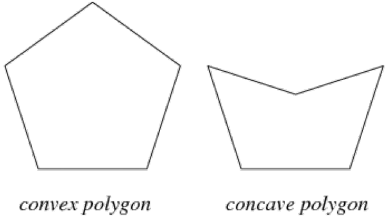

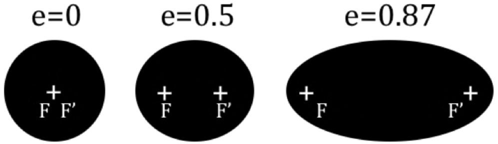

Guillaume Witz
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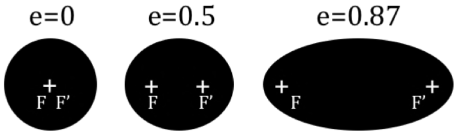
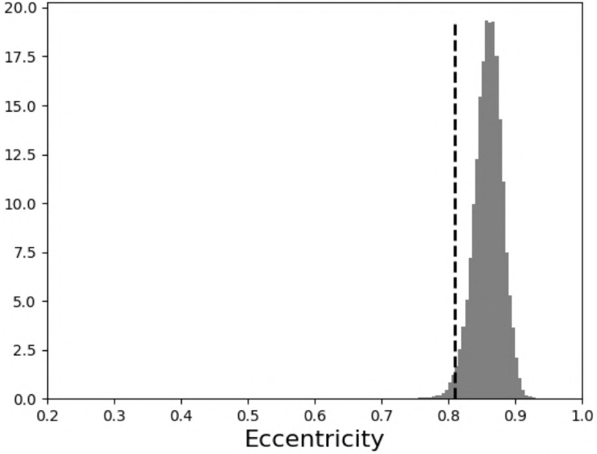
Roundspore assay: quantifying eccentricity



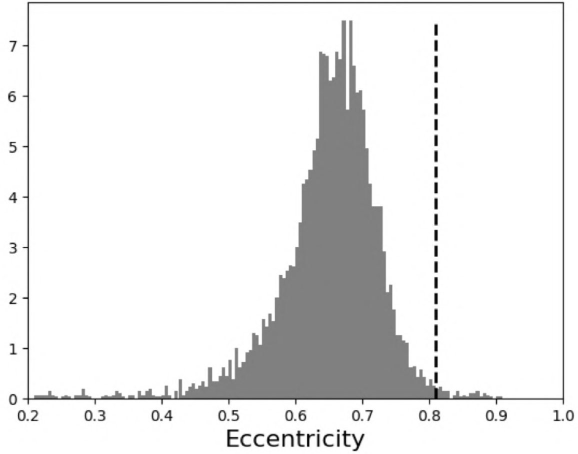
MSUD ✓

RSPC1, Round frac: 0.02



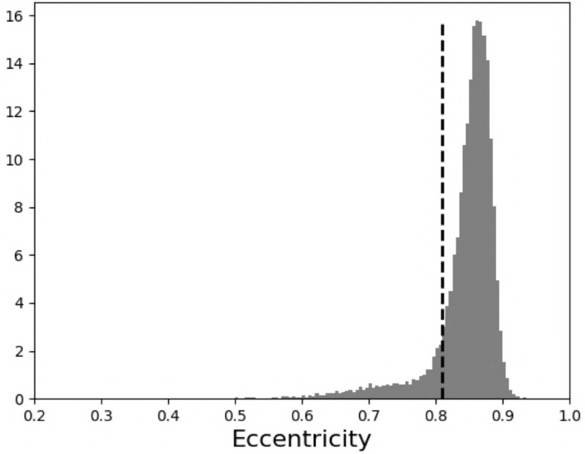
MSUD ✓

RSPC2, Round frac: 0.99

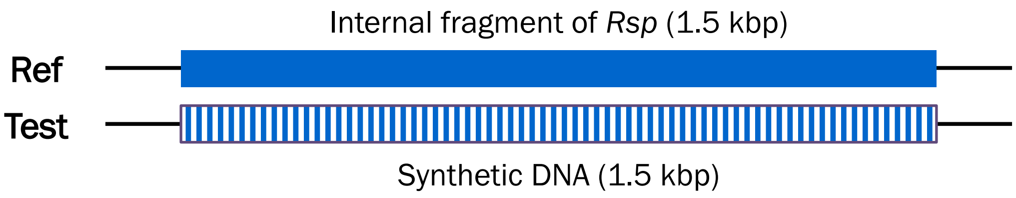


MSUD ~

RSPC3, Round frac: 0.13



Roundspore assay: designing synthetic test DNA



Ref

5’-GCCCGTAGCAT-3’
3’-CGGGCATCGTA-5’

Test

5’-GCCGCCTCGTC-3’
3’-CGGCGGAGCAG-5’

Ref

5’-**GCC**CGTAGCAT-3’
3’-**CGG**GCATCGTA-5’
| | | | | | | | | | |
5’-**GCC**GCCTCGTC-3’
3’-**CGG**CGGAGCAG-5’

Test

3 homologous base pairs (3H) followed by 8 non-homologous base pairs (8N)

Ref

Test

Schematic representation:

● ● ● ○ ○ ○ ○ ○ ○ ○ ○

3H-8N

● matching base pair
○ mismatching base pair

MSUD recognizes weak interspersed homology

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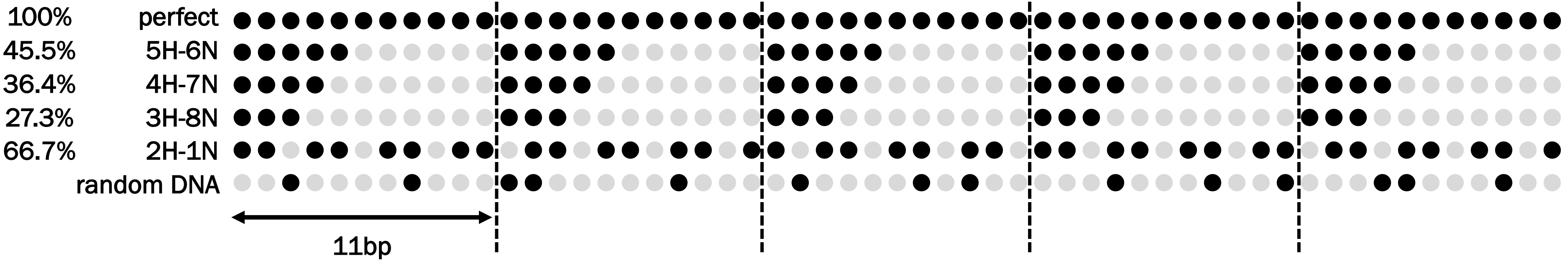
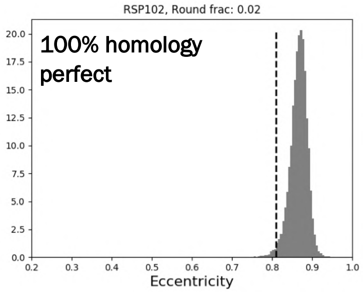
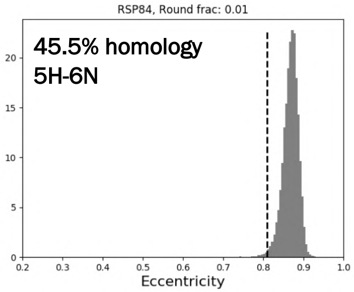
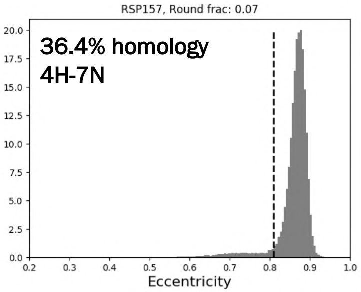
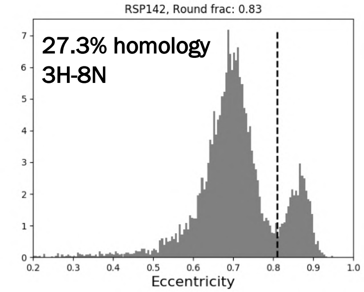
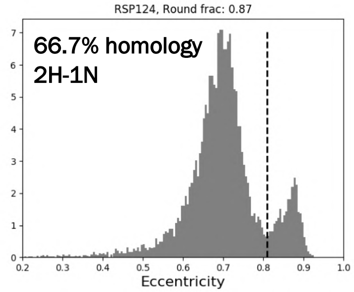


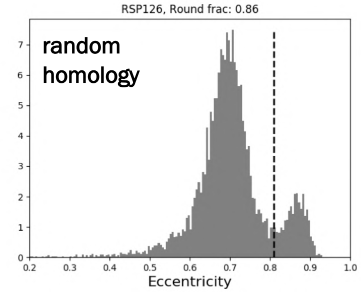






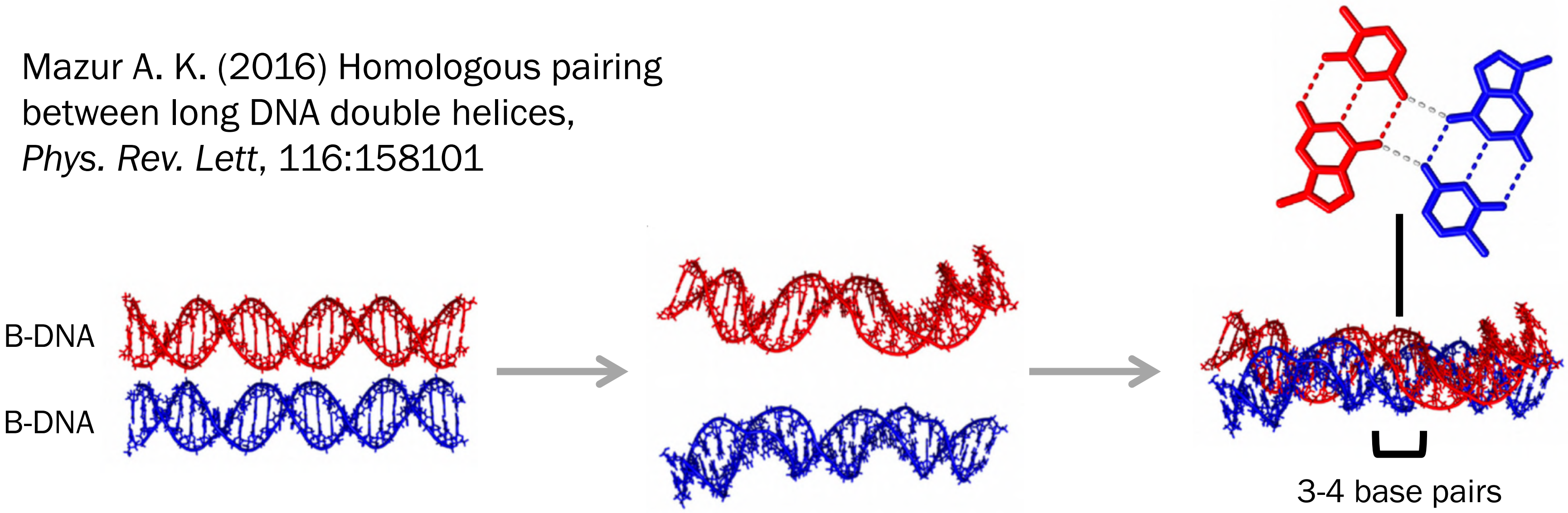






Biophysical features of DNA

Mazur A. K. (2016) Homologous pairing between long DNA double helices, *Phys. Rev. Lett*, 116:158101

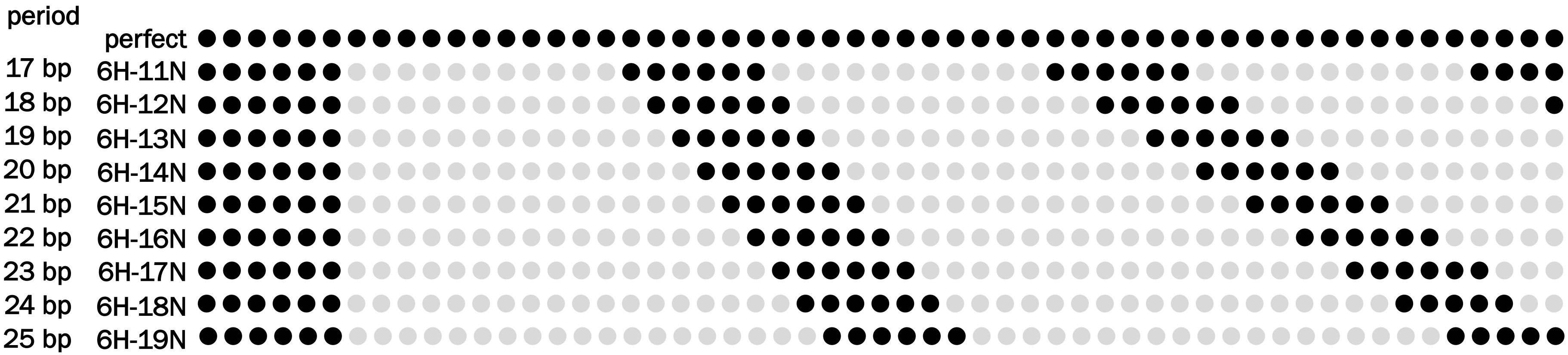


Paired B-DNA (PB-DNA)

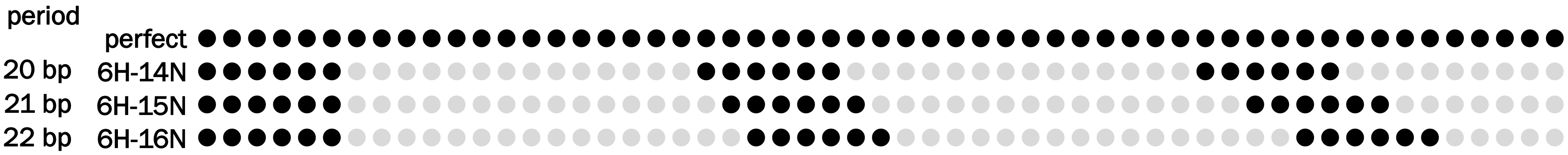
(Mazur *et al*., 2019)

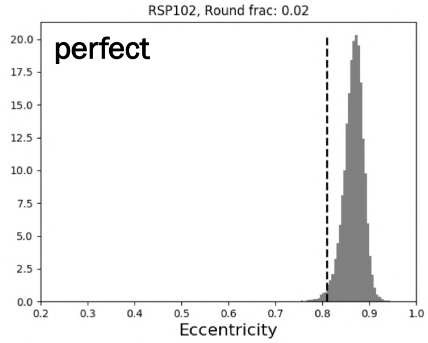
Prediction of direct dsDNA-dsDNA pairing with a period of ~ two B-DNA turns

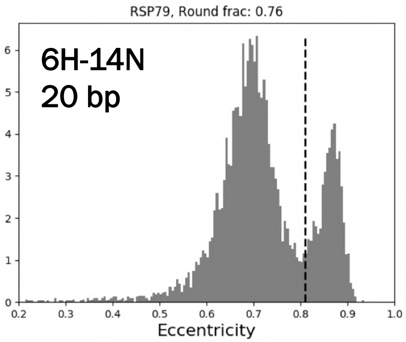
MSUD recognizes homology pattern equivalent to two B-DNA turns

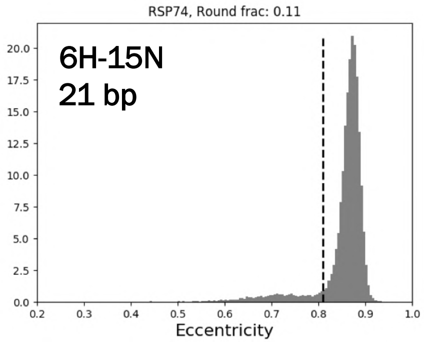


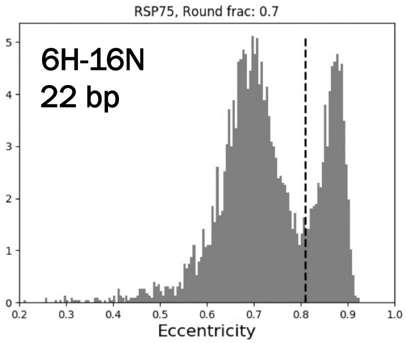
MSUD recognizes homology pattern equivalent to two B-DNA turns



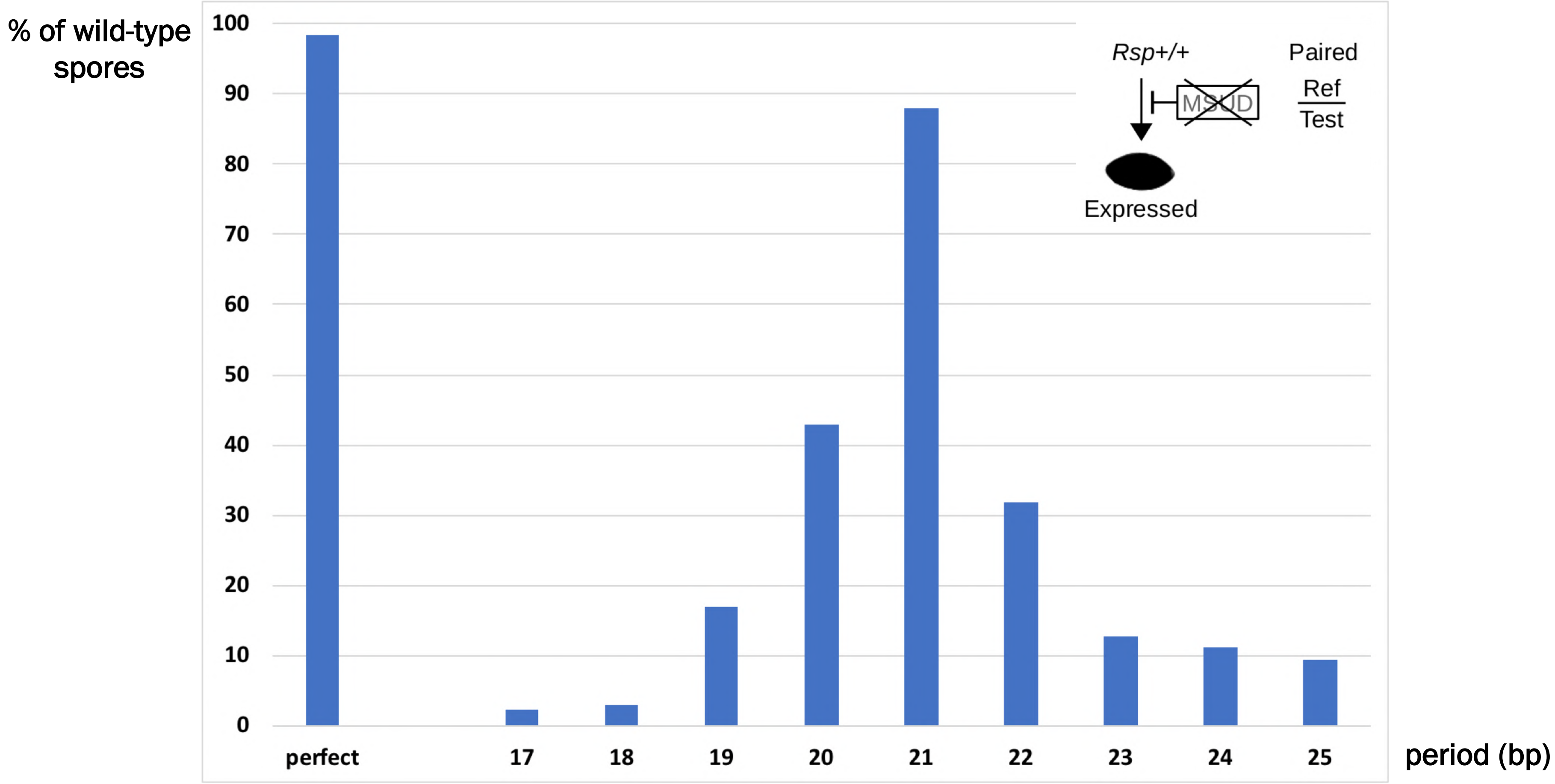






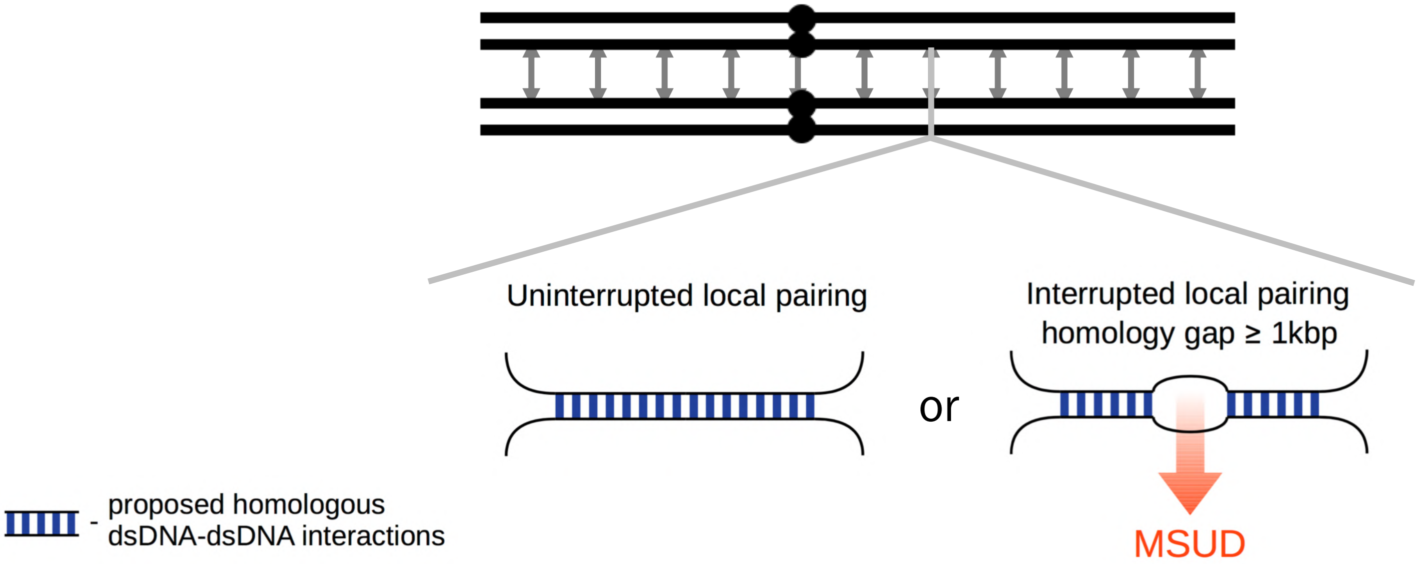


MSUD recognizes homology pattern equivalent to two B-DNA turns



Summary

- MSUD in *N. crassa* is a new approach to study recombination-independent homology recognition during meiosis
- Does not require neither MEI-3 (Rad51/Dmc1-like recombinase) nor Spo11
- Current results are consistent with the model of direct dsDNA-dsDNA pairing



Uninterrupted local pairing

or

Interrupted local pairing
homology gap ≥ 1kbp

MSUD

▥ - proposed homologous dsDNA-dsDNA interactions

Acknowledgments

Fungal Epigenomics Group

Eugene GLADYSHEV, head

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Alexey MAZUR, visiting scientist

Sébastian CASTRO RAMIREZ, graduate student

Blaise LI, research engineer

Illinois State University

Tom HAMMOND, head

Nick RHOADES, PhD student

Institut Pasteur

Federation of European Microbiological Societies

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AGENCE NATIONALE DE LA RECHERCHE
ANR