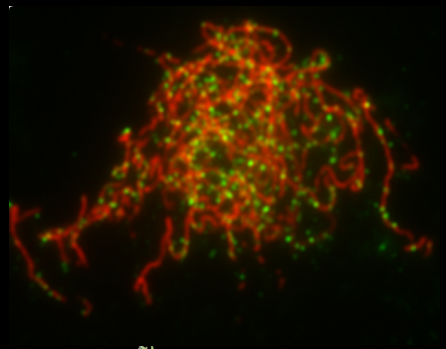
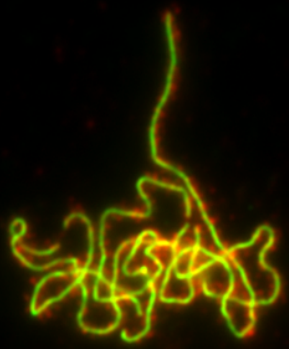
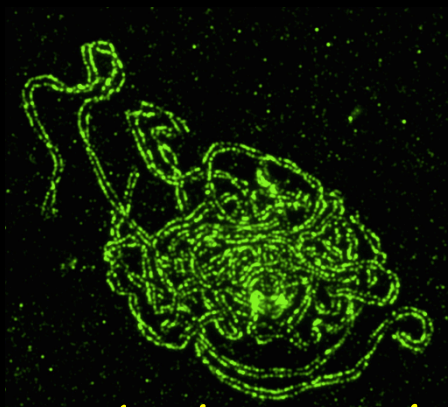
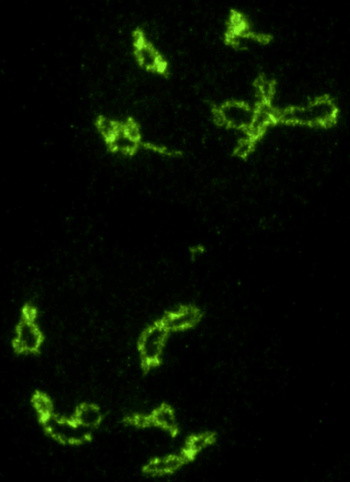




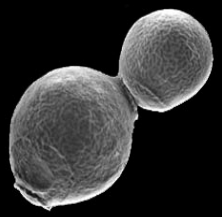


Recombinaison méiotique chez *Arabidopsis thaliana*: beaucoup de contraintes pour peu d'évènements

Institut Jean-Pierre Pierre Bourgin
UMR1318, ERL3559
INRA Agro Paris Tech
Versailles
France

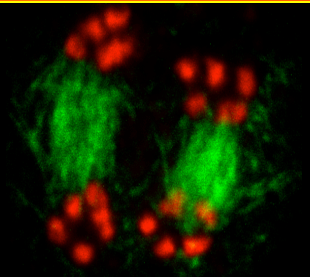
ijpb
SEVENTH FRAMEWORK PROGRAMME
MeioSys
AGENCE NATIONALE DE LA RECHERCHE ANR
INRA SCIENCE & IMPACT

Meiosis is a key step in the cell cycle of sexual reproduction

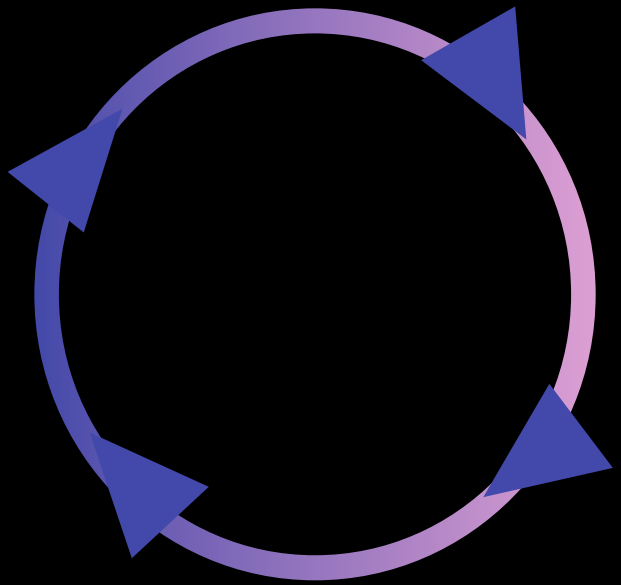





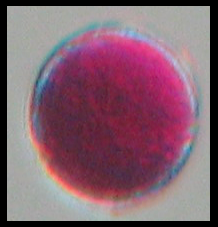
Diploid cells
(2N)



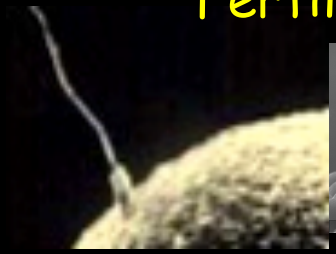
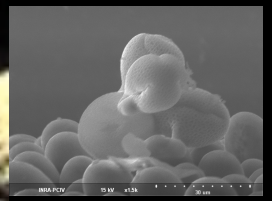
Meiosis



Gametes
(Haploid cells, N)

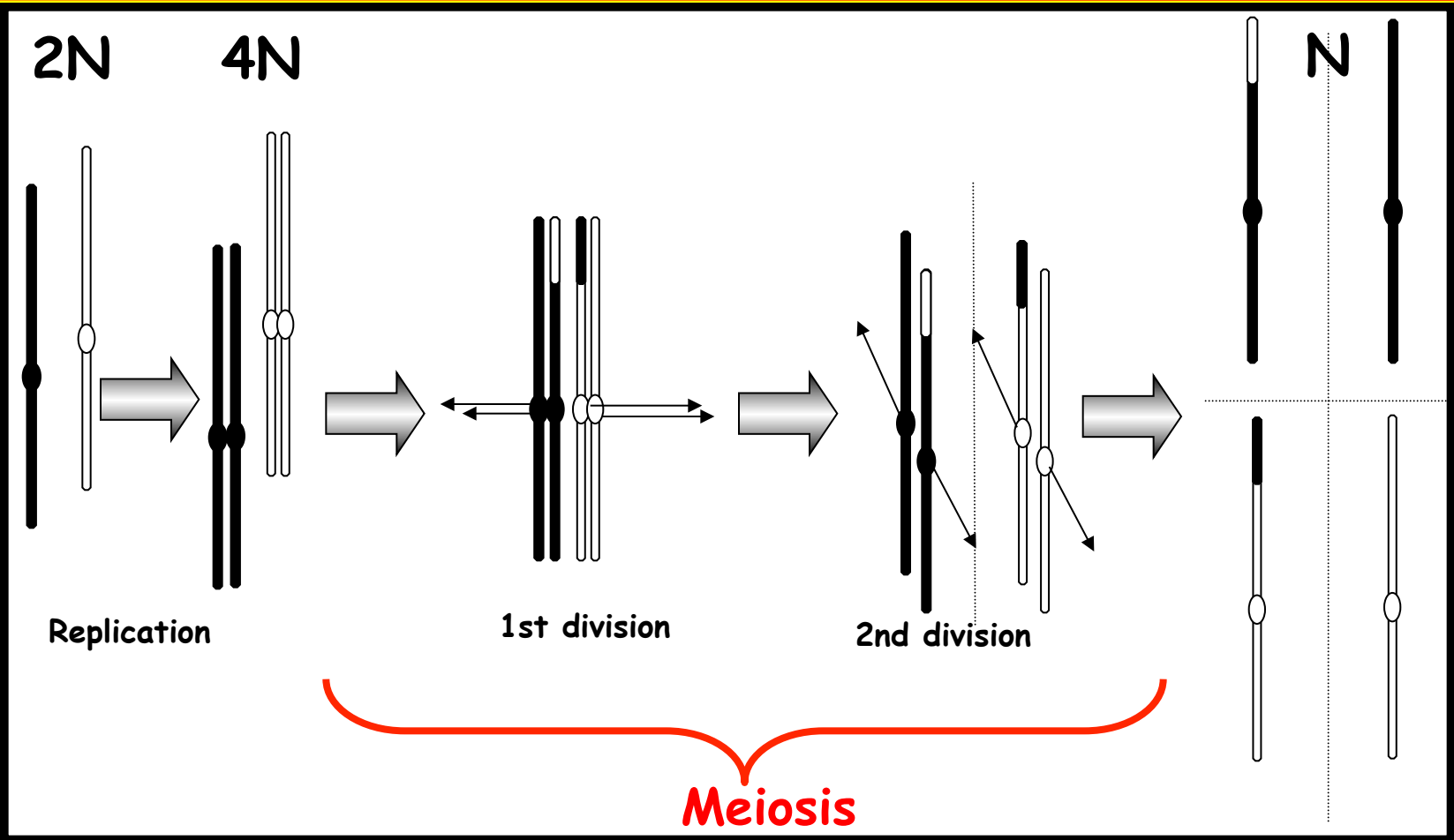


Fertilization

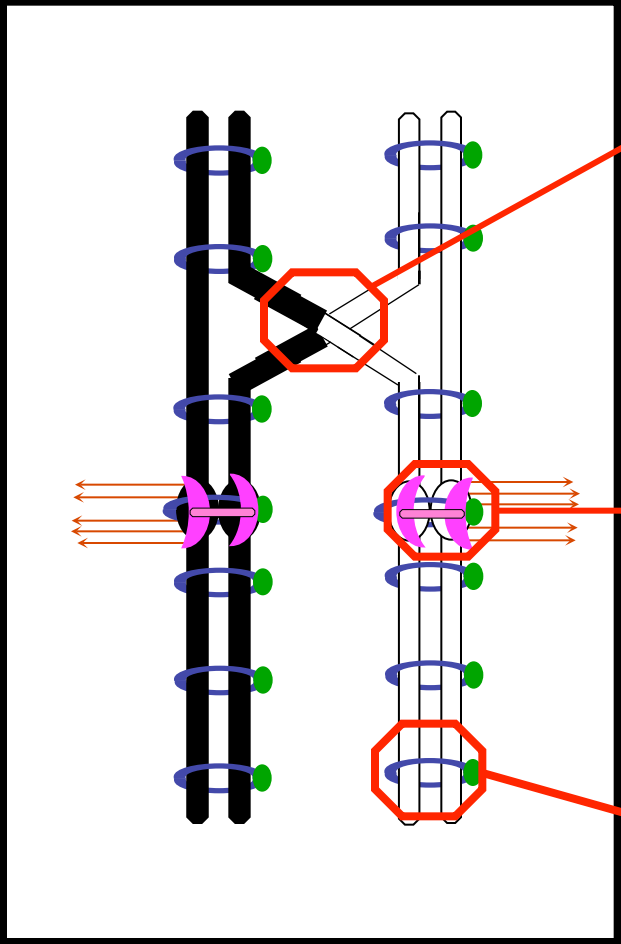



Meiosis : reduction of ploidy

One set of DNA replication followed by two cell divisions



The segregation of homologous chromosomes at 1st meiotic division depends on the formation of a stable bivalent

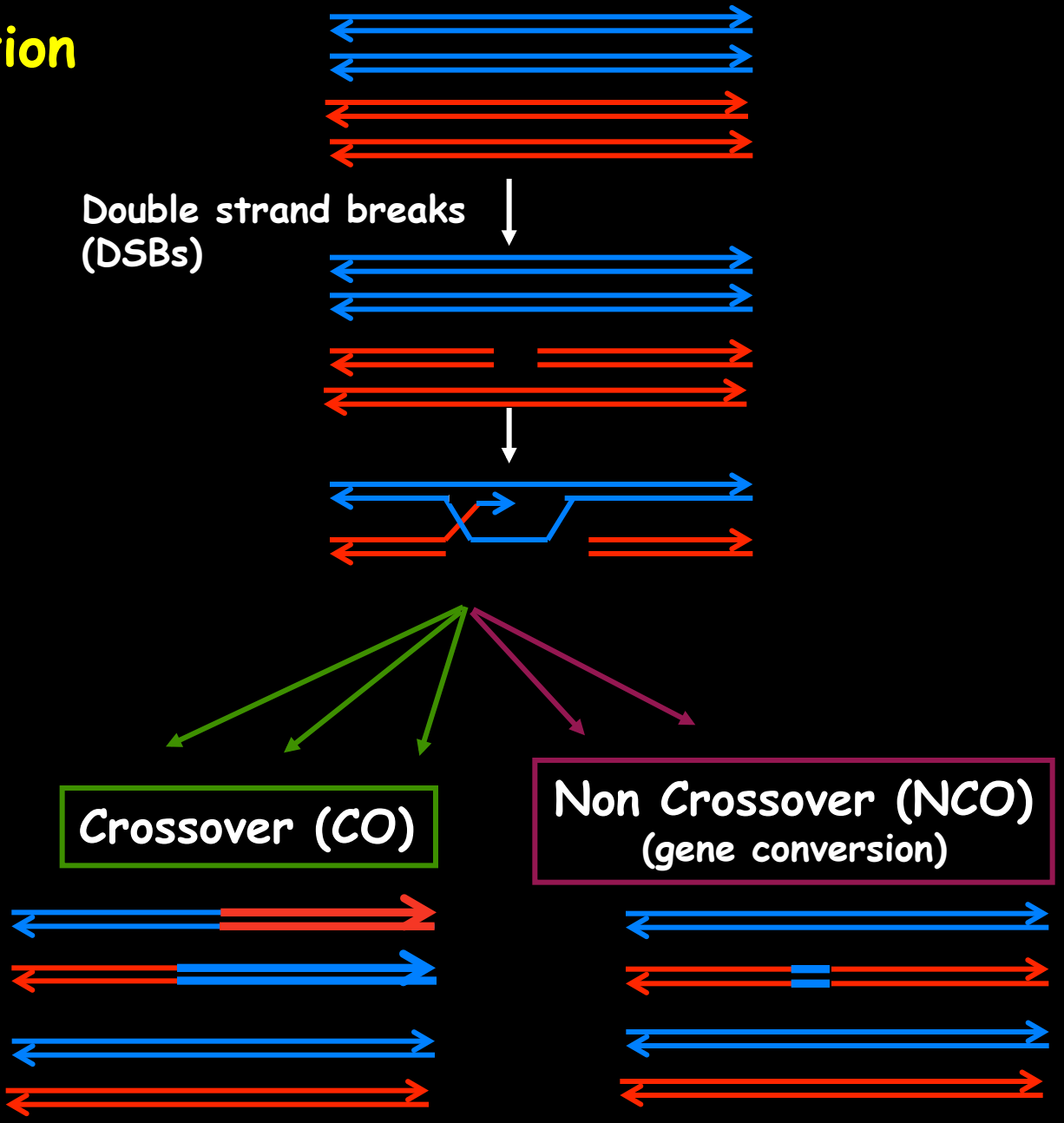


Recombination : at least one Crossing-Over/bivalent

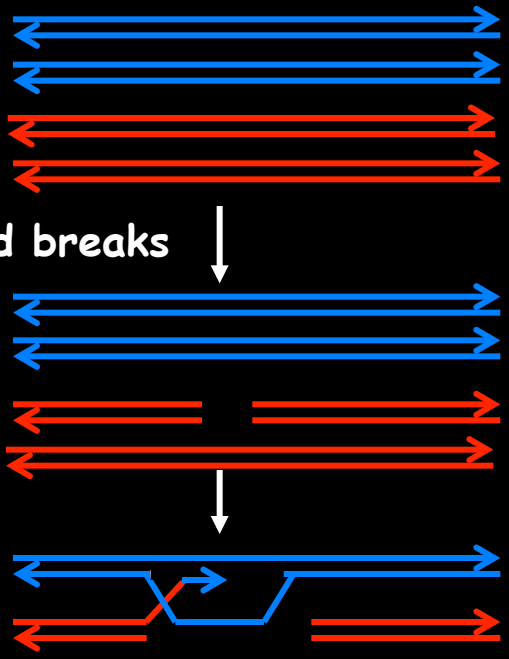
Monopolar orientation of the kinetochores

Sister chromatid cohesion

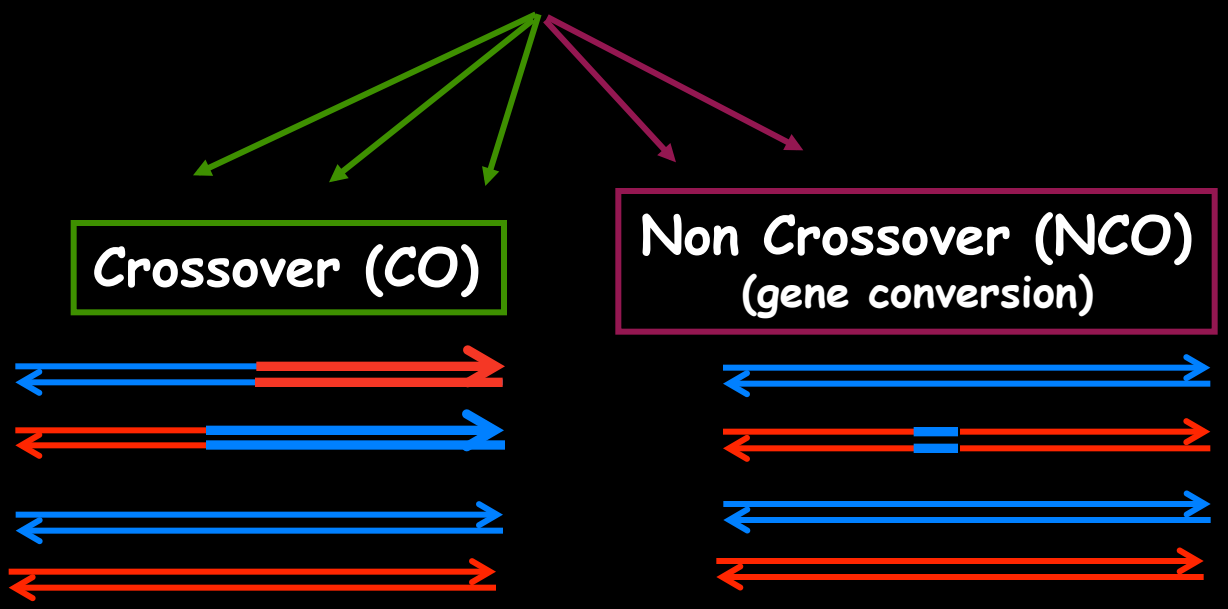
Meiotic Recombination



Meiotic Recombination



Where and how many ?



Recombination in meiosis : where and how many ?

3 strategies in parallel :

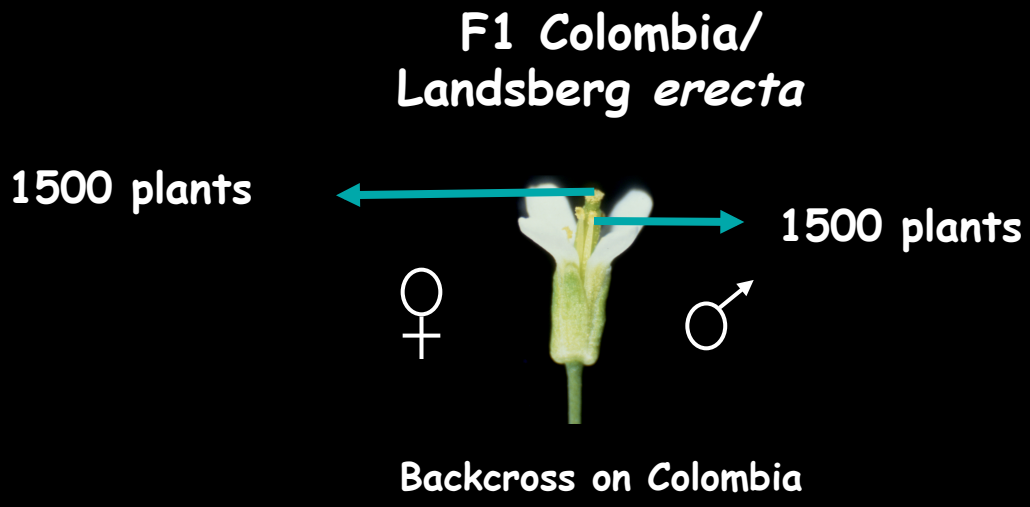
. Genome wide analysis of COs rate and distribution

. Hotspot of meiotic recombination

. Analysis of the number and the type of recombination events (CO and NCO) in ONE Single Meiosis

Genome wide analysis of COs rate and distribution

CO distribution in male and female meiosis



Choix technologique

Project (written in 2009)
Population of 3000 plants
1 marker each centimorgan thus each 250-300 Kb : ~ 480 markers needed

Choice of BeadXpress Illumina : 384 SNPs (1SNP/ 315 kb

Test with a first series of markers:
around 10 % of Markers did not worked
new set of markers tried but problemes with some of the markers that were OK before

A few SNPs were genotyped with Taqman
Singletons were verified by standard PCR (around 450)

Conclusion:+ good results
- a long way to get them and technique not so flexible …

There are more COs in male meiosis than in female meiosis

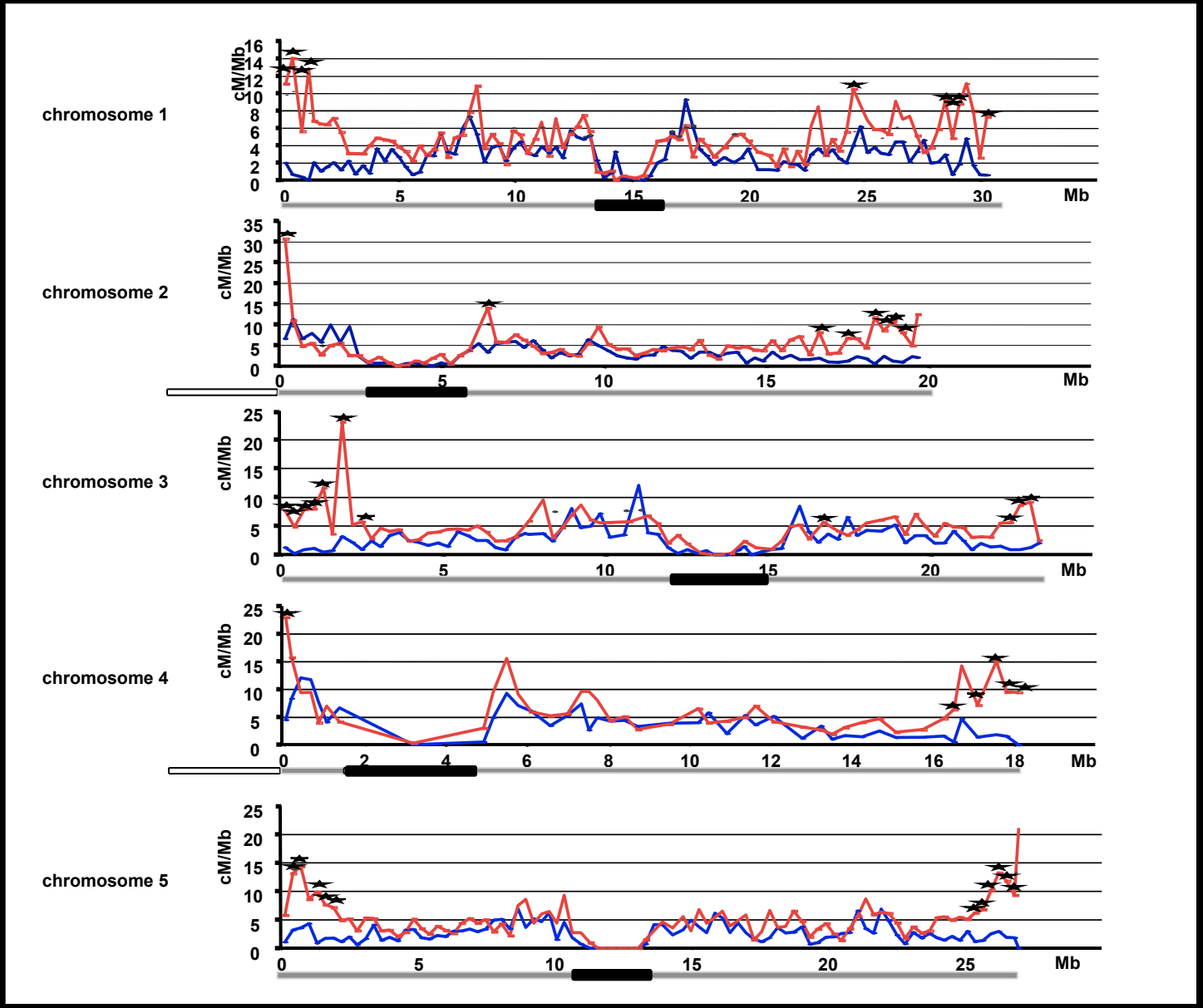
	Female	Male	Pool
number of COs detected	8532	13535	
COs per meiosis	6.6	11.1	8,9
1 cM	361 kb	209 kb	285 kb
Genetic Map cM	332	575	

M /F CO rate : 1.7

Giraut et al, 2011

CO distribution is different in male and female meiosis

Giraut et al, 2011



Conclusions part 1

Giraut et al, 2011

More COs in Male than in Female meiosis

Distribution of COs varies between Male and Female meiosis

In Female Meiosis: CO rates correlate (-) with GC %, genes
(+) TEs

In Male Meiosis: CO rates correlate (+) with CpG

Interference varies between male and female meiosis, between chromosomes (Basu-Roy et al, 2013)

?????

Recombination in meiosis : Where and How Many events ?

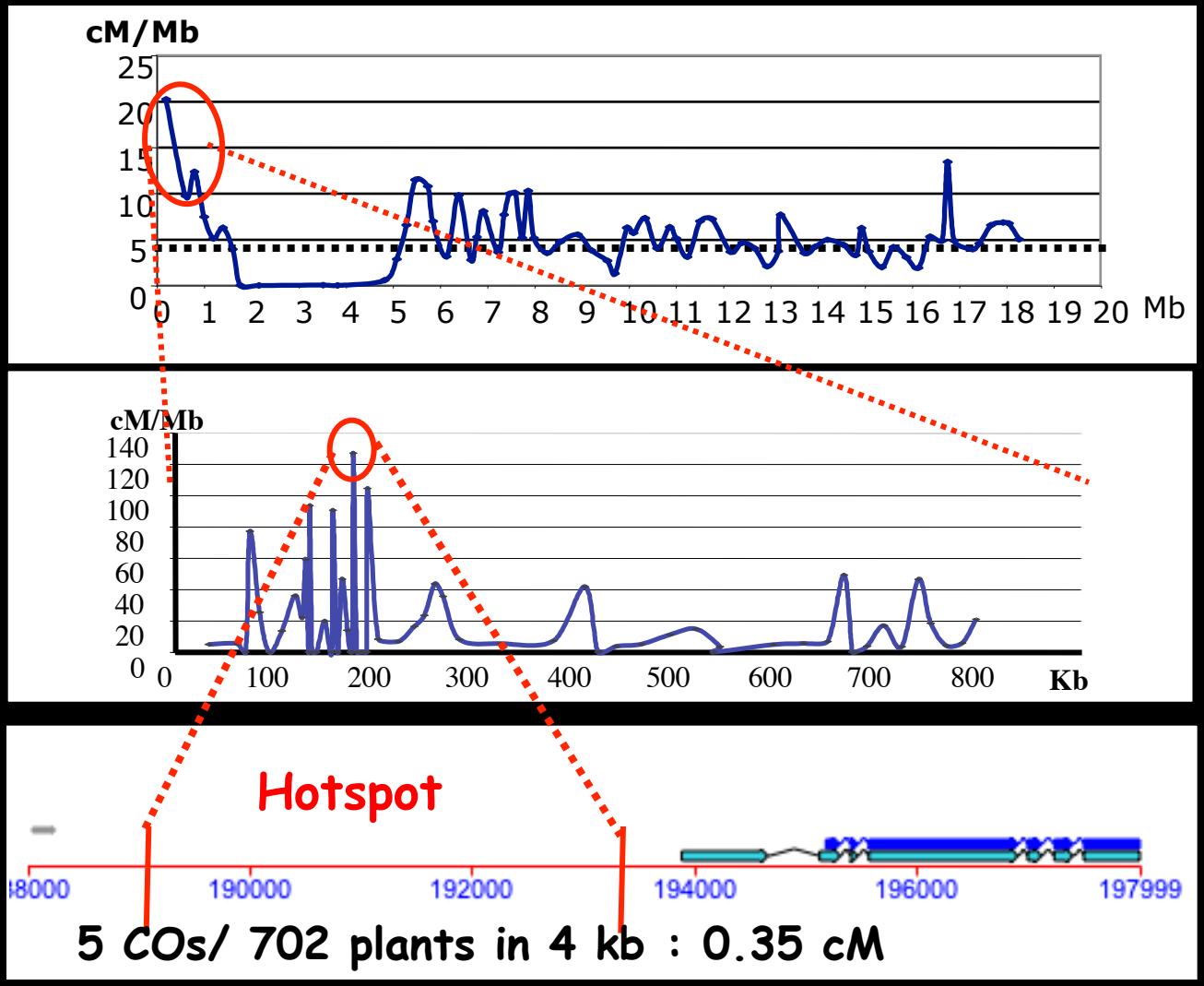
3 approaches in parallel :

. Genome wide analysis of COs rate and distribution

. Hotspot of meiotic recombination

. Analysis of the number and the type of recombination vents (CO and NCO) in A Single Meiosis

The distribution of COs is not homogeneous at any scale



Chromosome 4

Pieces of Chromosome

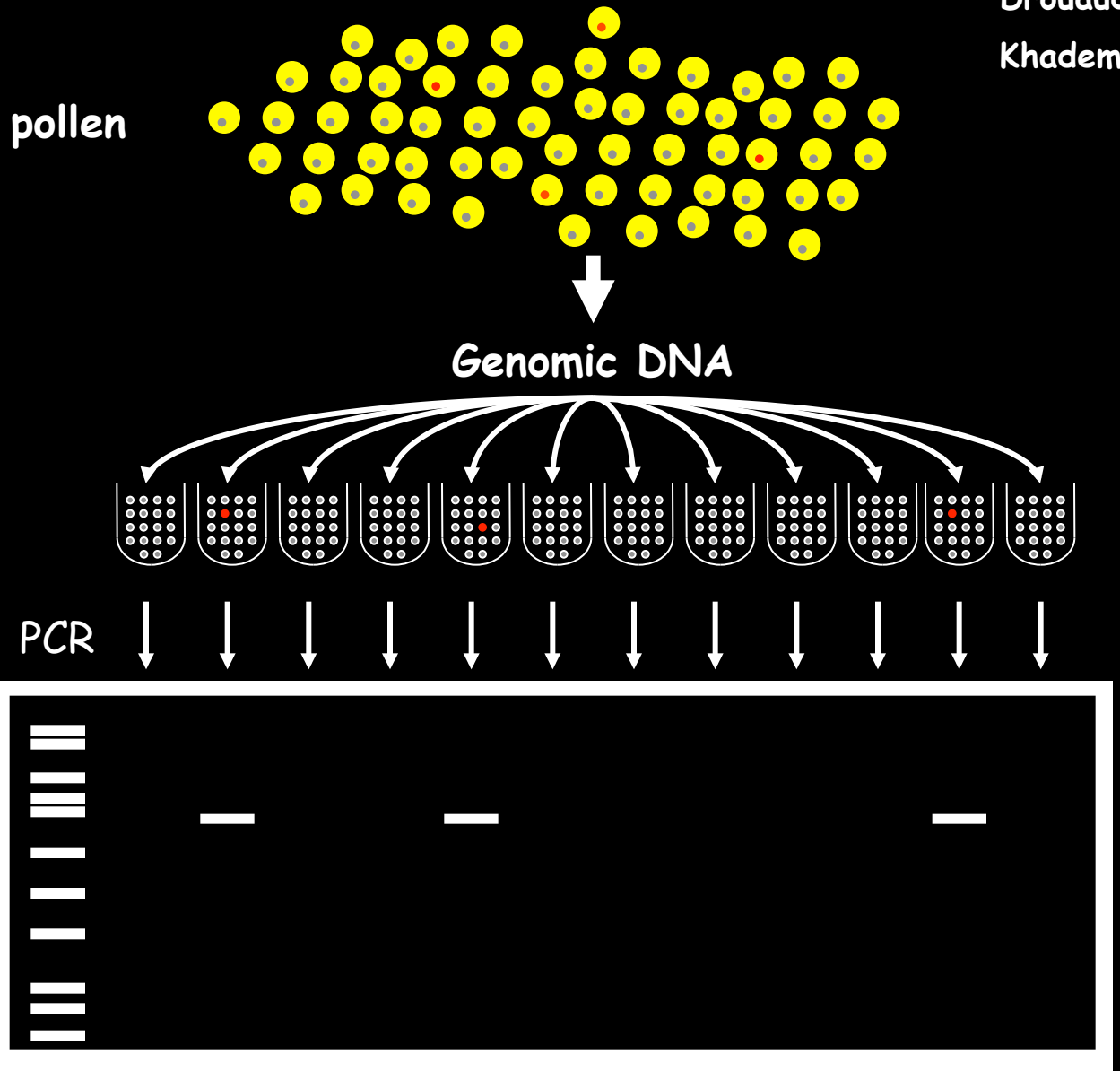
Kilobases

Drouaud et al., 2013

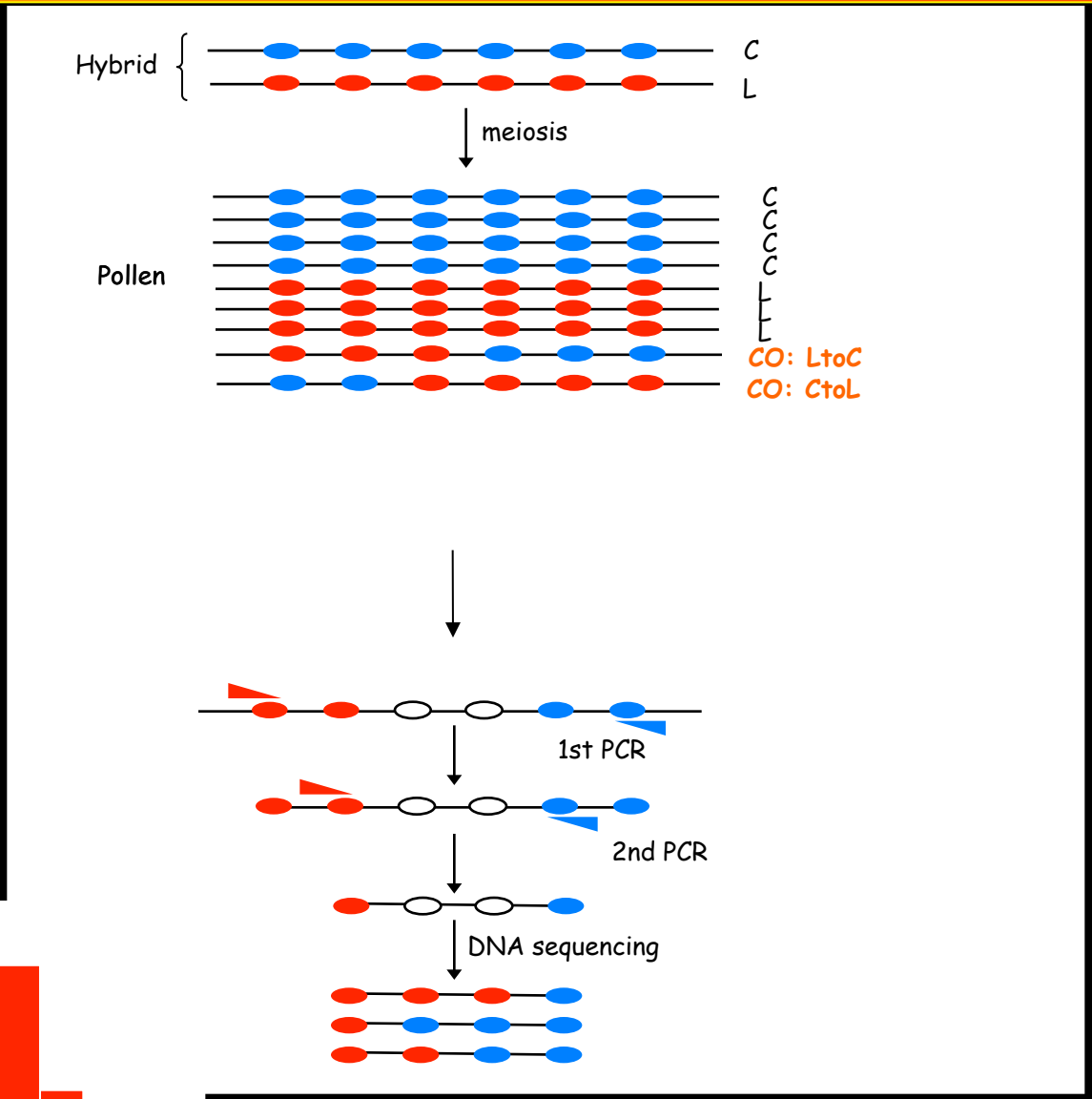
An out line of the "Pollen typing"approach

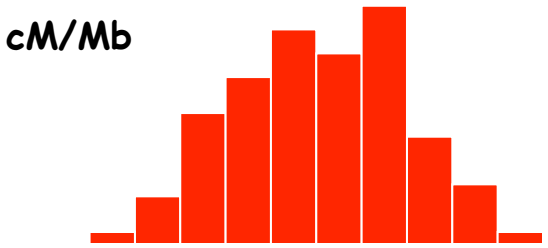
Drouaud et al., 2011

Khademian et al., 2012



CO detection: PCR based strategy

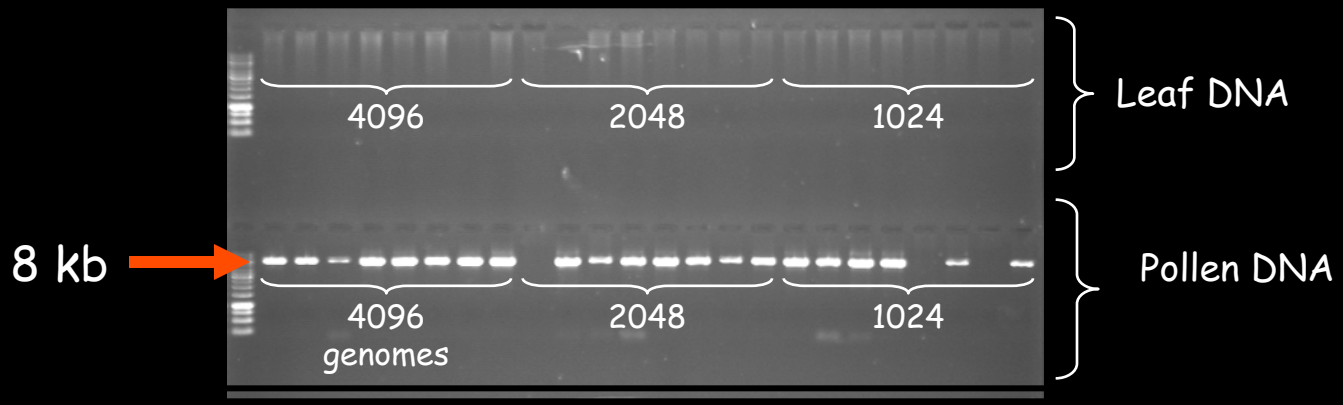




(Drouaud and Mézard, 2011)

CO detection by pollen typing: example at 130x

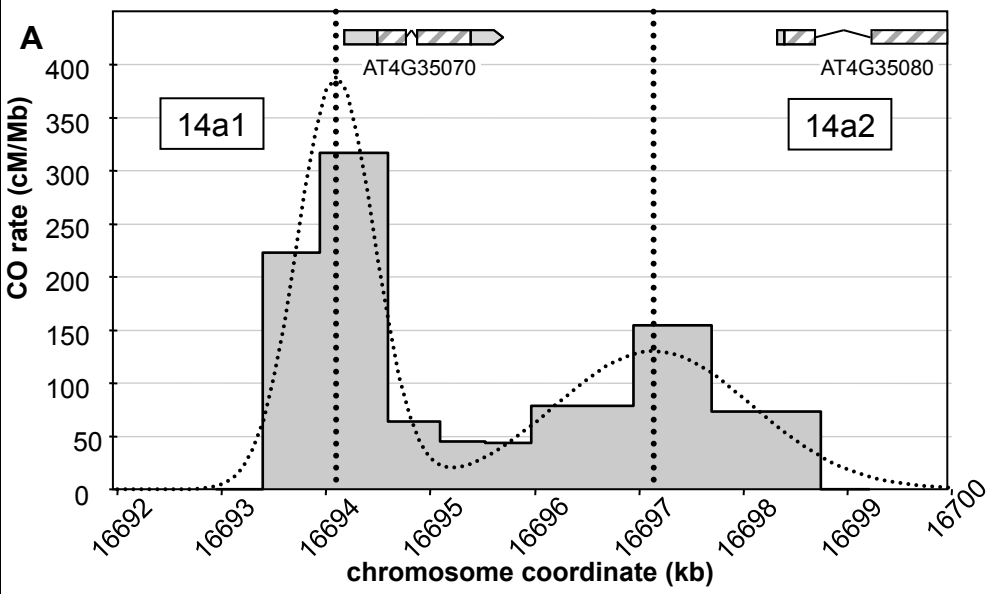
Recombination rate: 1 / 200 genomes



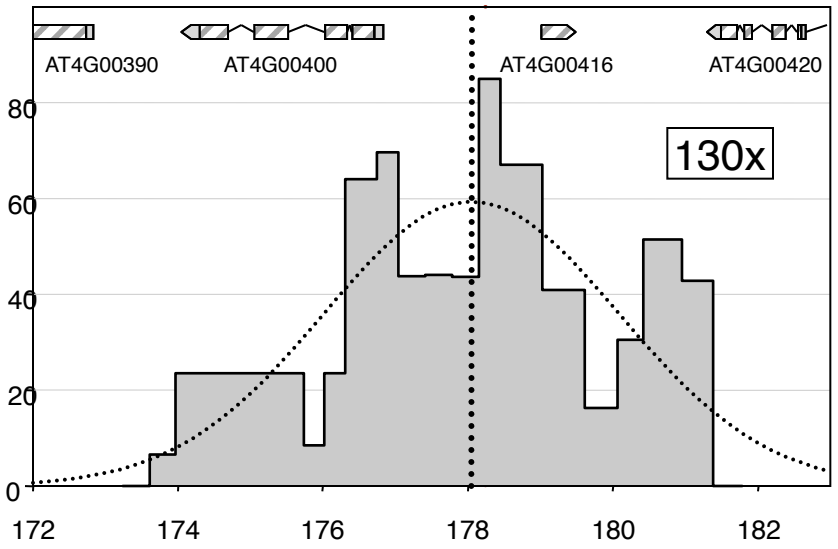
Main difficulty

Specific Long PCR (up to 14 kb……) on single recombinant molecules diluted in hundreds of non recombinant molecule recognized by the same primers ……

Clusters of CO at hotspots of meiotic recombination



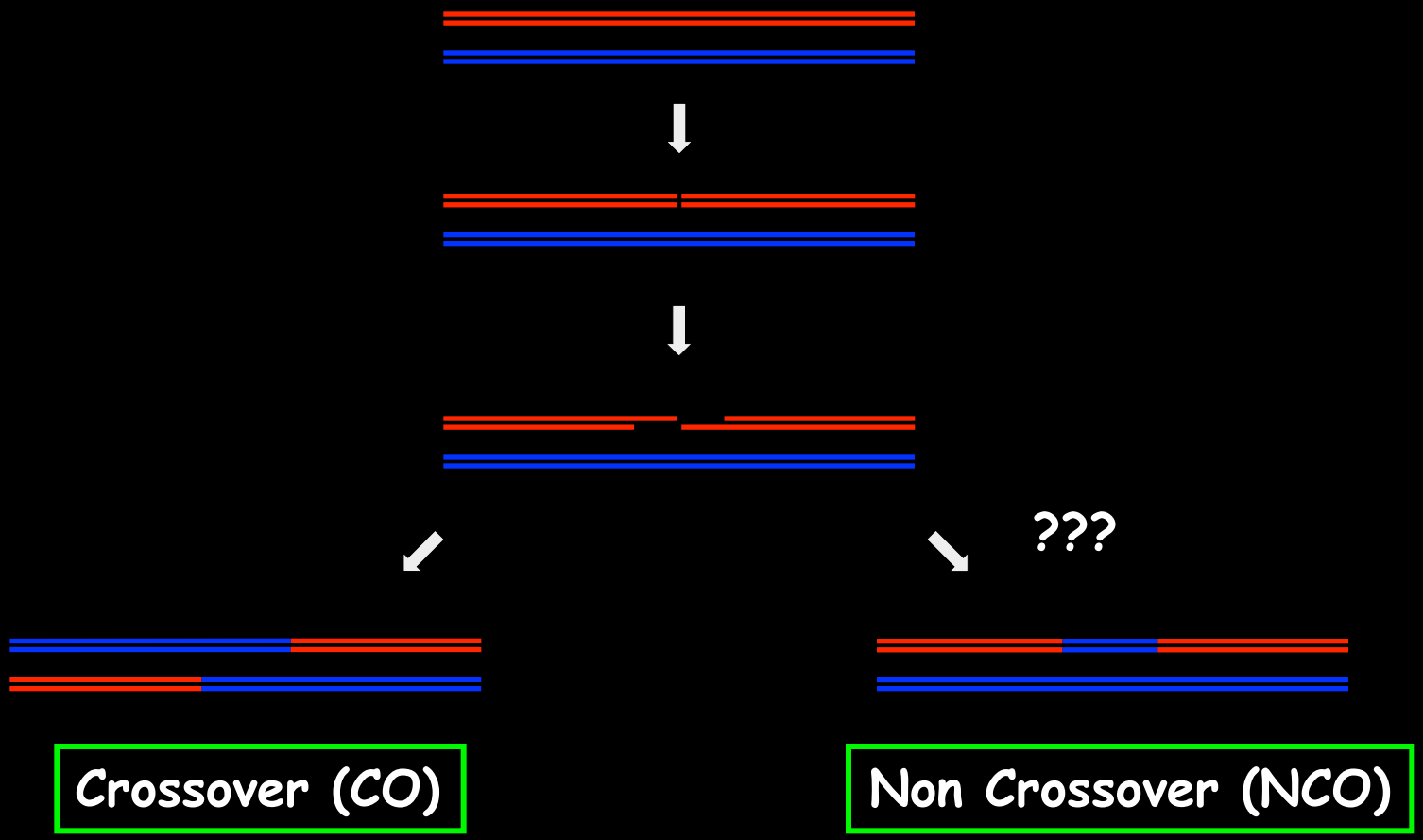
104 COs 0.55% (0,29-0,95)



167 COs 0.53% (0,37-0,78)

Drouaud et al., 2013

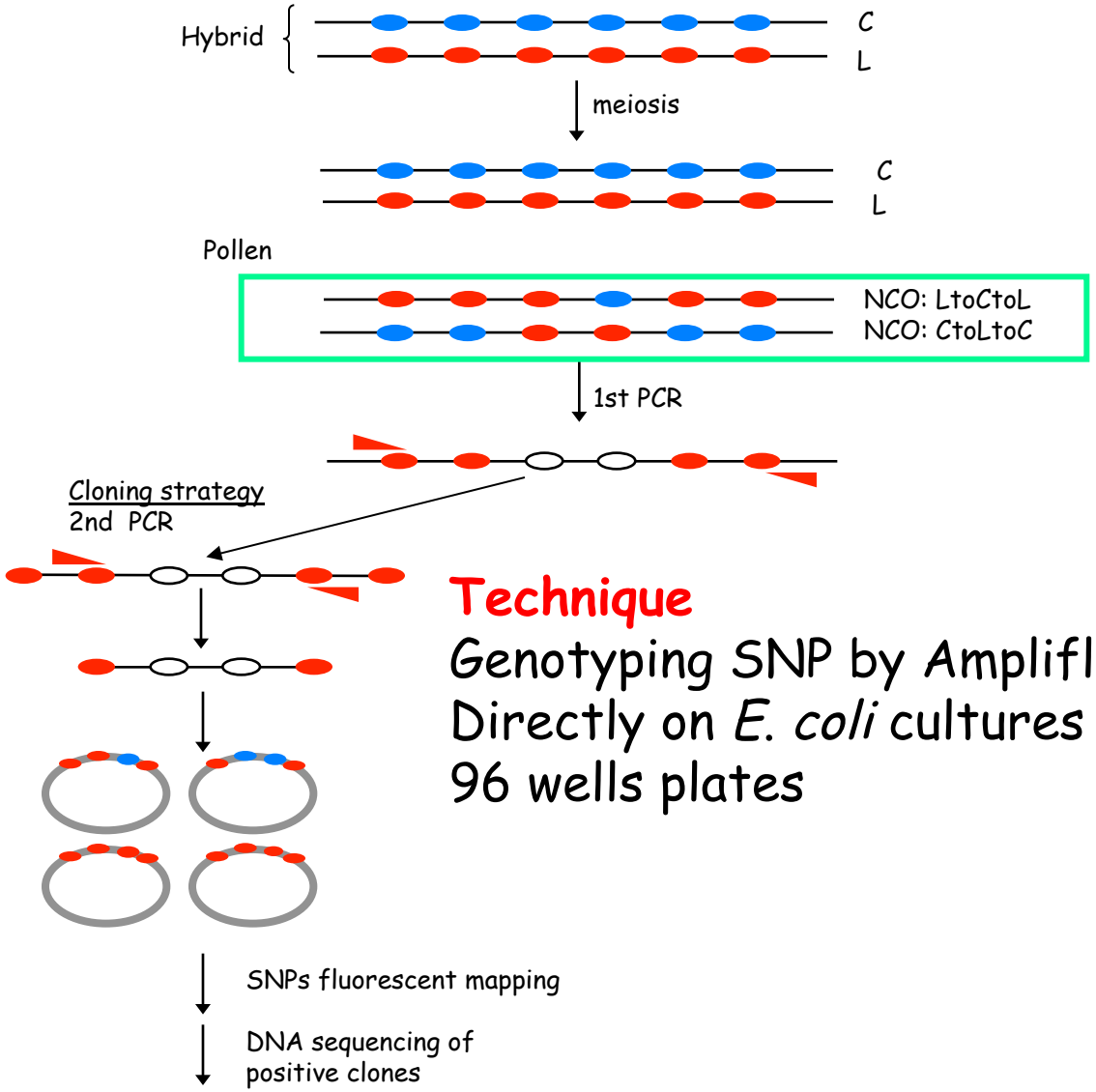
NCOs at meiotic recombination hotspots



Only four NCOs had been detected in *Arabidopsis*

(Francis et al., 2007; Lu et al, 2012)

NCO detection: PCR based strategies

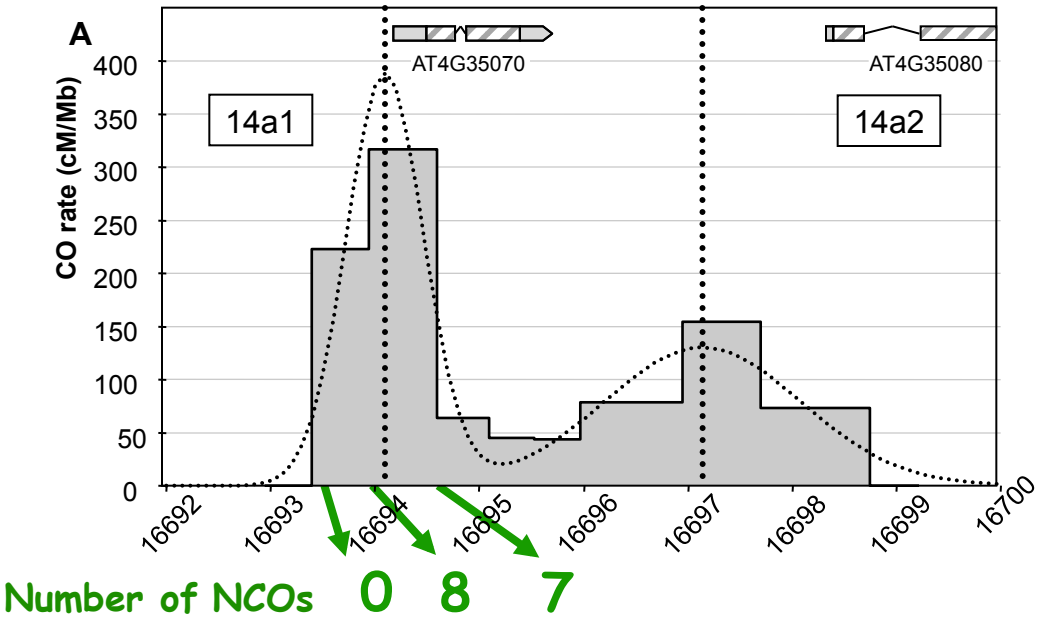


Technique

Genotyping SNP by Amplifluor Directly on *E. coli* cultures in 96 wells plates

(Ng and Paigen, 2008; Khademian et al, 2012)

NCOs at 14a1



3000 clones tested

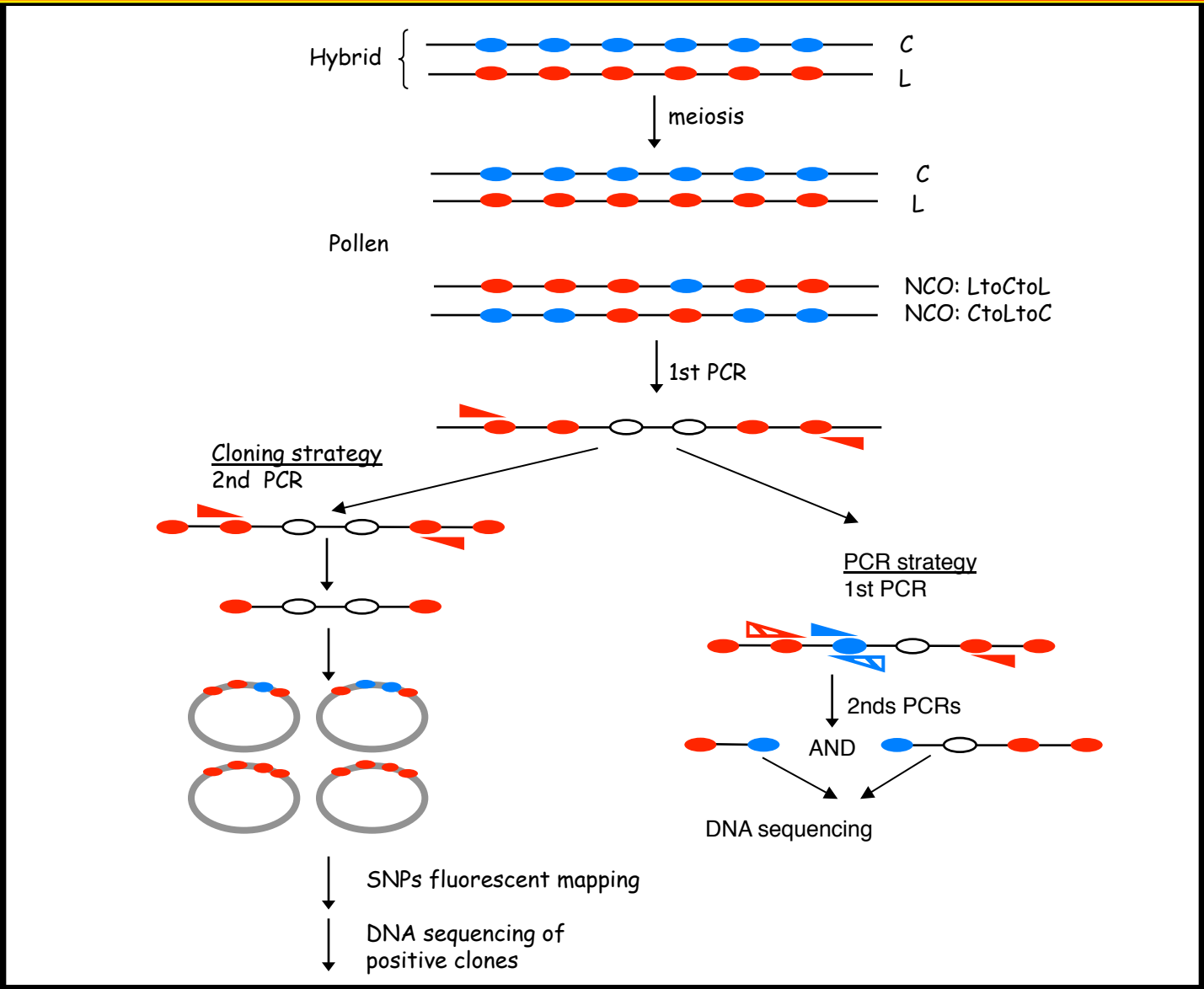
NCOs Rate: 0.5% (0.28-0.82)

CO/NCO 1.7 : 1

Tract length: 1 bp to 480 bp (conversion of a single SNP)

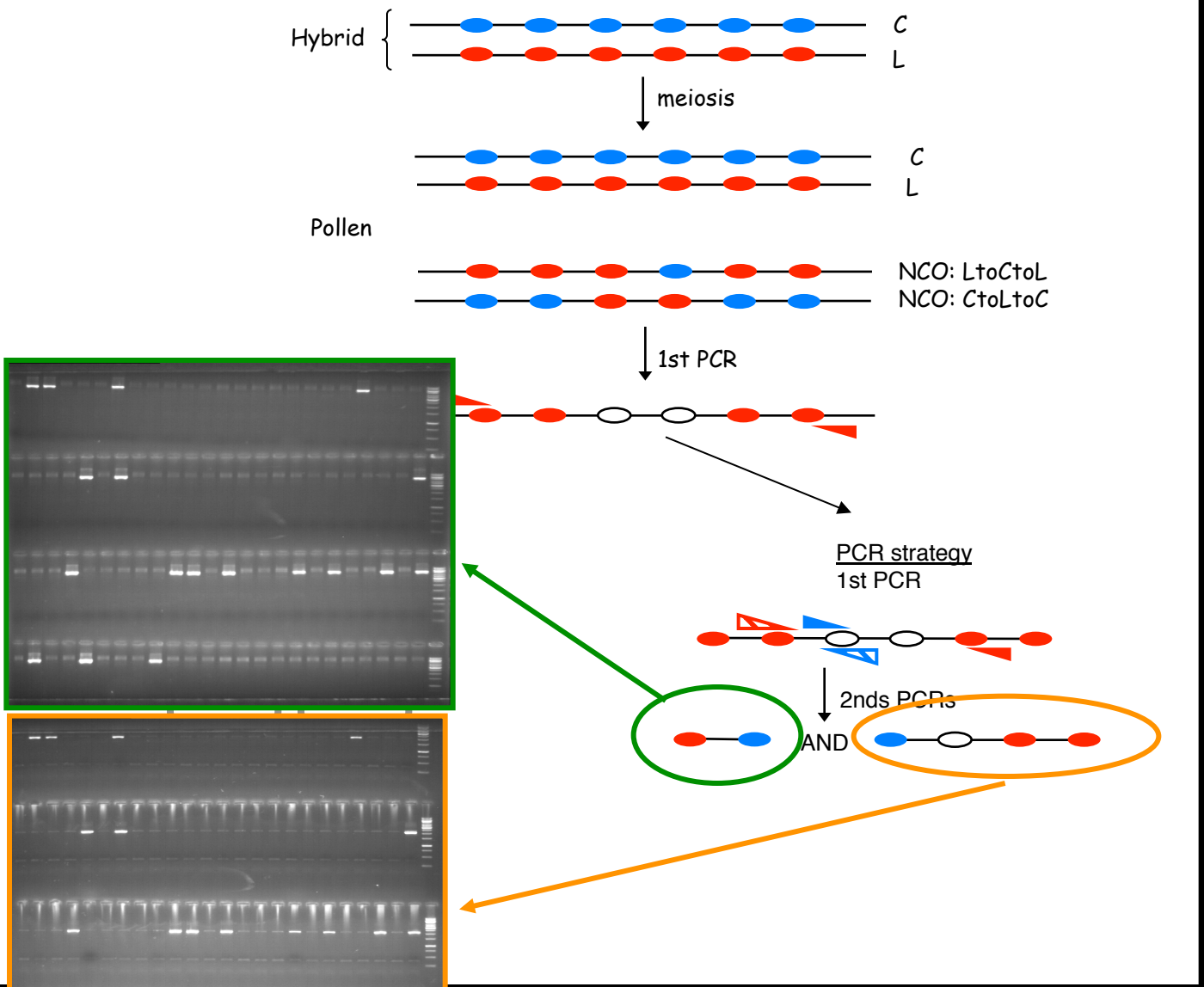
Average: 277 bp

NCO detection: PCR based strategies



(Khademian et al, 2012)

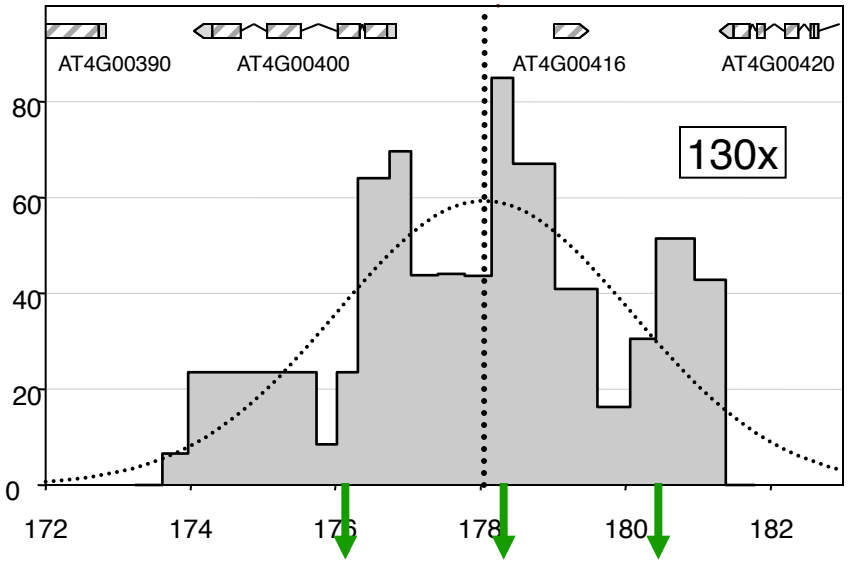
NCO detection: PCR based strategies



(Khademian et al, 2012)

Detection of NCOs at 130x

398,000 genomes tested
Pollen typing based detection



Number of NCOs 30 29 4

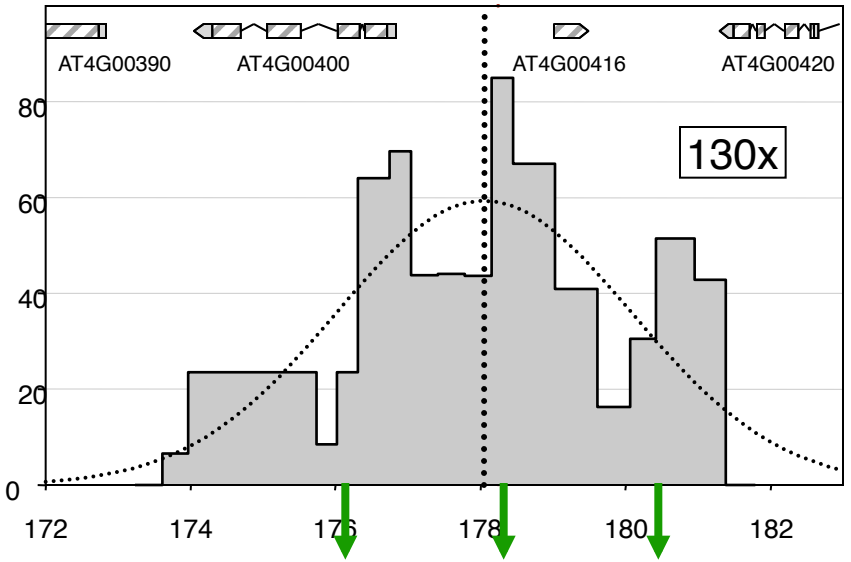
Rate: 1 /13,267
0.008% (0.005-011)

Rate: 1 /13,724
0.007% (0.005-0.010)

Rate : 1 /99,500
0.001% (0.0004-0.0033)

Detection of NCOs at 130x

398,000 genomes tested
Pollen typing based detection



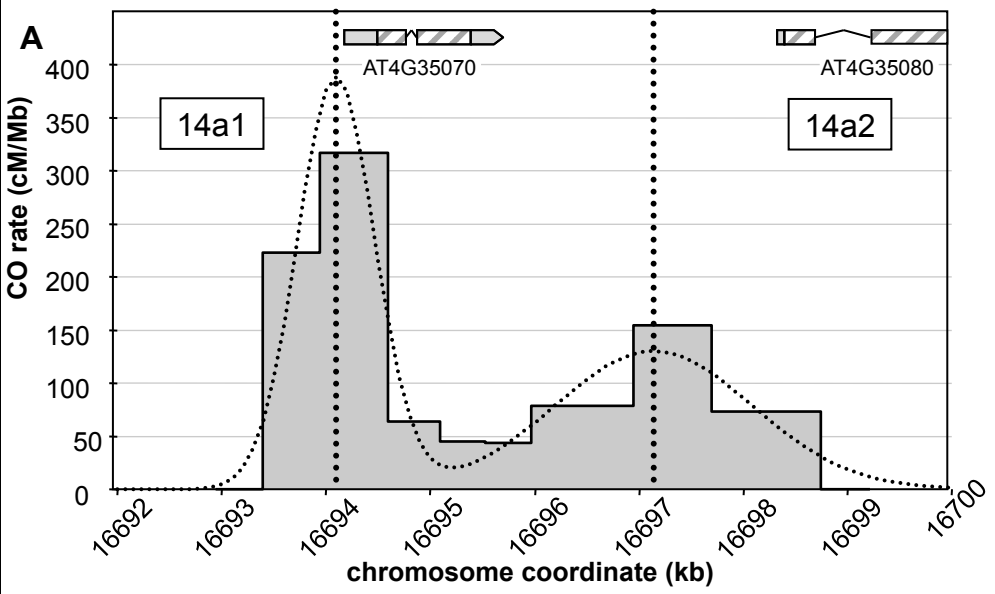
Number of NCOs 30 29 4

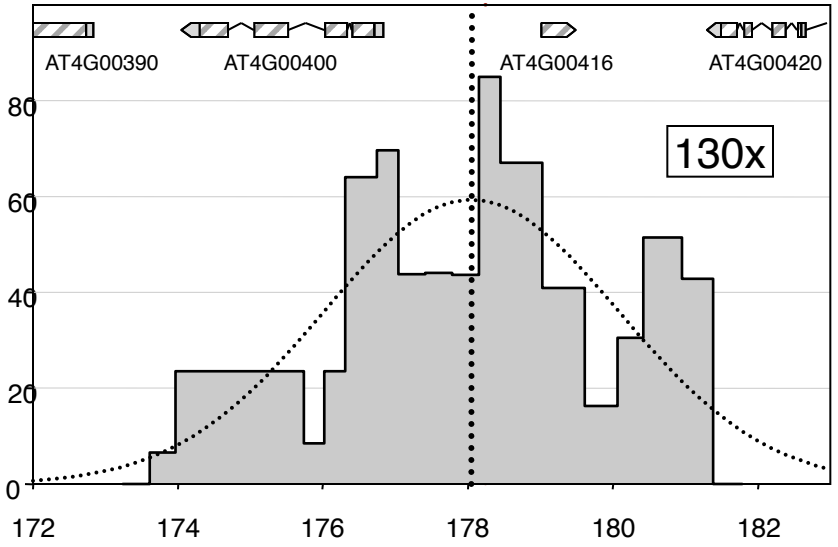
Rate: 1 /13,267
0.008% (0.005-011)
Length: 13 tp 3271 bp
Average: 979

Rate: 1 /13,724
0.007% (0.005-0.010)
Length: 5 to 1615 bp
Average: 491 bp

Rate : 1 /99,500
0.001% (0.0004-0.0033)
Length: 14 to 3045 bp

Clusters of CO and NCOs at hotspots of meiotic recombination





104 COs 0.55%

15 NCOs/ 6000 0.5%

CO/NCOs: 1.7

Average: 277 bp

167 COs 0.53%

63 NCOs / 3980000 0.016%

COs/NCOs : 20

Average: 808 bp

Drouaud et al., 2013

Conclusions part 2

Drouaud et al, 2013

There are hotspots of meiotic recombination in *A. thaliana*

Hotspots tend to cluster in small regions

NCO rates are extremely variable from one Hotspot to another one

Recombination in meiosis : Where and How Many events ?

3 approaches in parallel :

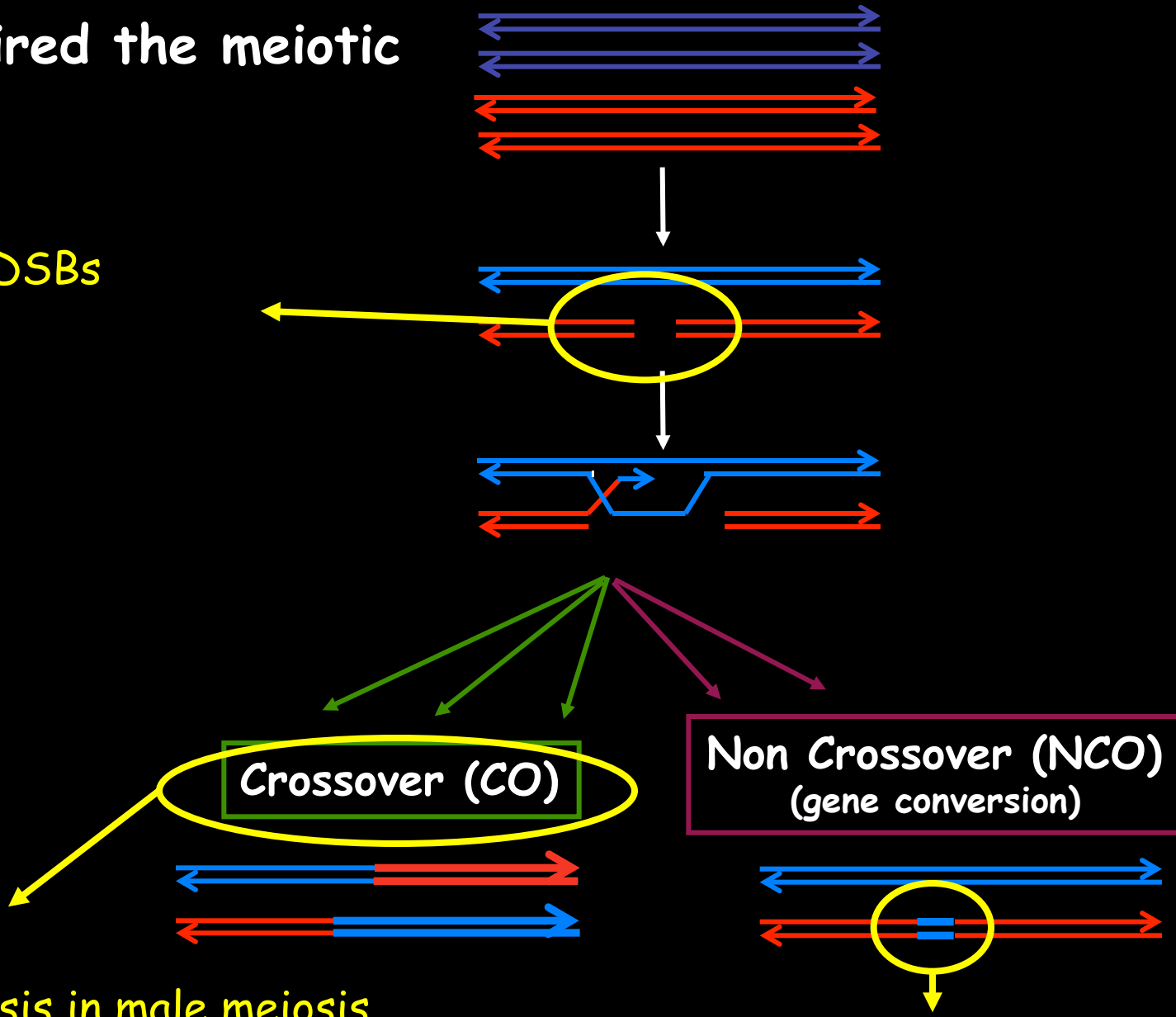
. Genome wide analysis of COs rate and distribution

. Local analysis of COs and NCOs rate and distribution

. Analysis of the number and the type of recombination vents (CO and NCO) in ONE single meiosis

How are repaired the meiotic DSBs ?

From 210 to 290 DSBs



11.1 COs per meiosis in male meiosis

And at least one per bivalent

????

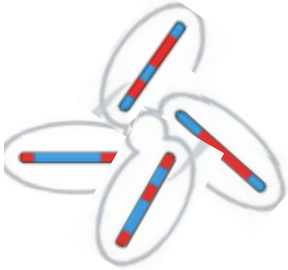
Plan de bataille

Col *quartet*

Diploid hybrid

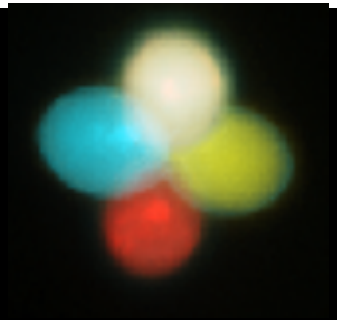
Col x Ler *quartet*

Haploid pollen grains attached in tetrads



Meiosis

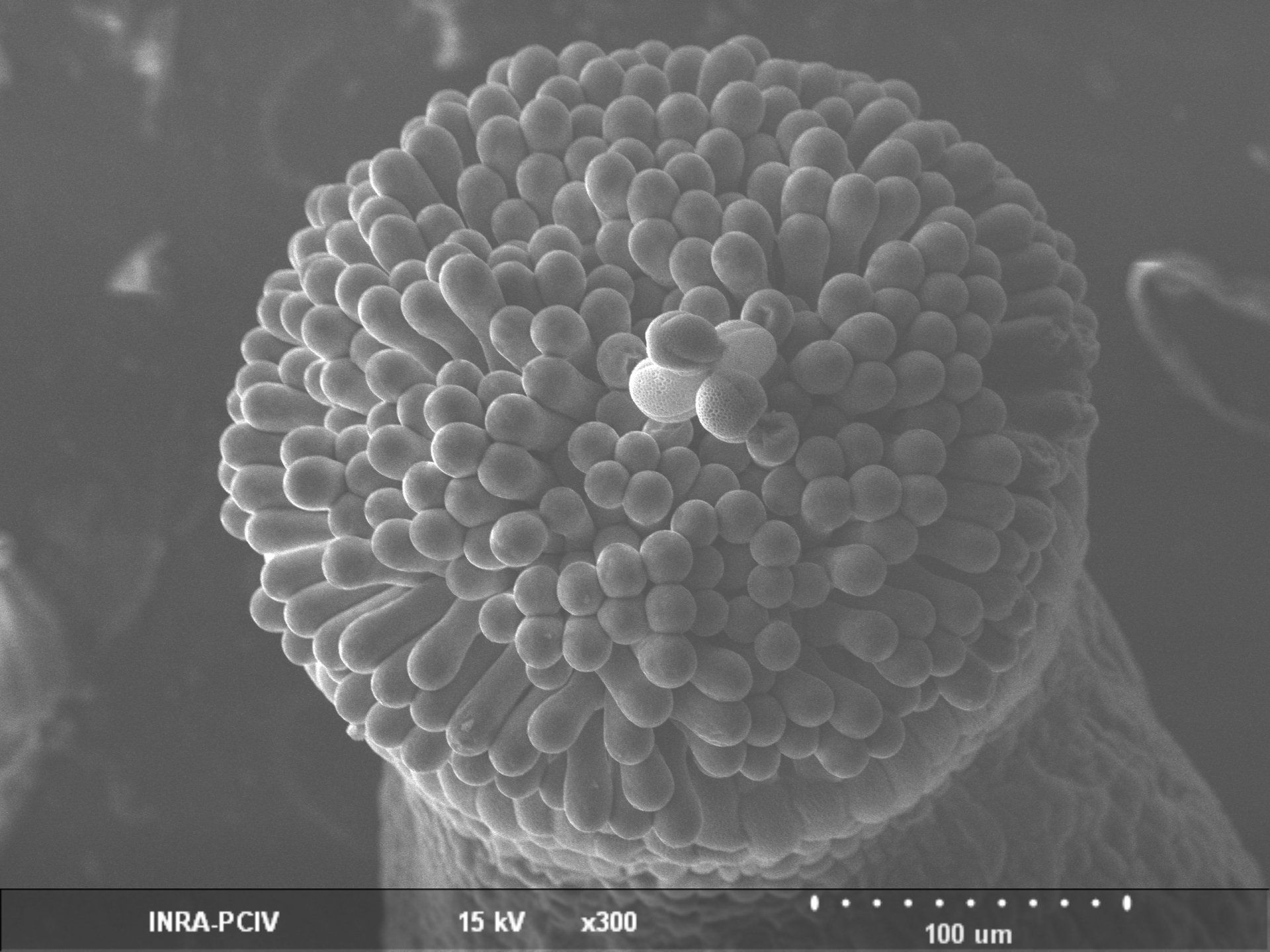
Ler *quartet*

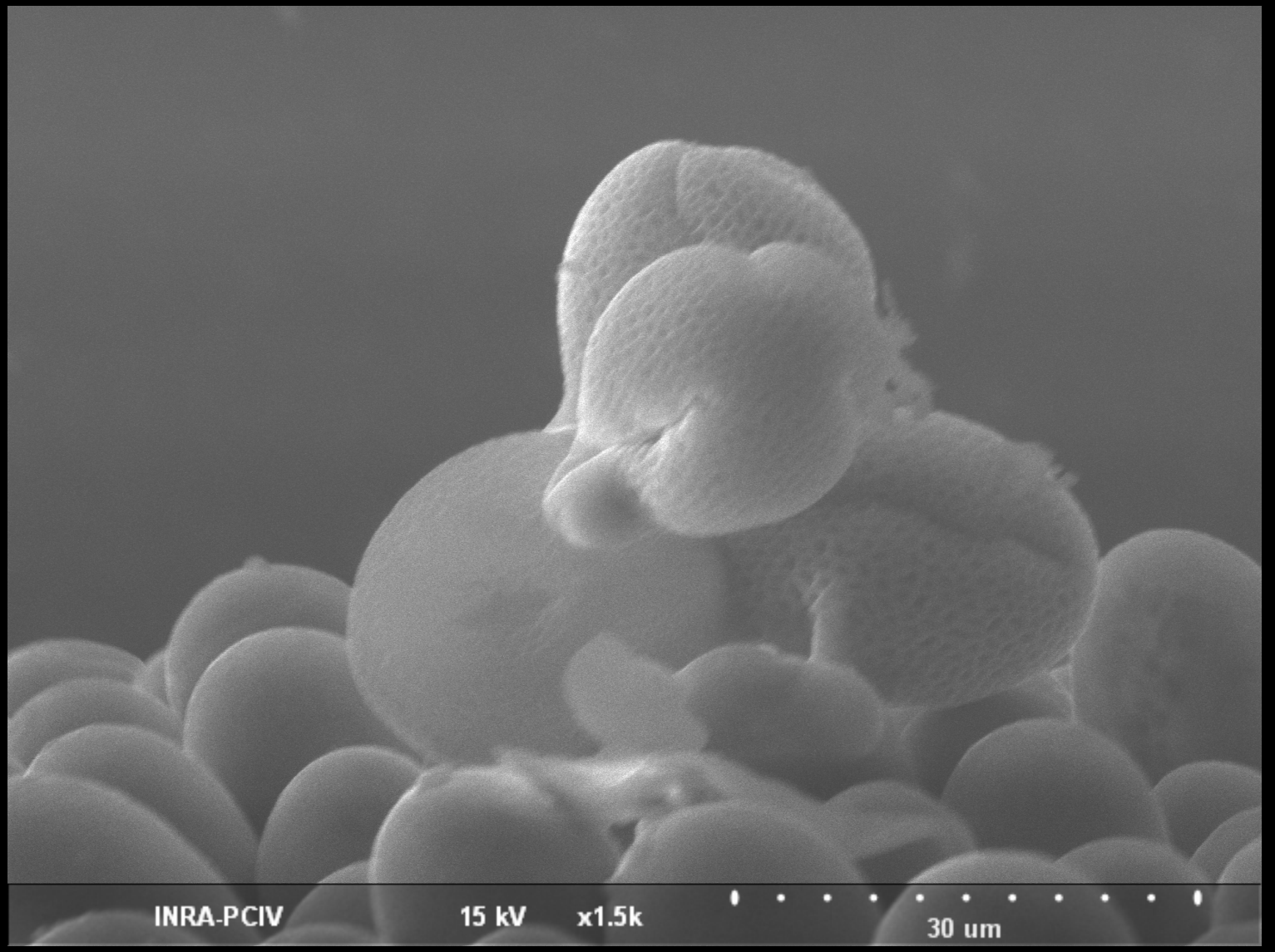


Fertilization on Col *quartet*

Fruits with four seeds !!

DNA extraction and Hi Seq sequencing


INRA-PCIV
15 kV
x300
100 um


INRA-PCIV
15 kV
x1.5k
30 um

Plan de bataille (suite)

Not very Efficient : 2000 fertilizations
12 fruits with 4 seeds
4 "true" tetrads

Sequencing done at EPGV, Evry : NGS, Illumina , 2x 100 bp pair-end , Coverage between 35 to 50x

Bio-informatic analysis performed by Delphine Charif, Bioinformatic unit, IJPB, Versailles

"Theoretical" Polymorphisms : 690 522 SNPs + 174 638 indels

Polymorphisms / tetrad : 370 442 to 390 582

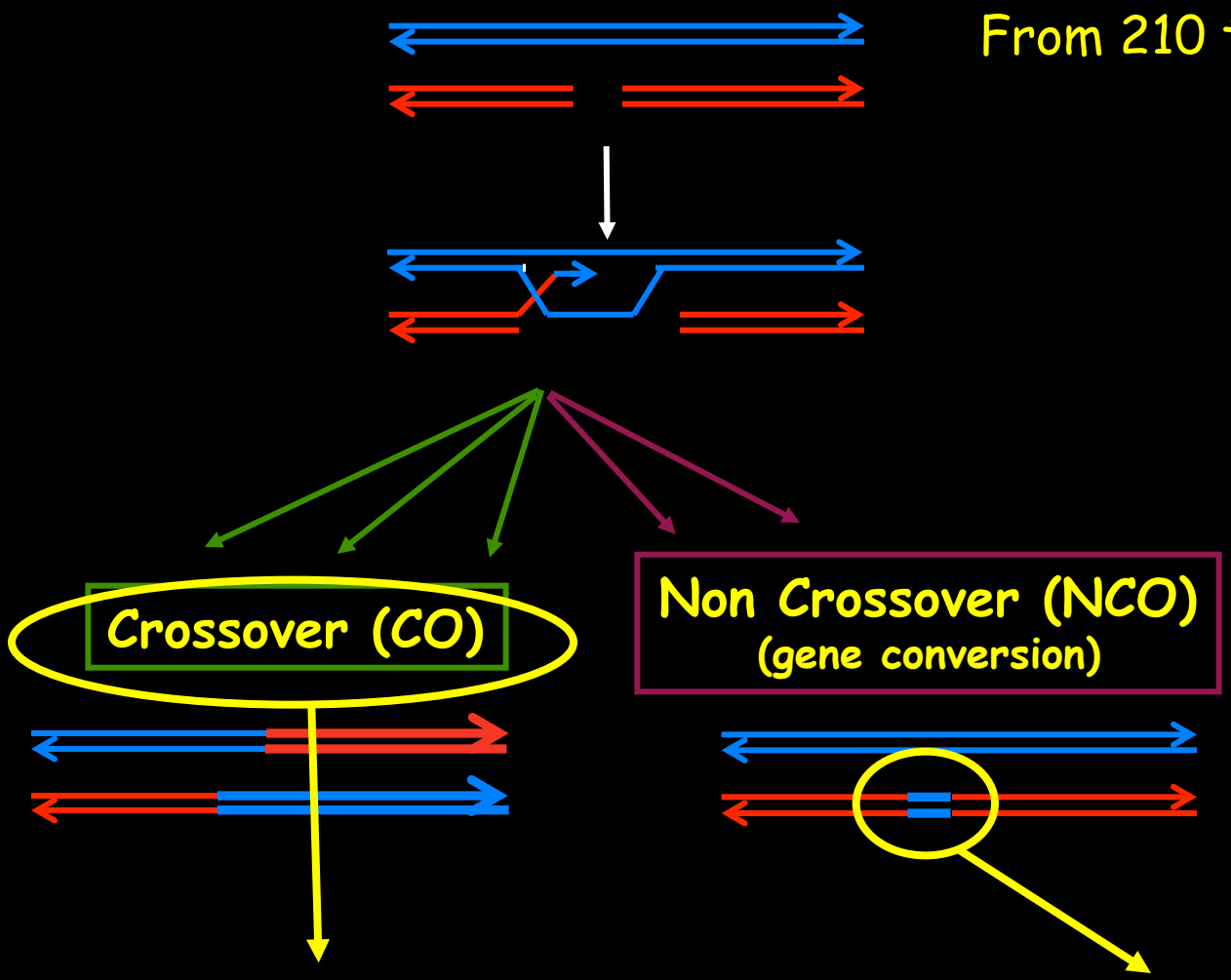
0,3% of divergence

	T1	T2	T3	T4
COs	10	9	10	11
Verified by Sanger	10	9	10	11

	T1	T2	T3	T4
NCOs	40	15	29	20
Verified by Sanger	4	1	3	2 ? ongoing

Length from 1 bp to 6,3 kb (1 SNP to 7 SNPs)

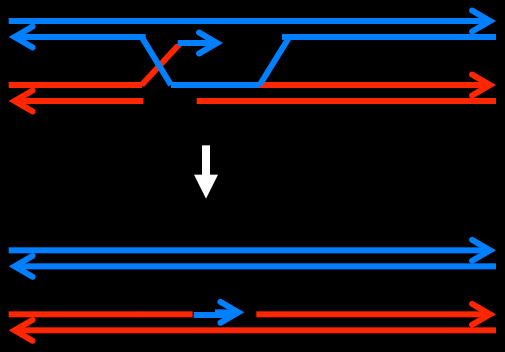
Very few NCOs detected in a single meiosis……..!!!


From 210 to 290 DSB sites
Crossover (CO)
Non Crossover (NCO)
(gene conversion)
To 9 to 11 COs per meiosis
To 1 to 4 NCOs per meiosis
(Liu et al., 2012; Wijnker et al., 2013)

How are repaired the DSBs ?

From 210 to 290 DSB sites

To 9 to 11 COs per meiosis plus 1 to 4 NCOs

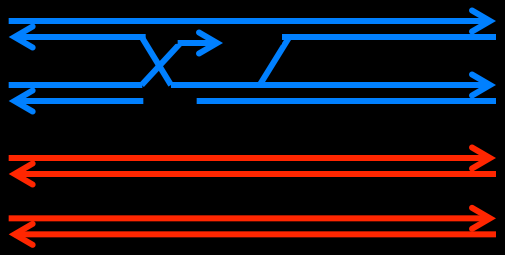


NCOs ? If so very small conversion tracts that rarely involve a SNP !!! (a few bp….)

How are repaired the DSBs ?

From 210 to 290 DSB sites

To 9 to 11 COs per meiosis plus 1 to 5 NCOS

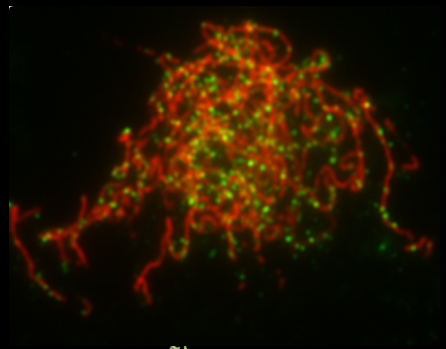


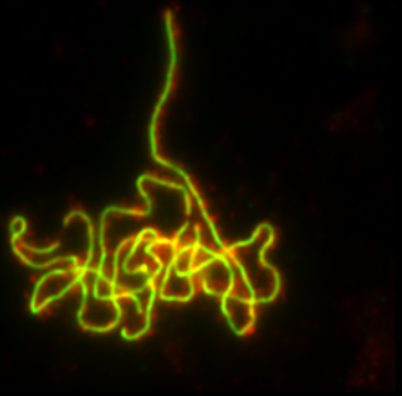
NCOs ? If so very small conversion tracts that rarely involve a SNP !!! (a few bp….)

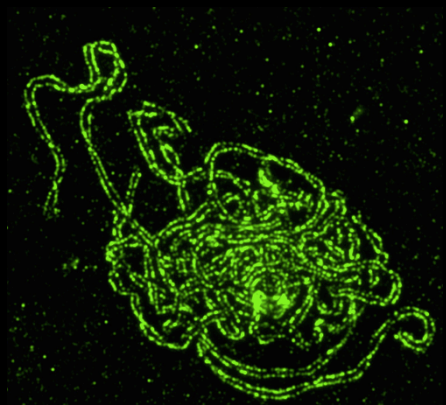
Sister Chromatid ? May be but how many ?

Other: Mismatch repair, ligation ?

Ongoing:
Genetic Maps, Hotspots and Tetrads analyses being done in various mutant context







Is there recombination in meiosis ?

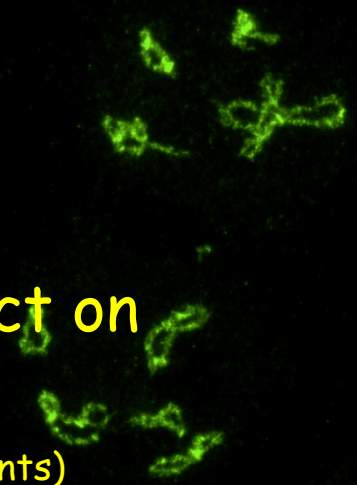
YES !

And it is essential ….

But it is mainly COs

and NCOs have a very limited impact on haplotype landscape

(at least in *Arabidopsis* and perhaps more generally plants)



Ces résultats ont été obtenus grâce à





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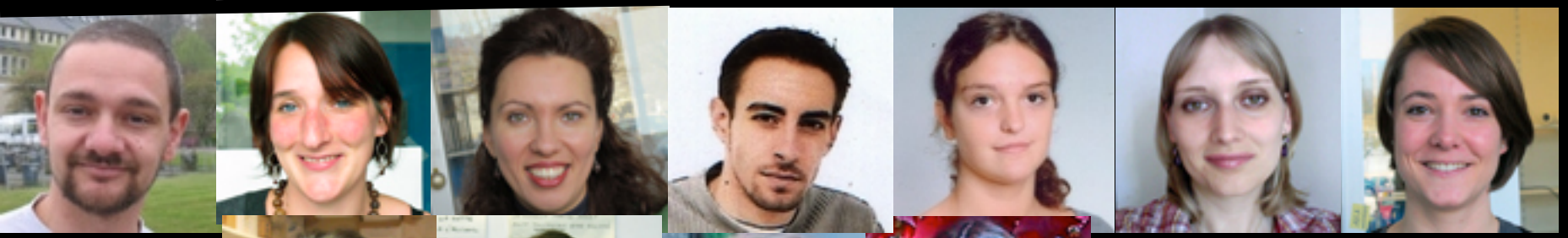
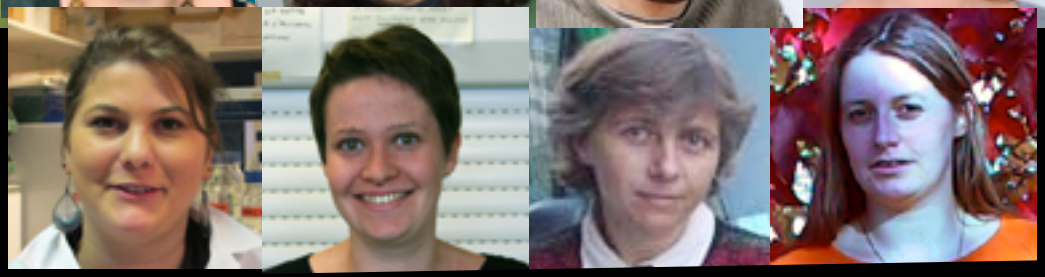
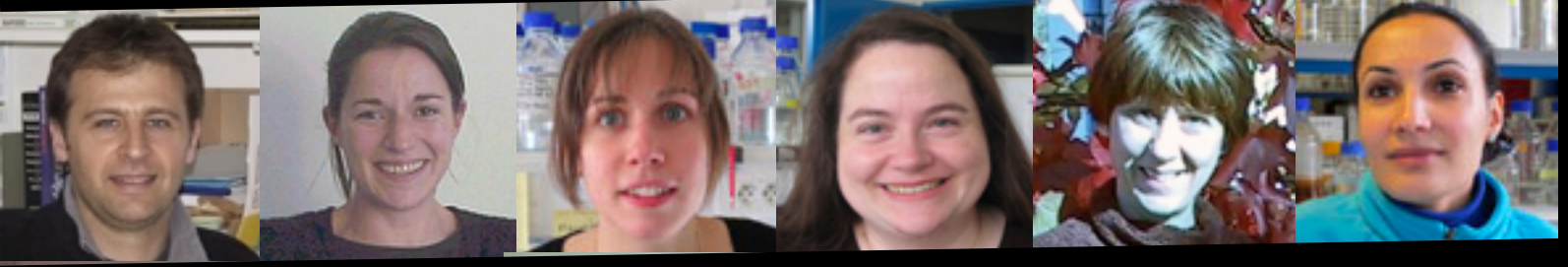
Equipe EPGV, CNG, Evry

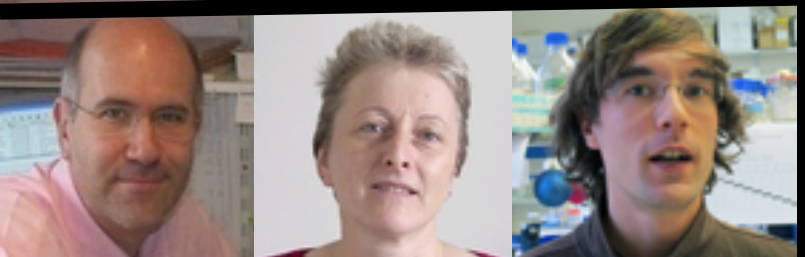
Fundings

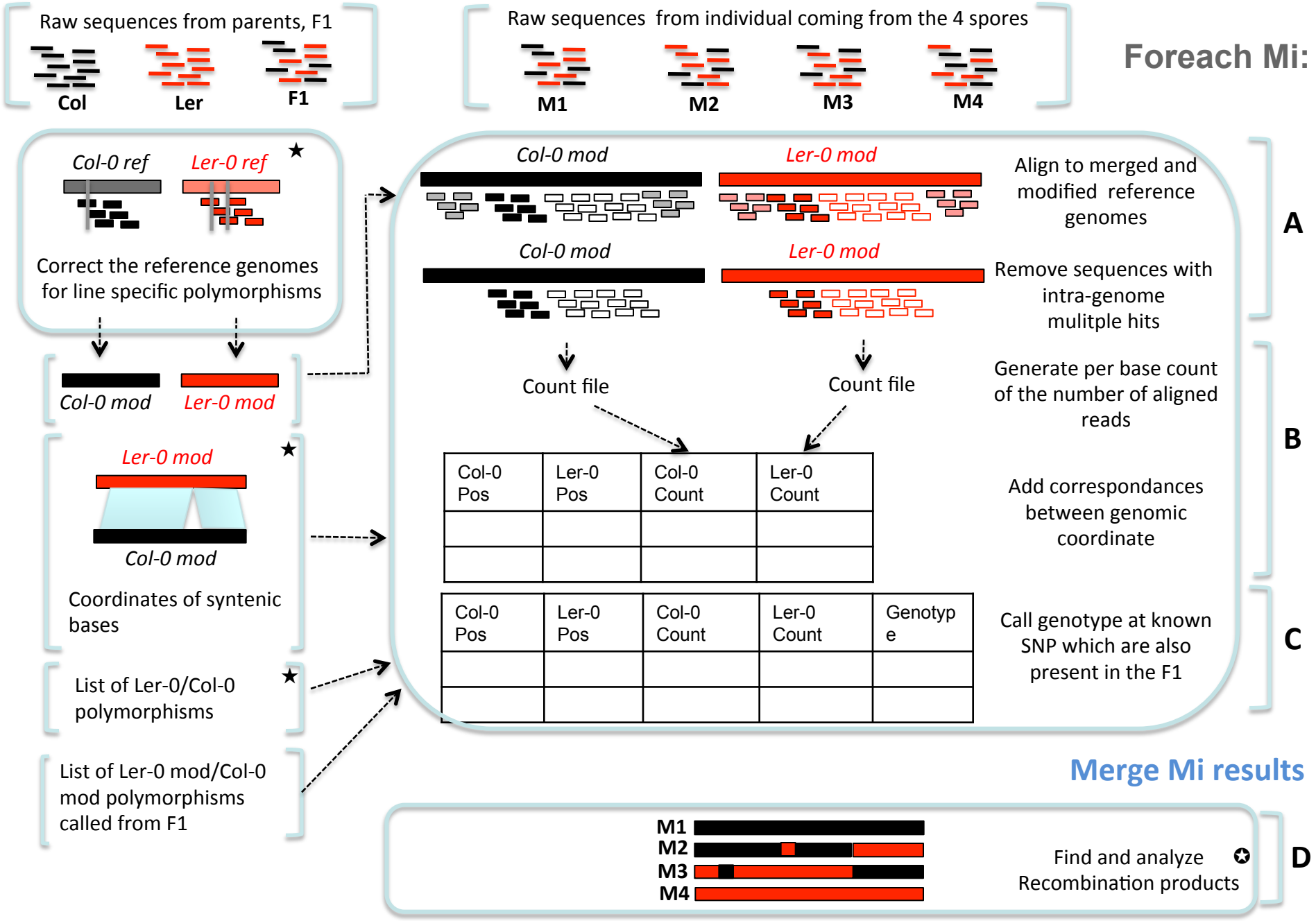
Groupe Méiose et Recombinaison







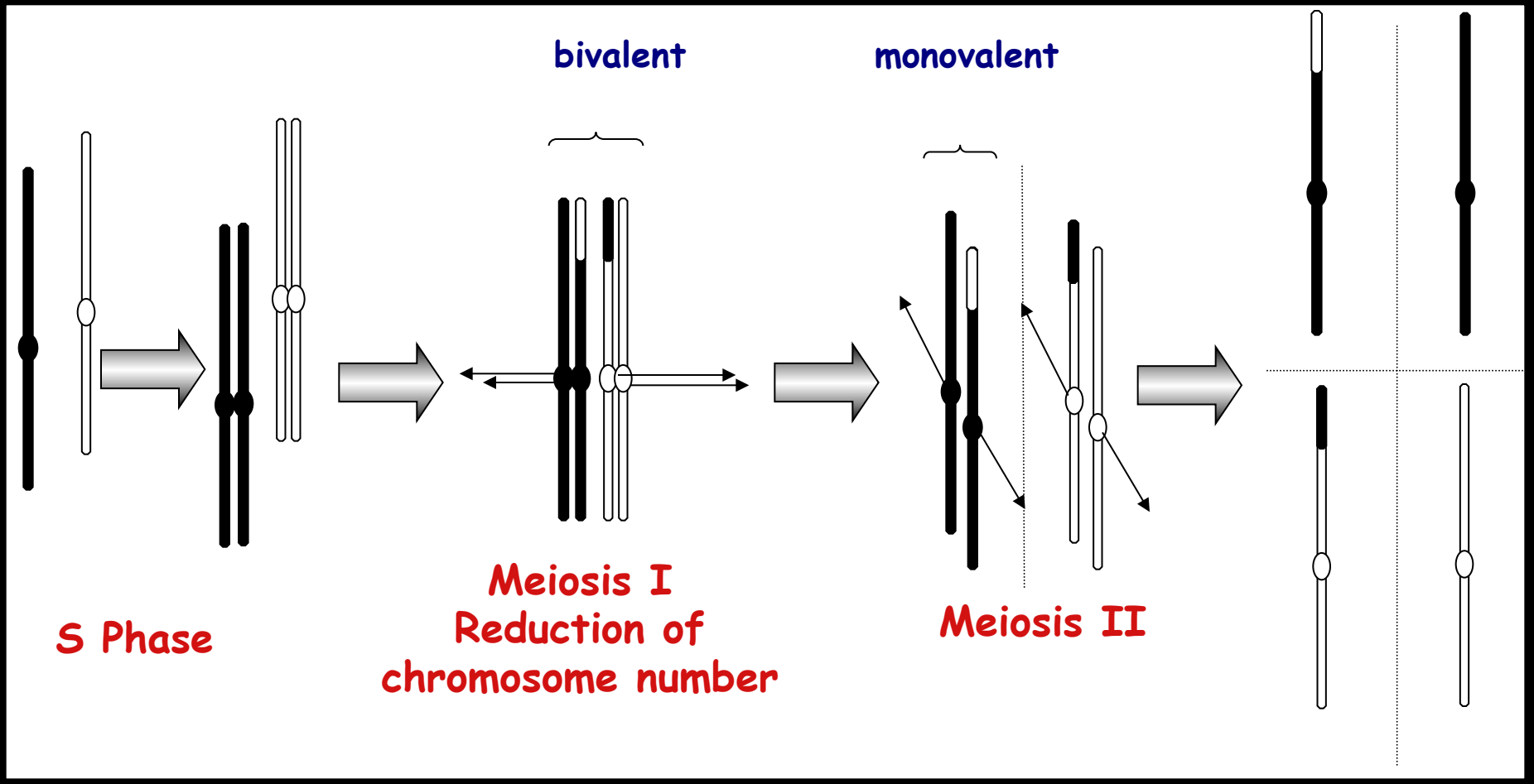




★ Gan, X et al. (2011). Multiple reference genomes and transcriptomes for Arabidopsis thaliana. Nature.

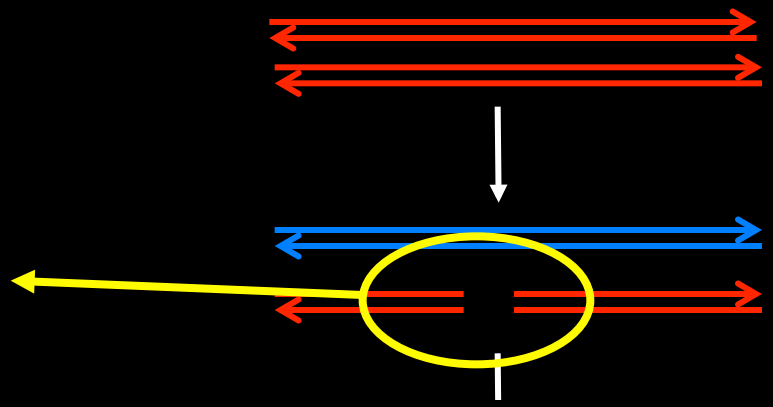
✪ Anderson, C. M. et al. (2011). ReCombine: A Suite of Programs for Detection and Analysis of Meiotic Recombination in Whole-Genome Datasets. PLoS ONE.

The aim of meiosis: reduction of ploidy

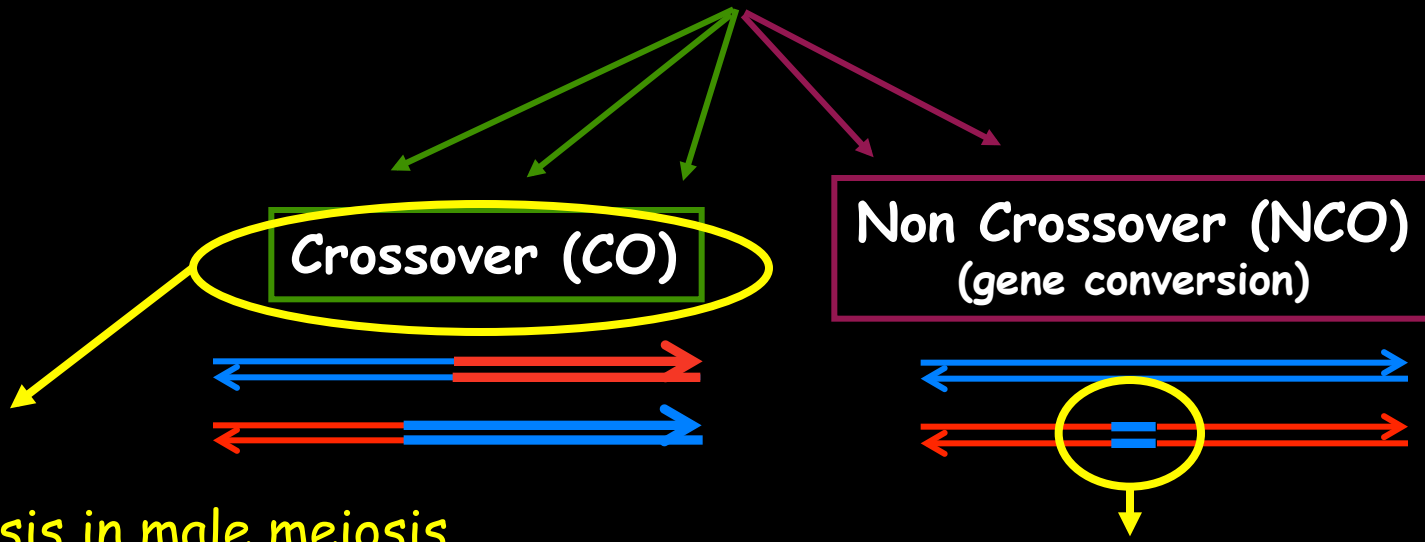


How many Recombination events in one meiosis ?

From 210 to 290 DMC1 foci
(DSBs repair sites)

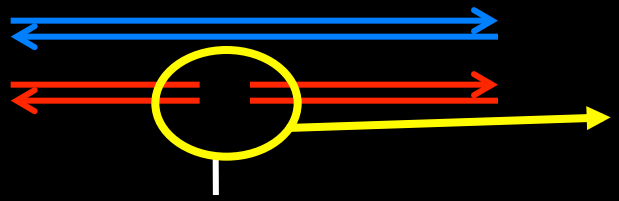


How are repaired the DSBs ?



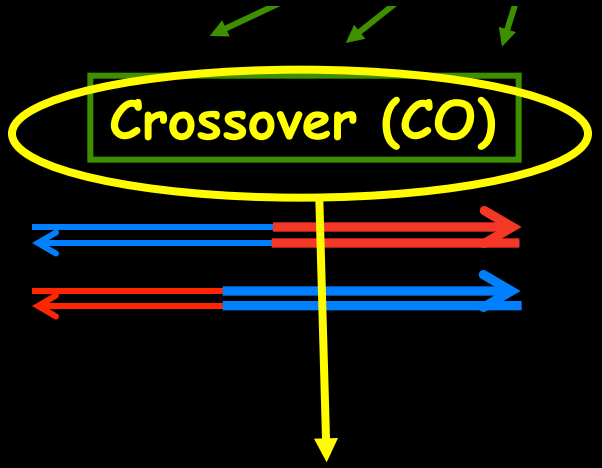
11.1 COs per meiosis in male meiosis
And at least one per bivalent

????

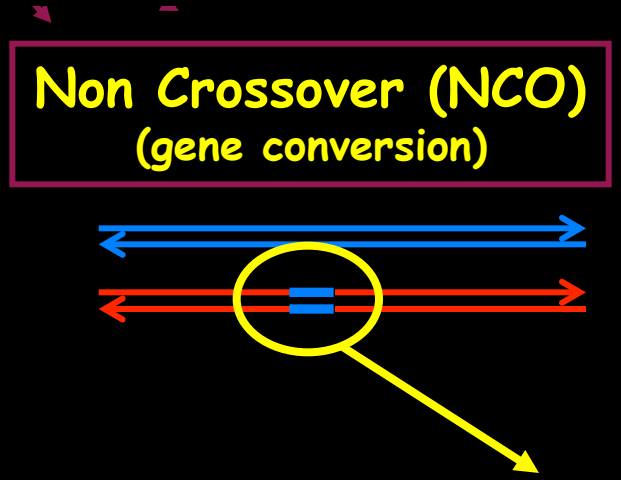


From 210 to 290 DSB sites

How are repaired the other DSBs ?

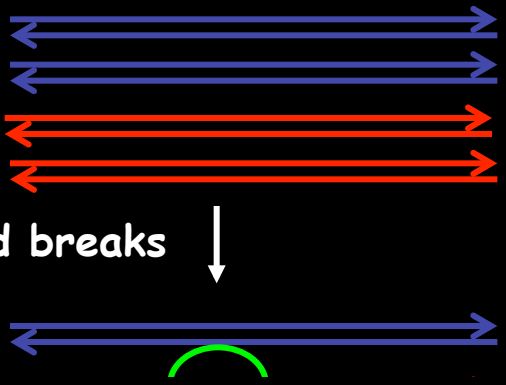


To 9 to 11 COs per meiosis



To 1 to 5 NCOs per meiosis
(Liu et al., 2012; Wijnker et al., 2013)

Meiotic Recombination



Is there recombination in meiosis ?

YES !

And it is essential ….

But where ?

And how many events ?