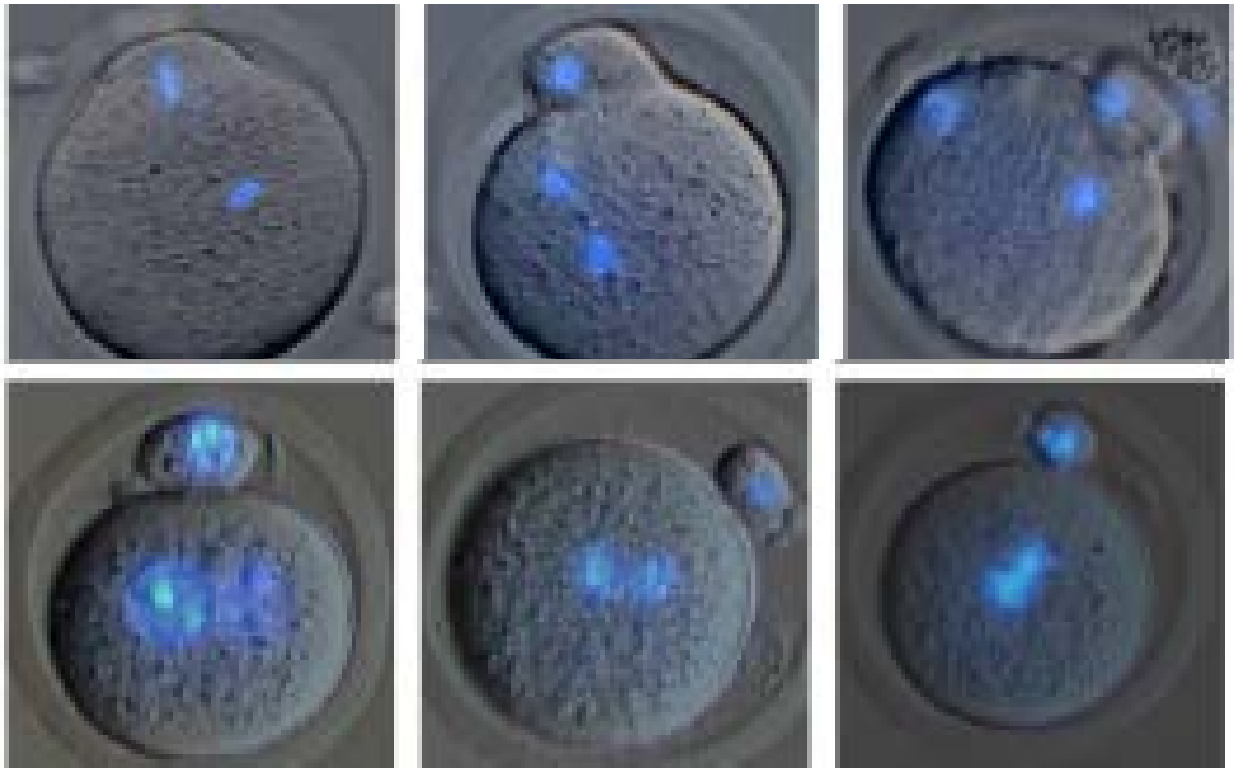
# Gene editing in the embryo & double-strand break repair

Maria Jasin, PhD

Memorial Sloan Kettering Cancer Center
New York

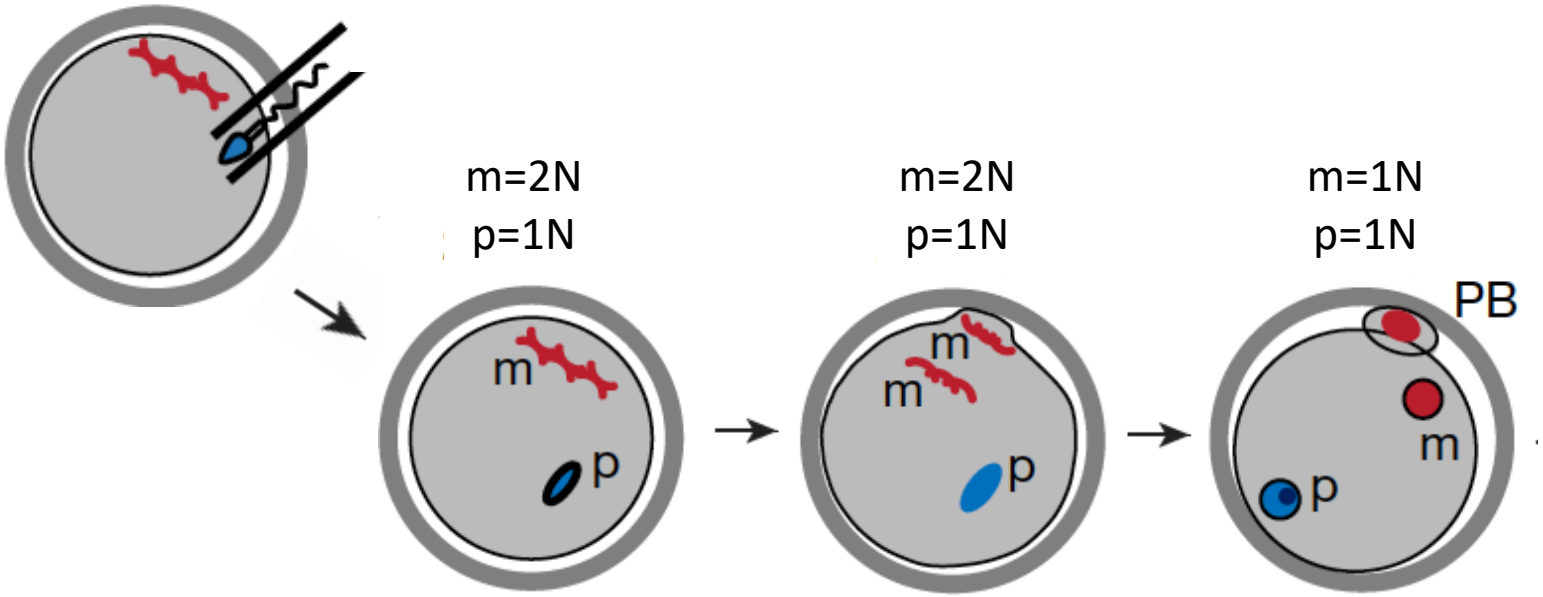


Overview:

1. Unique features of the first embryonic cell cycle

2. The complexities of double-strand break repair

# Unique features of the first embryonic cell cycle




**Ploidy**:
Maternal genome begins as 2N, paternal as 1N

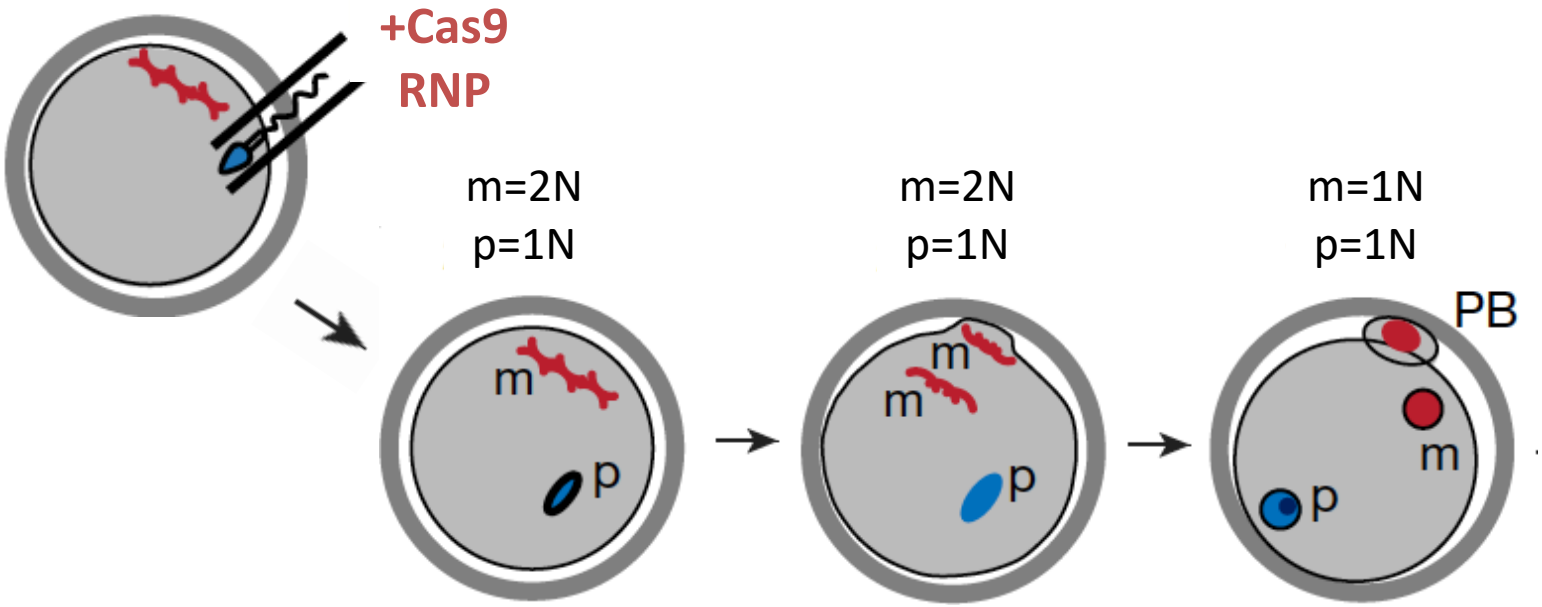
**Chromatin:**
Paternal genome undergoes protamine to histone transition

**Division**:
Maternal genome undergoes 2nd meiotic division

**Pronuclei**:
Maternal & paternal genomes in separate pronuclei

Egli et al Nature 2018

# Unique features of the first embryonic cell cycle

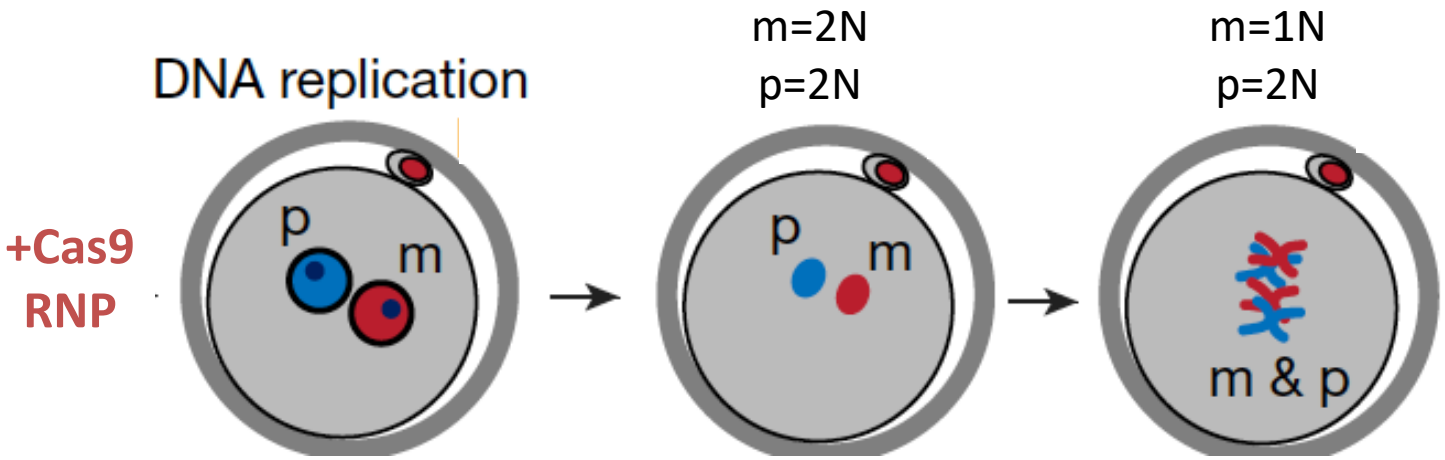



**Advantage of CRISPR-Cas9 injections at this stage**:
Immediate gene editing would lead to embryos without **mosaicism.**

**Possible complications:**
-Differential maternal/paternal genome editing due to distinct chromatin.
-Cas9 cleavage of maternal genome during MII division.

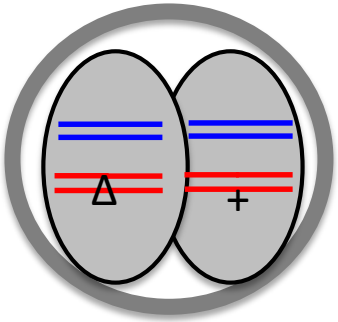
Egli et al Nature 2018

# Unique features of the first embryonic cell divisions



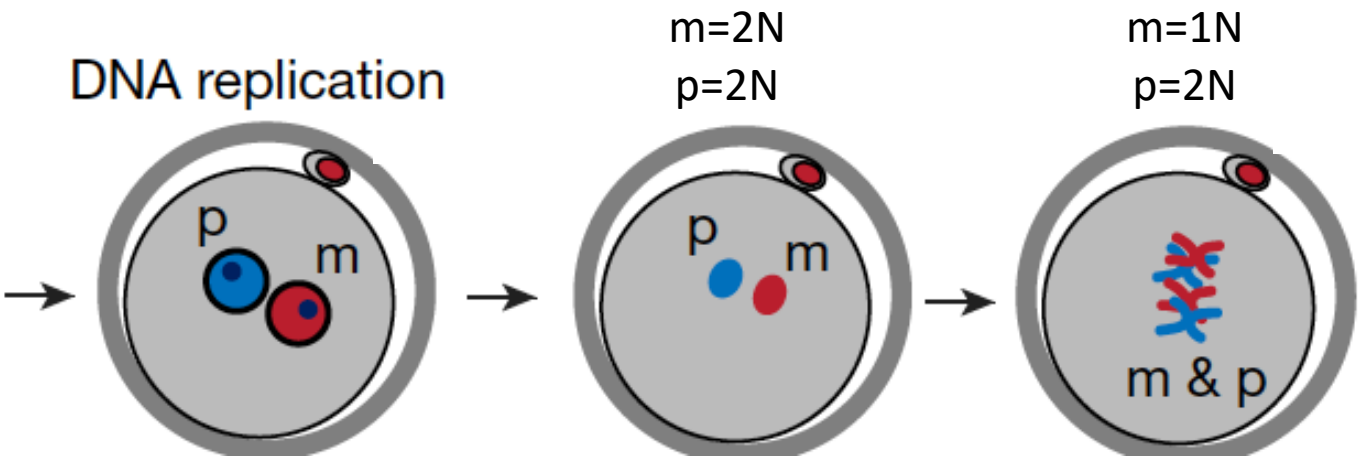

**Disadvantage of CRISPR-Cas9 injections at this stage**:

**Mosaicism**




Egli et al Nature 2018

# Unique features of the first embryonic cell divisions



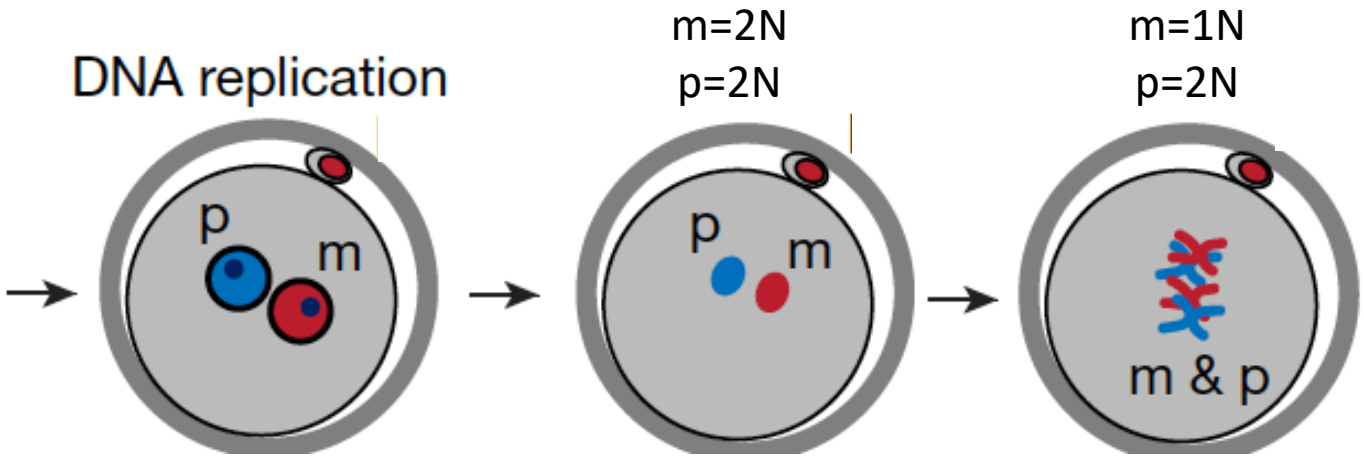

**Pronuclei**:
Maternal & paternal genomes are in separate pronuclei

**Nuclear envelope breakdown**:
Distinct maternal & paternal genomes

**Metaphase**:
Maternal & paternal genomes remain distinct

Egli et al Nature 2018

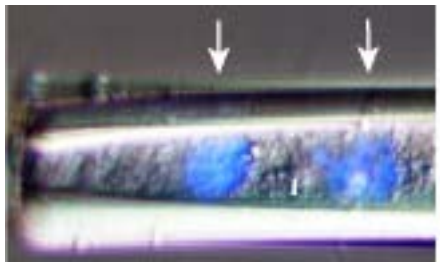
# Unique features of the first embryonic cell divisions




**Pronuclei**:
Maternal & paternal genomes are in separate pronuclei

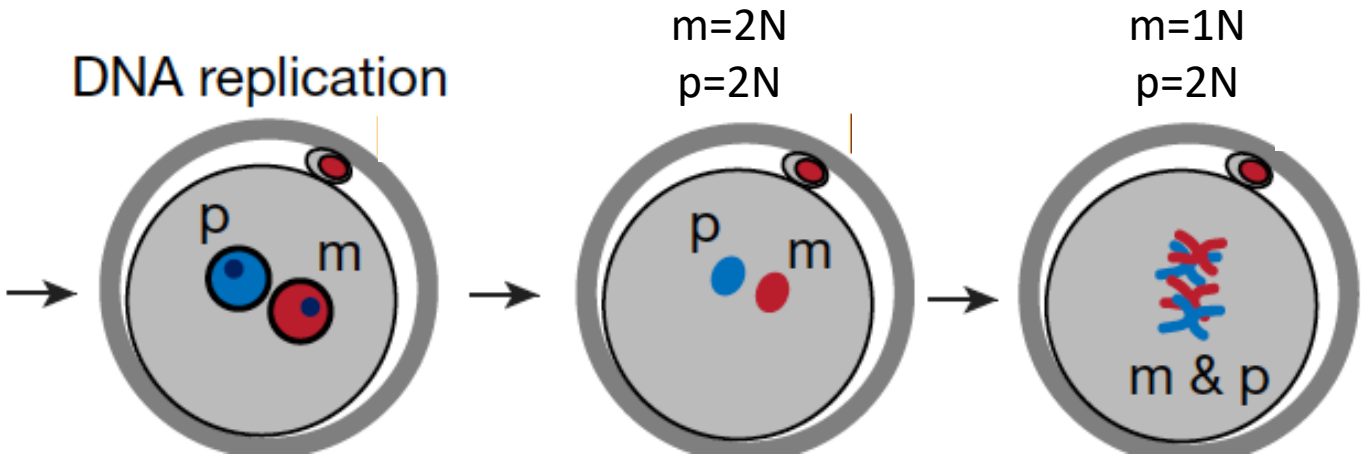
**Nuclear envelope breakdown**:
Distinct maternal & paternal genomes

**Metaphase**:
Maternal & paternal genomes remain distinct



Egli et al Nature 2018

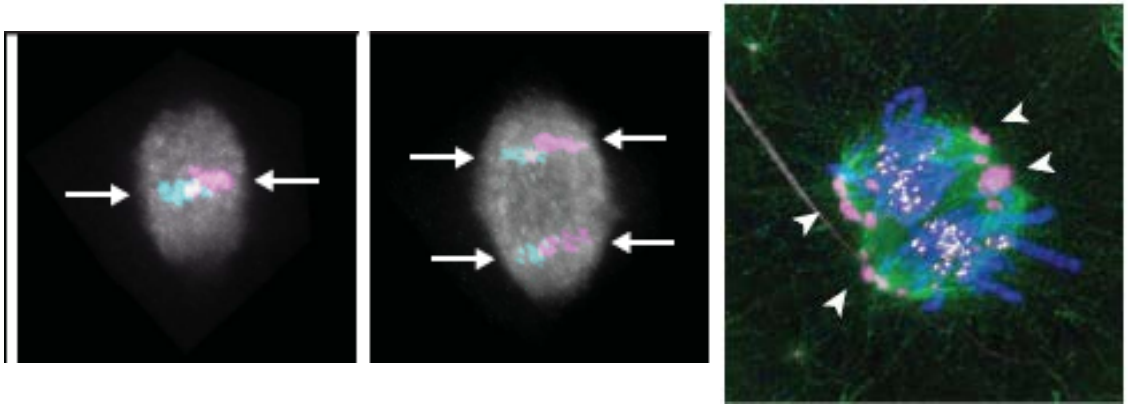
# Unique features of the first embryonic cell divisions




**Pronuclei**:
Maternal & paternal genomes are in separate pronuclei

**Nuclear envelope breakdown**:
Distinct maternal & paternal genomes

**Metaphase**:
Maternal & paternal genomes remain distinct



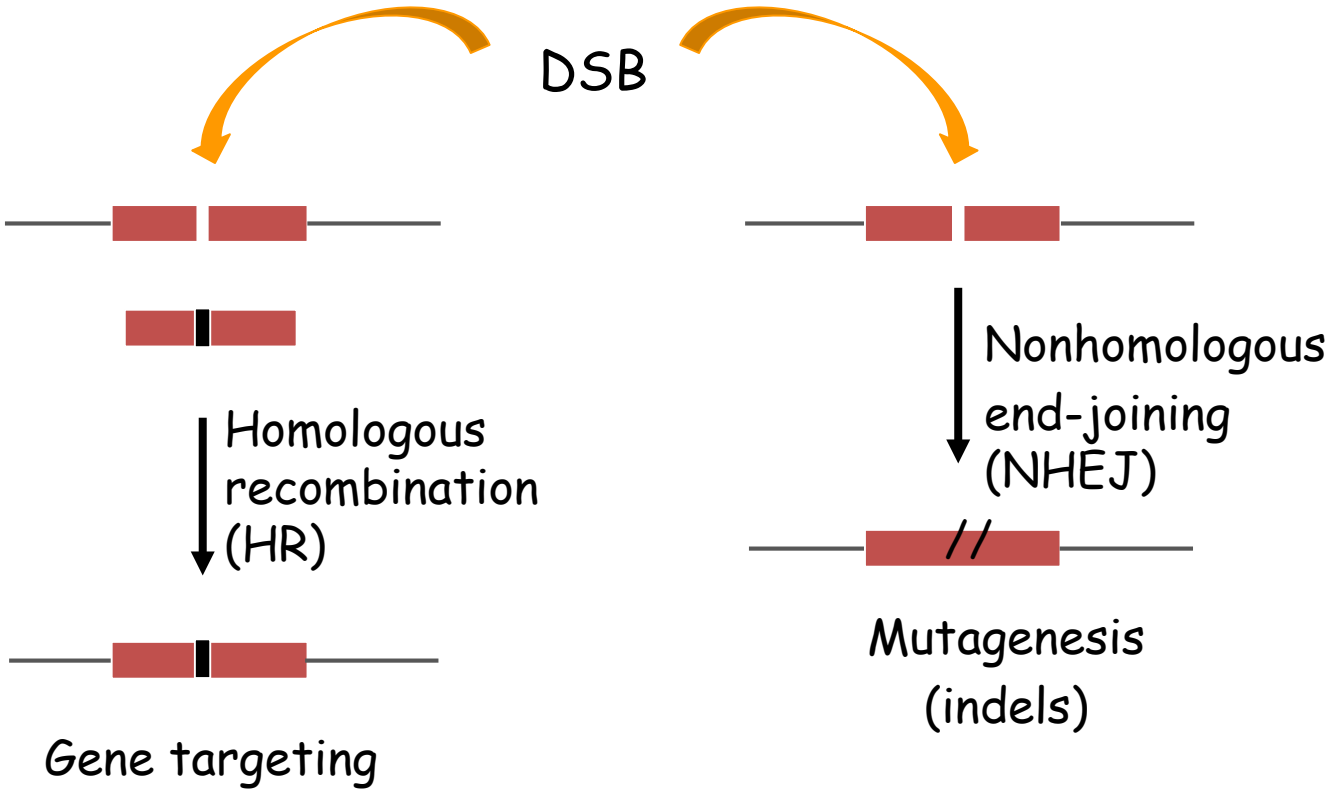
Ellenberg and colleagues Nature 2018

Overview:

1. Unique features of the first embryonic cell cycle

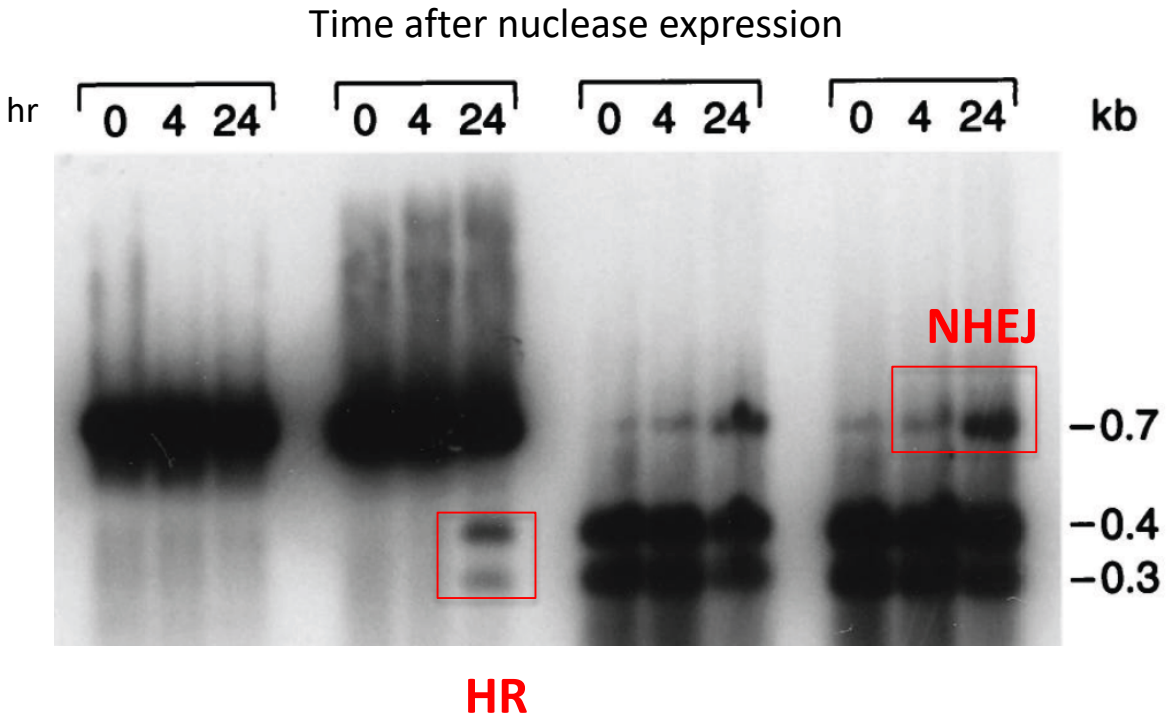
2. The complexities of double-strand break repair

# Gene editing – 1994 and onward:
# DNA double-strand break (DSB) centric




Rouet et al, 1994

# Two major DSB repair pathways

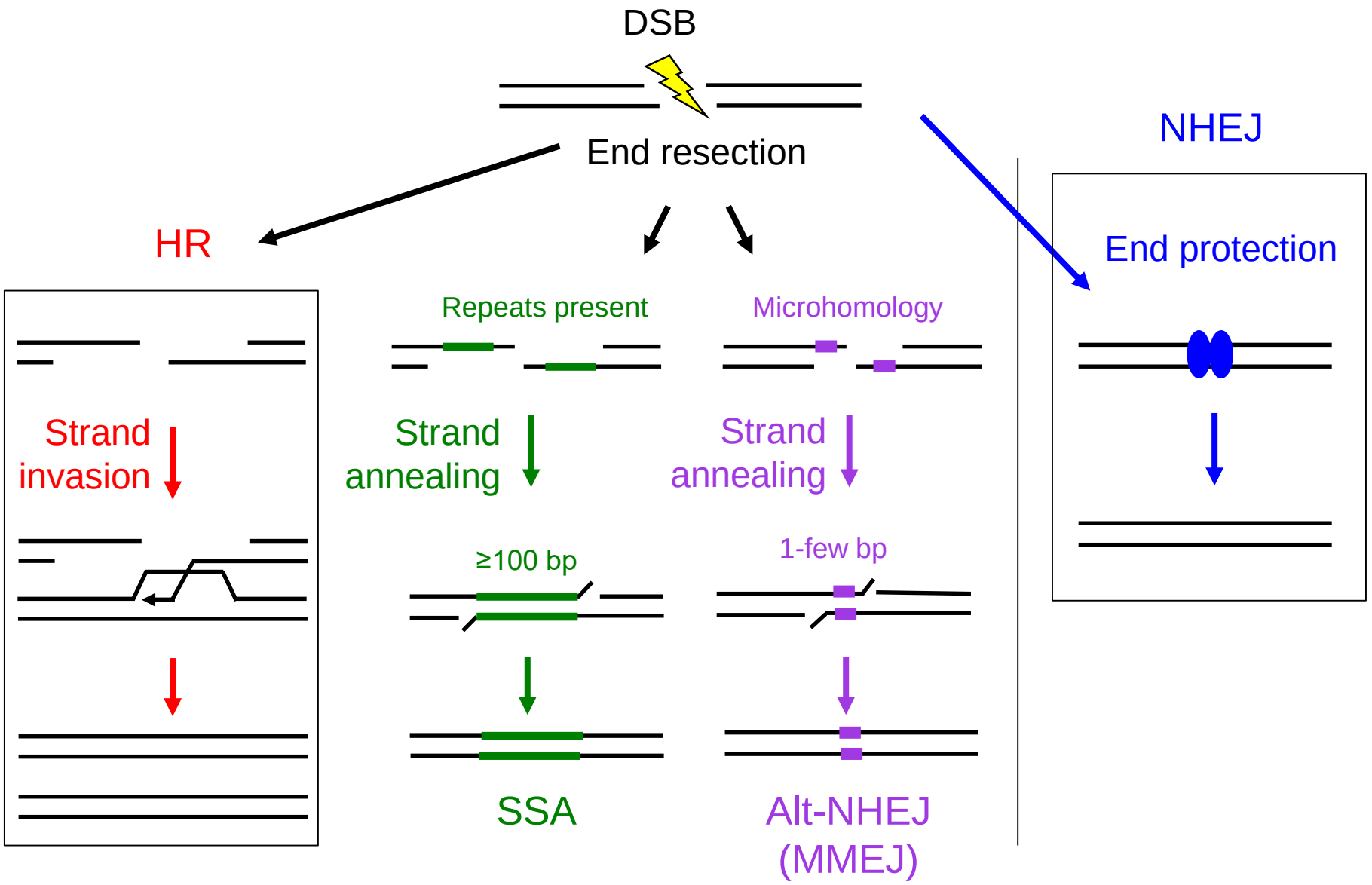



HR: Homologous recombination (= HDR)

NHEJ: Nonhomologous end-joining

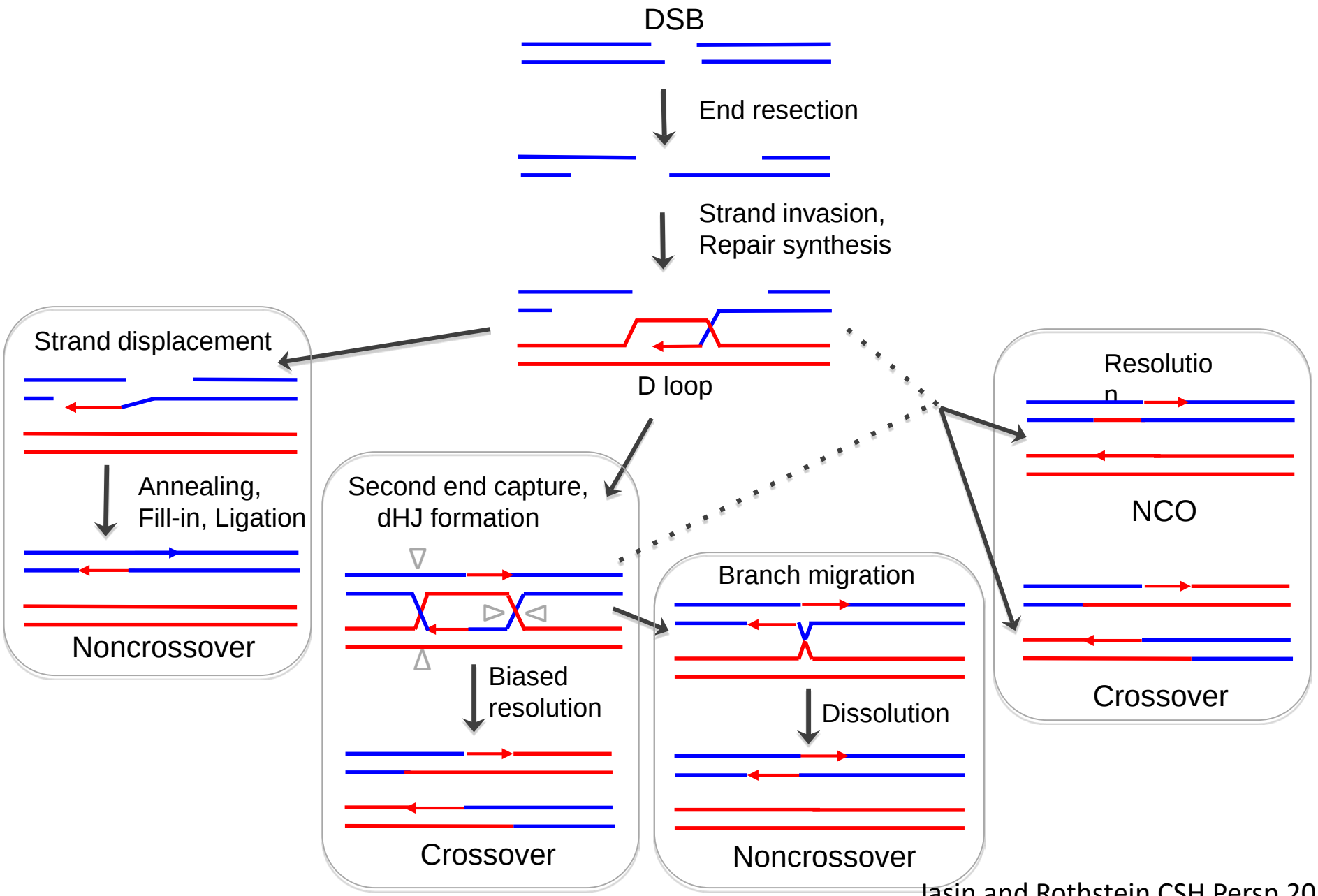
Liang et al 1998

# Additional DSB repair pathways



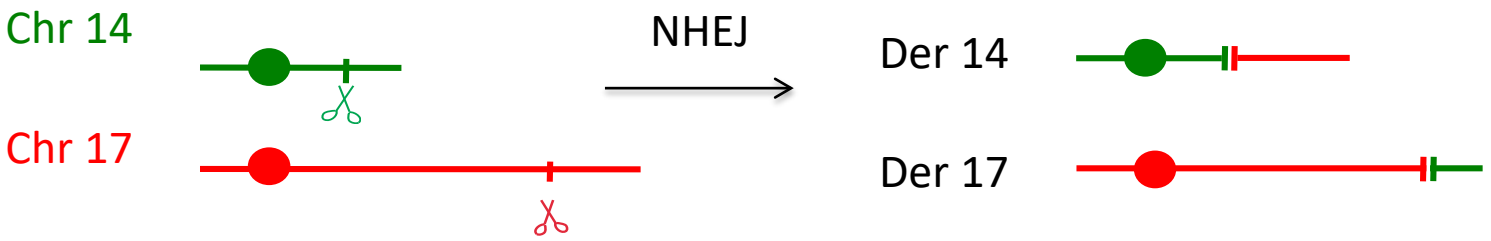

Jasin and Rothstein CSH Persp 2013

# HR is actually quite complex

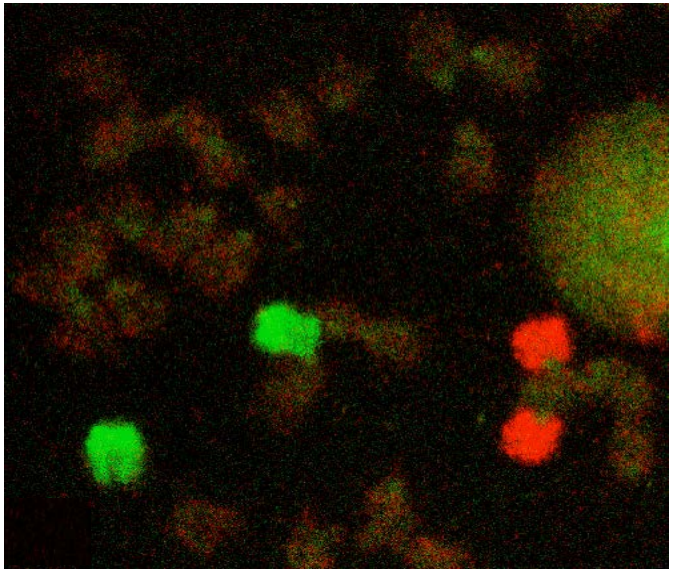



Jasin and Rothstein CSH Persp 2013

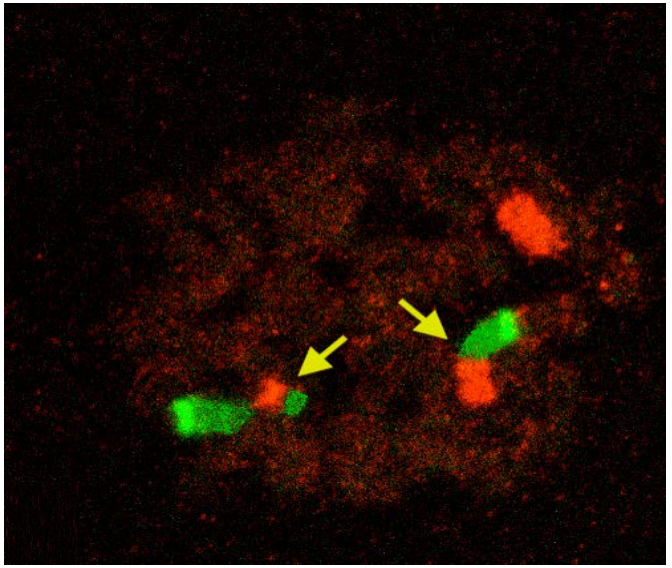
# DSBs induce chromosomal rearrangements in mammalian cells




Normal chromosomes

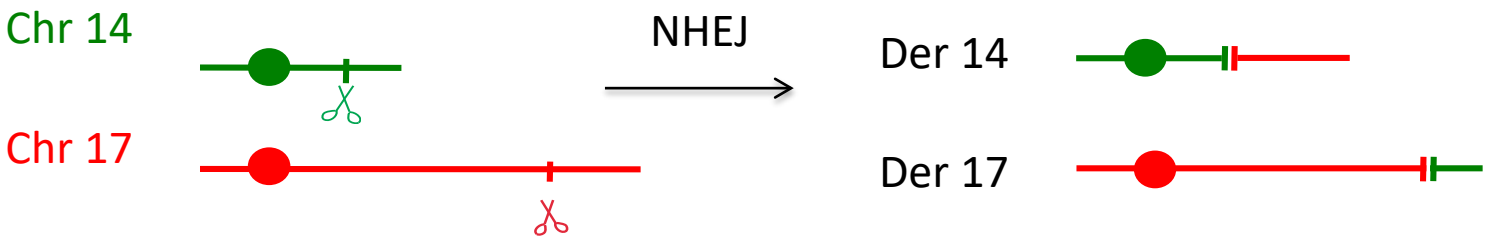


Chromosomal translocation

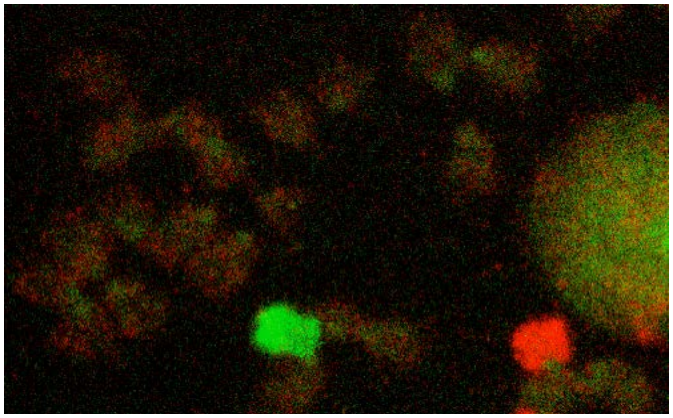


Richardson and Jasin, Nature 2000

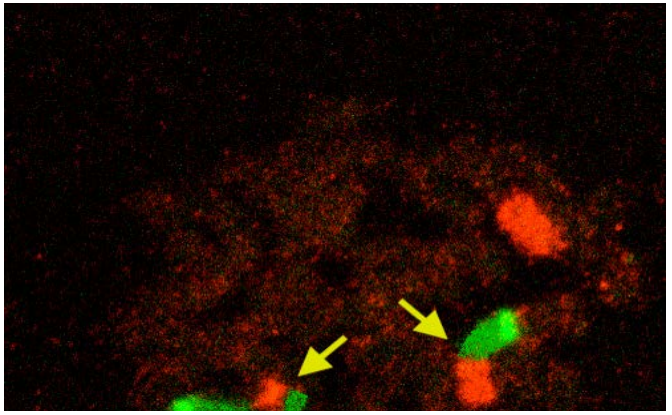
# DSBs induce chromosomal rearrangements in mammalian cells




Normal chromosomes

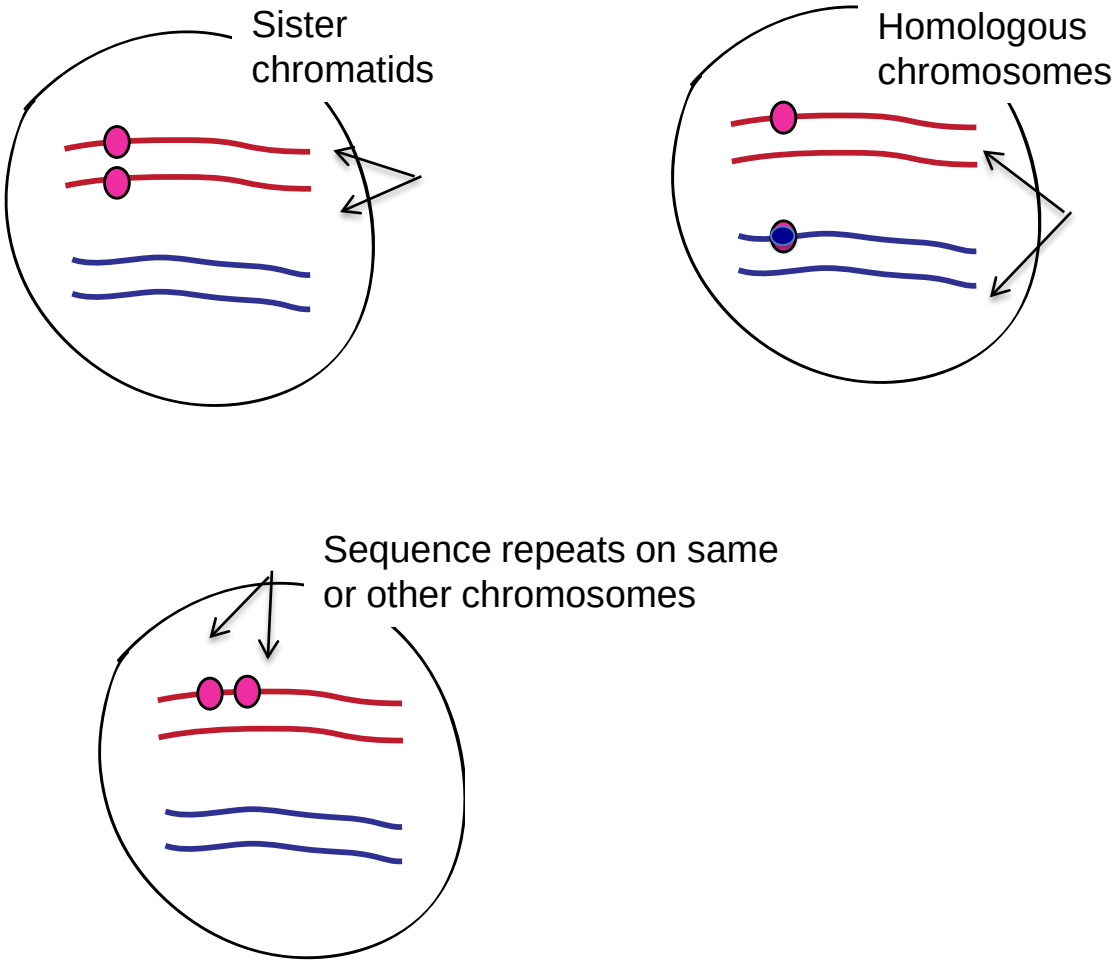


Chromosomal translocation

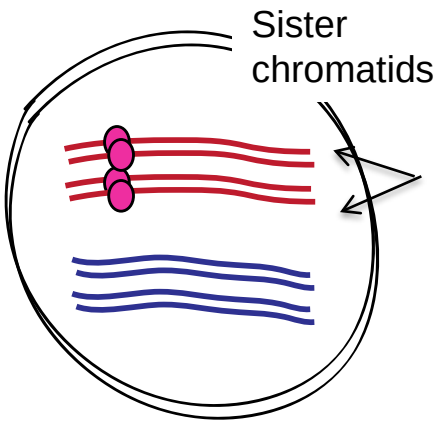


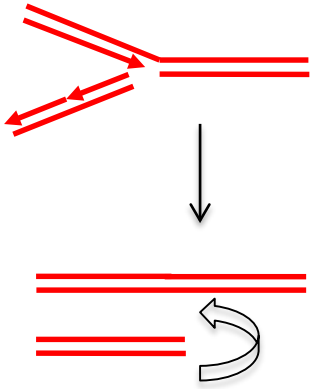
Other rearrangements: Deletions, insertions, chromosome fusions, etc

# Multiple HR templates



# Intersister HR is important during DNA replication



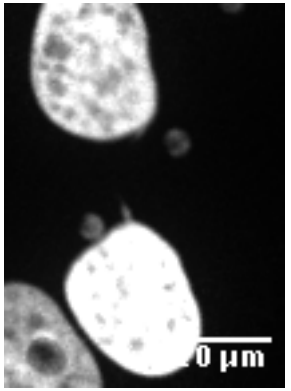



HR to re-establish
Replication forks

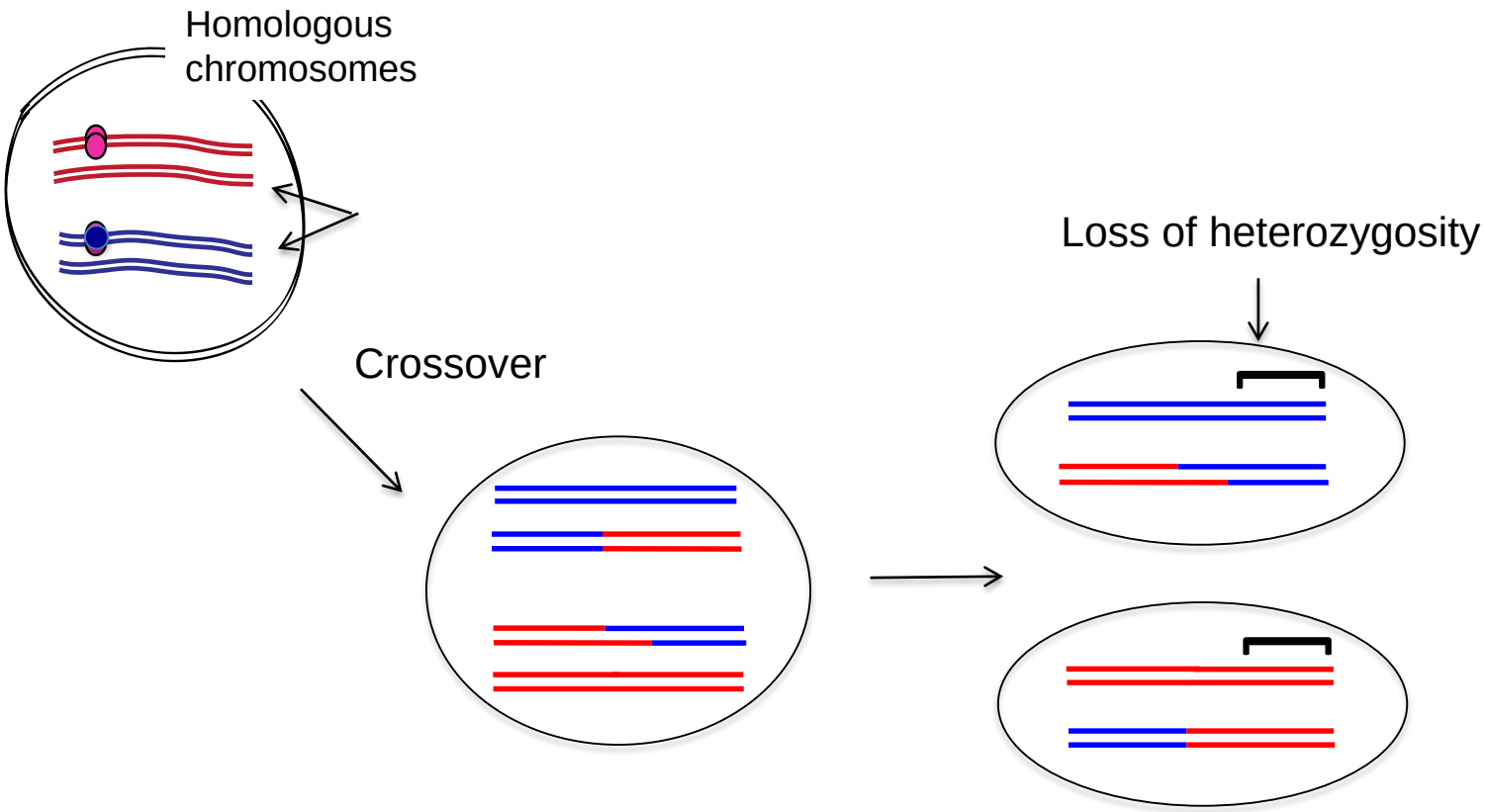
HR-deficient cells

Chromosome fragments

Micronuclei

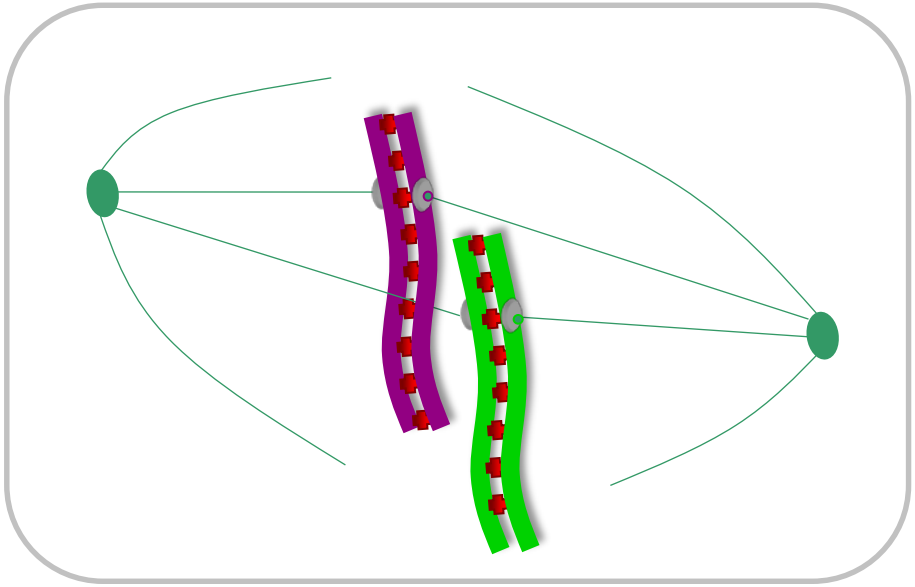


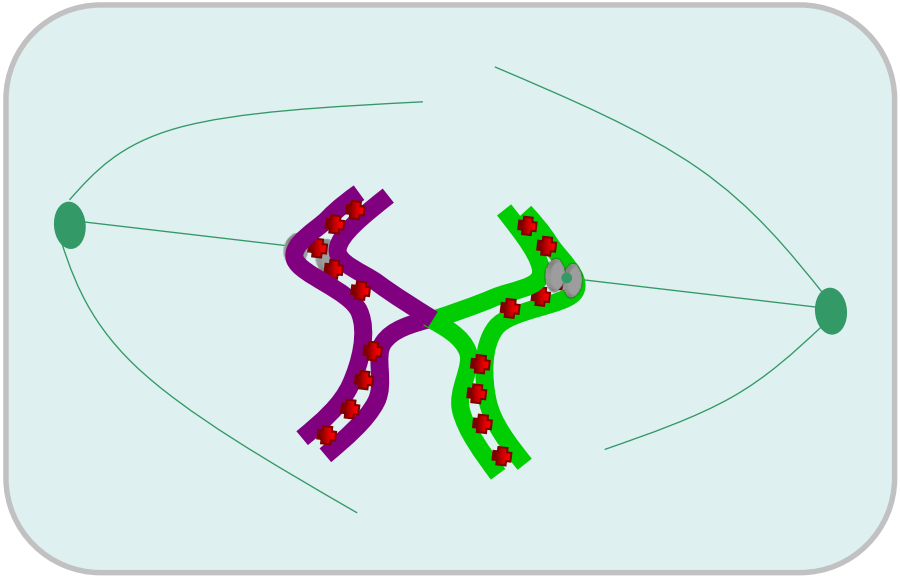
# Interhomolog HR: genetic risk from loss of heterozygosity



# In germ cells, interhomolog HR has an essential mechanical role (sperm and egg)

Mitosis vs Meiosis I



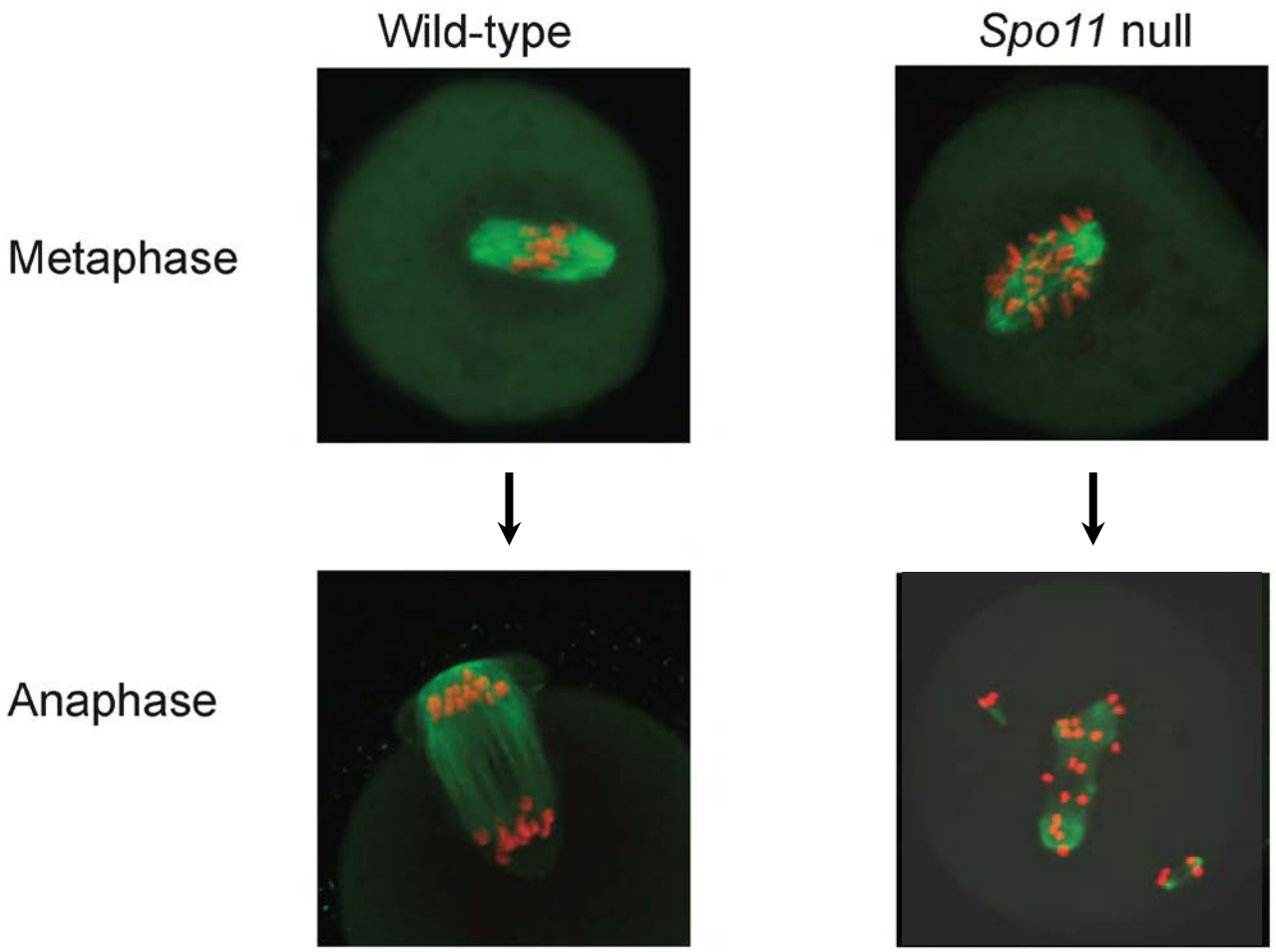


Cohesin release directs disjunction of sister chromatids

Crossovers *with* cohesin direct disjunction of homologs

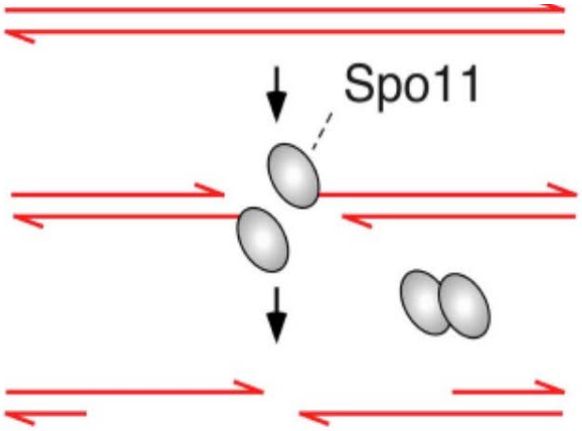
*animation courtesy of Neil Hunter*

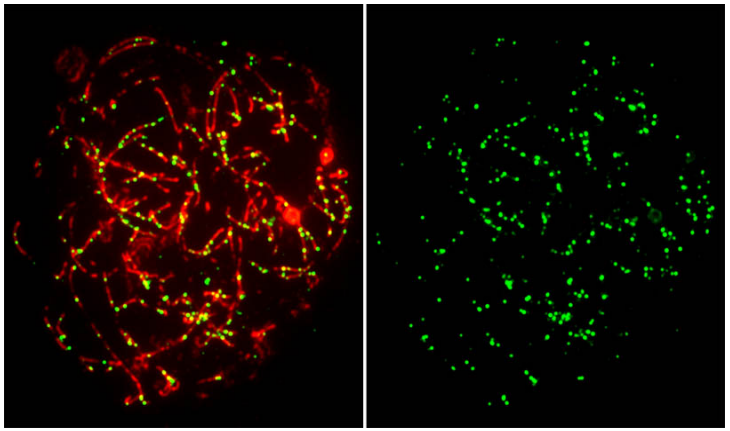
# Meiotic catastrophe in the absence of HR




Monica Di Giacomo

# Meiotic HR: Programmed DSBs introduced into the genome

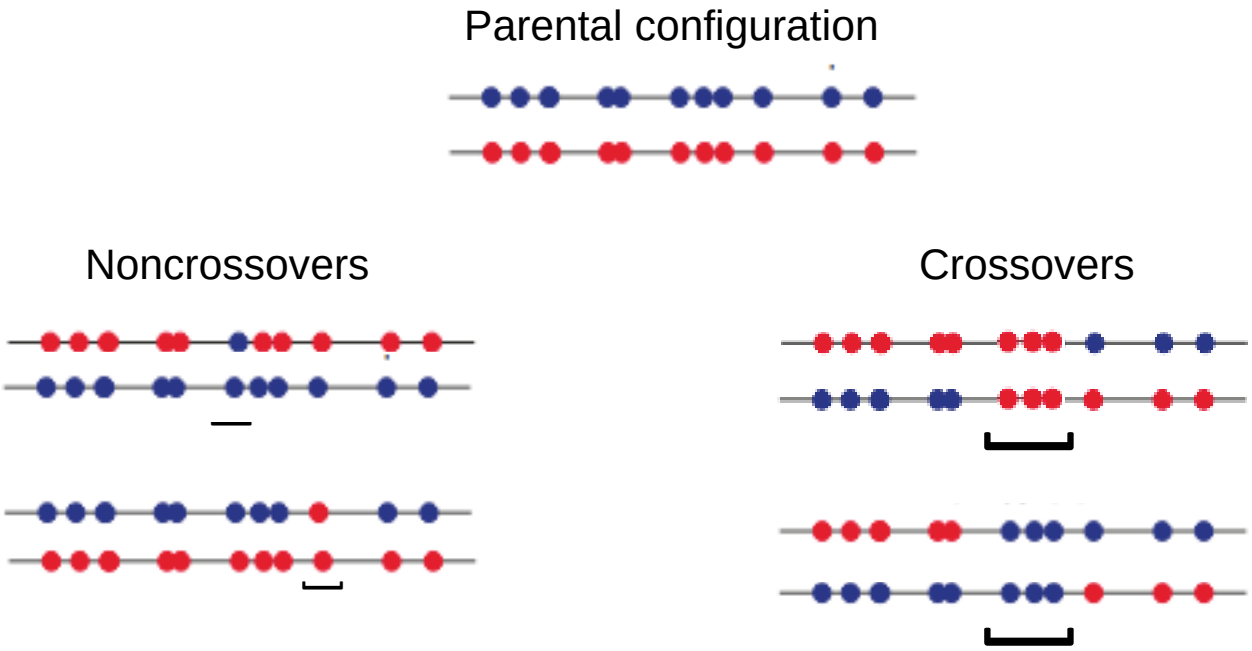


~300 to 400
DSBs per cell

Cells that transmit the genome undergo massive,
but controlled, genome damage

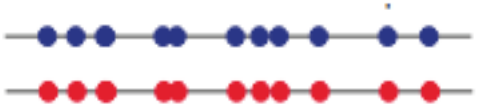
# HR between homologs involves transfer of genetic information



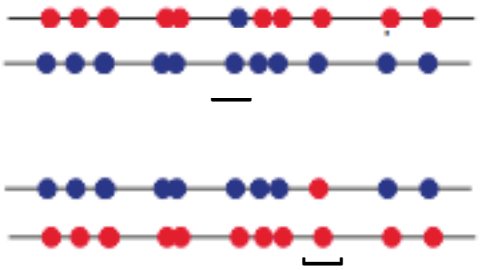

Need for high confidence genotyping

# HR between homologs involves transfer of genetic information

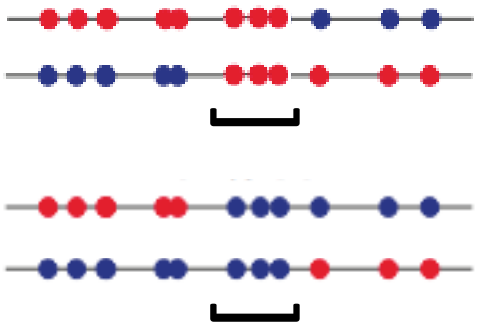
Parental configuration



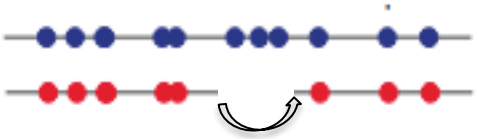
Noncrossovers



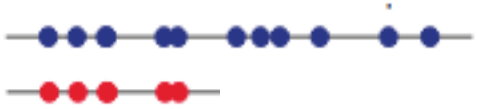
Crossovers



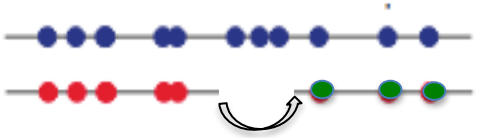
Need for high confidence genotyping



Deletion



Deletion



Translocation