

Identification of sister chromatids by their DNA template strands

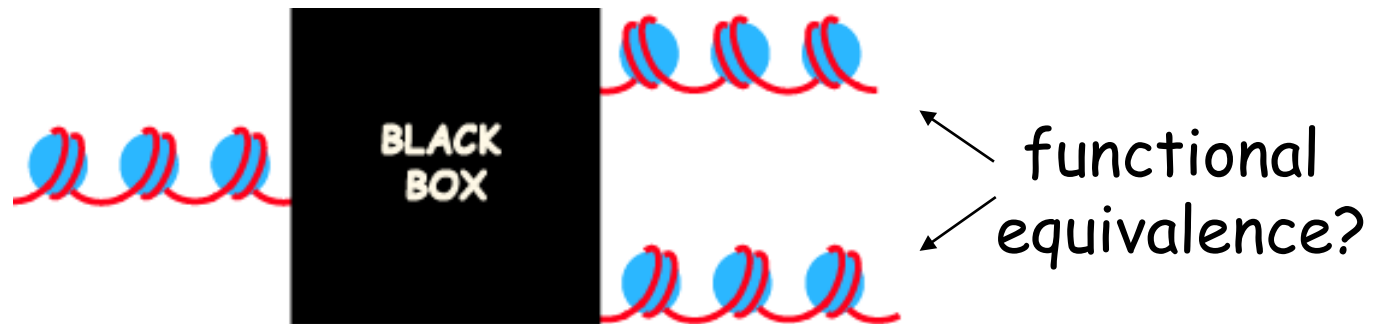
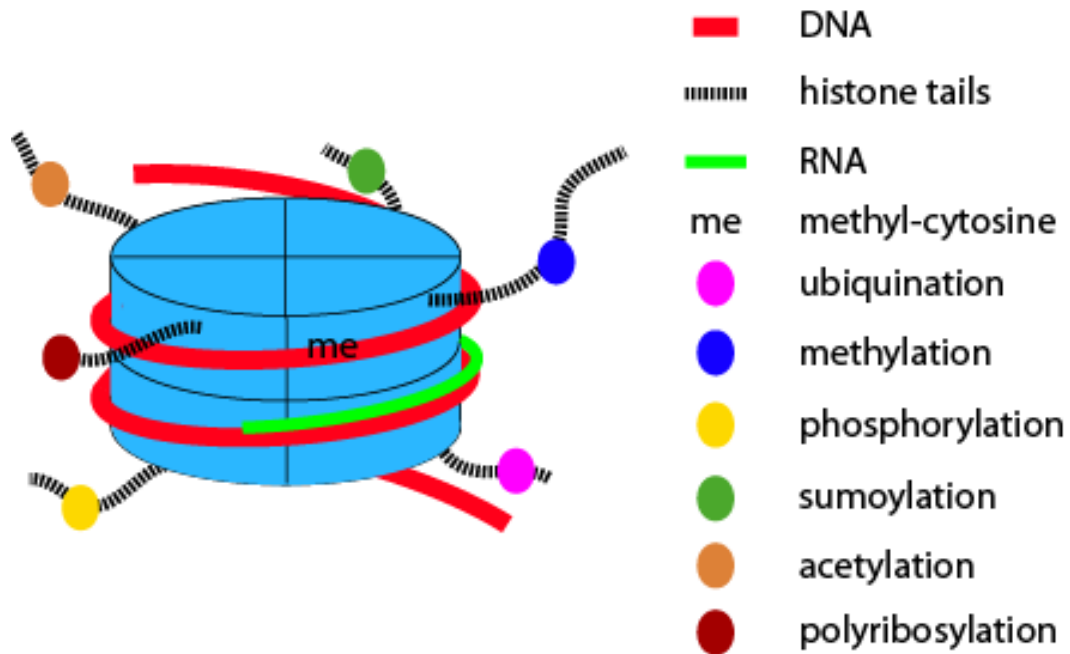
Peter Lansdorp

Terry Fox laboratory, BCCRC

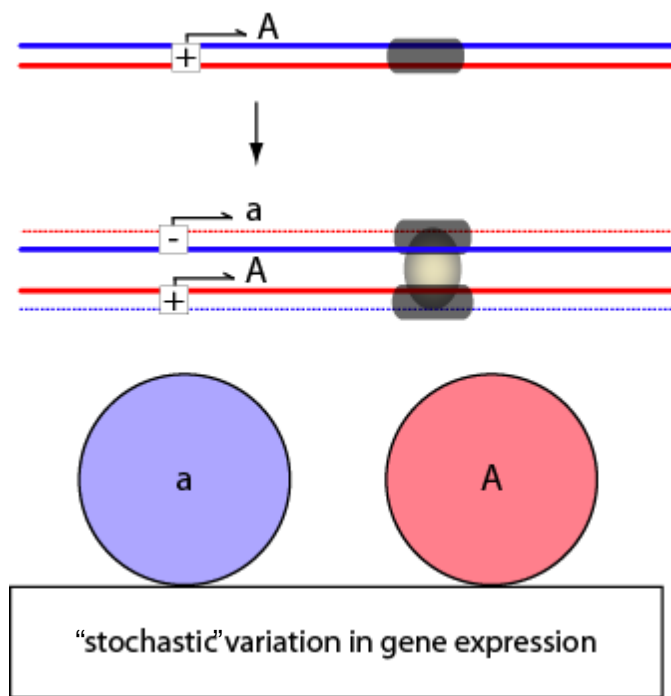
AND

European Institute on the Biology of Ageing, Medical
Centre Groningen, the Netherlands

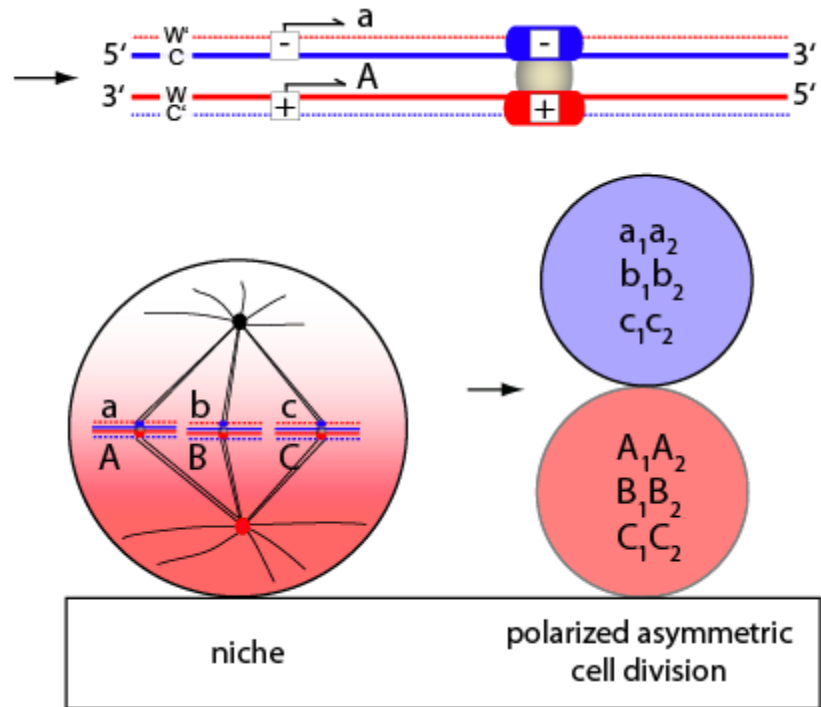
Chromatin: the replication challenge



Regulation of cell fate via random or non-random segregation of sister chromatids?

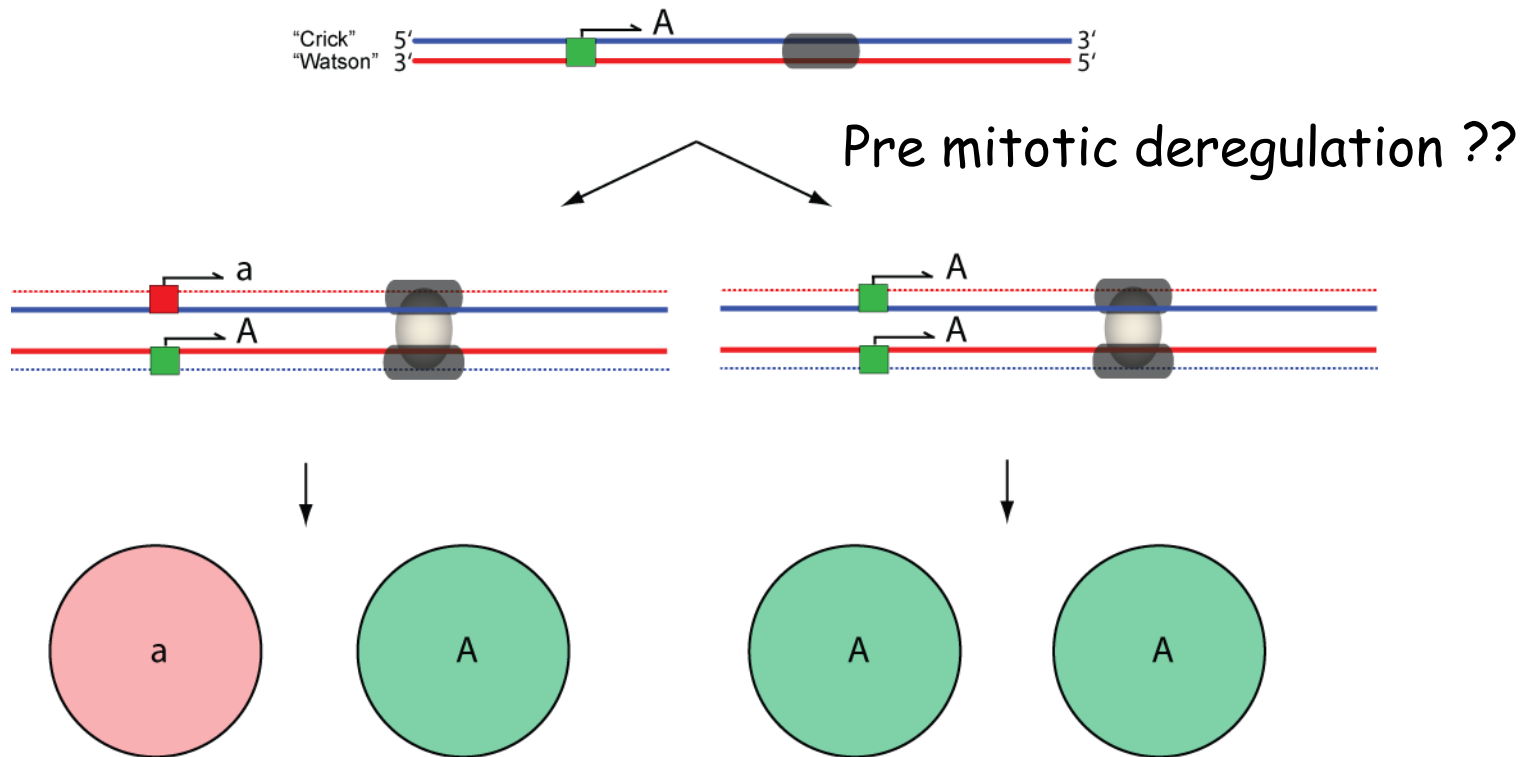


The "silent sister" hypothesis



Lansdorp, *Cell*, 2007

The silent sister hypothesis and cancer: pre or post mitotic deregulation of "master" genes?

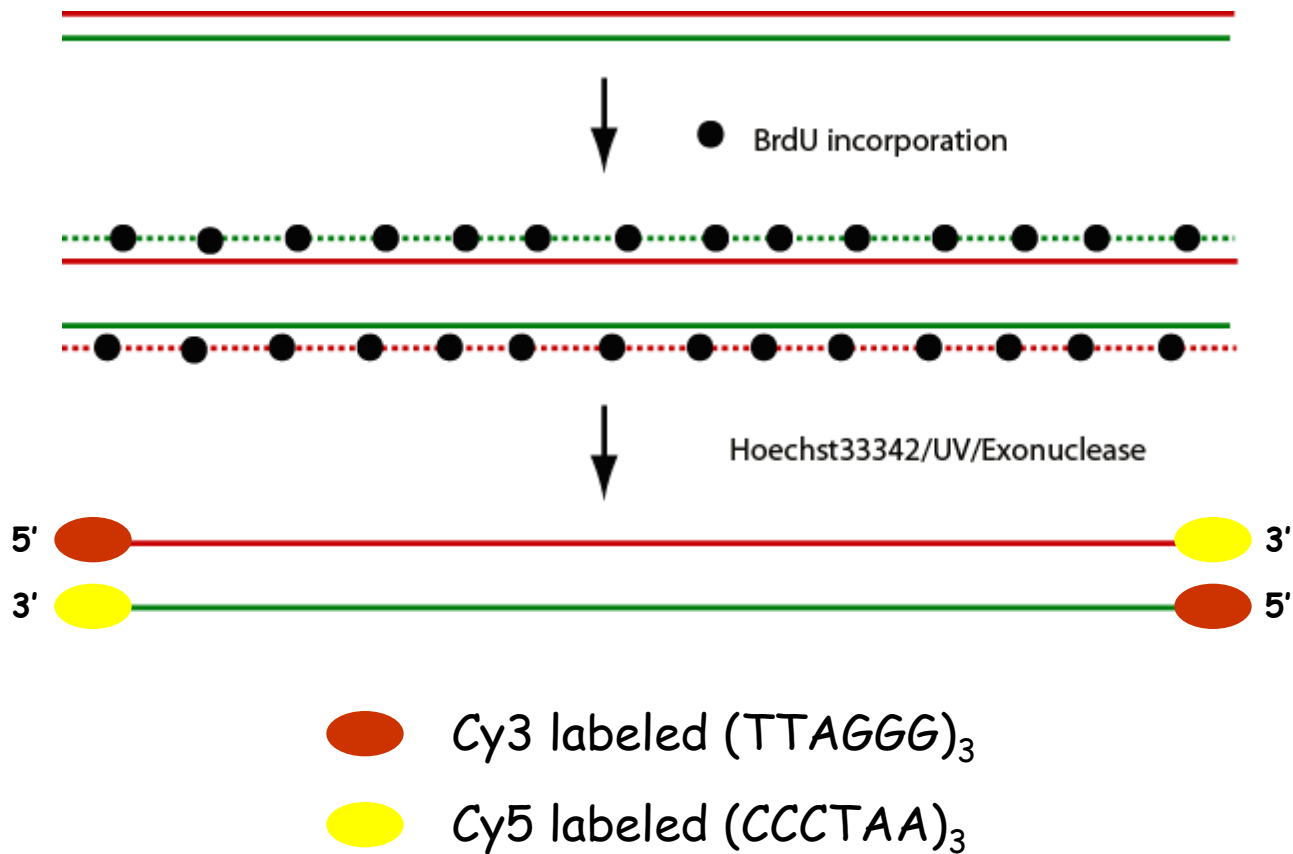


> STUDY SISTER CHROMATIDS SEPARATELY

Identification of sister chromatids by DNA template strand sequences

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Sister chromatids can be identified using Chromosome Orientation Fluorescence In situ Hybridization: CO-FISH

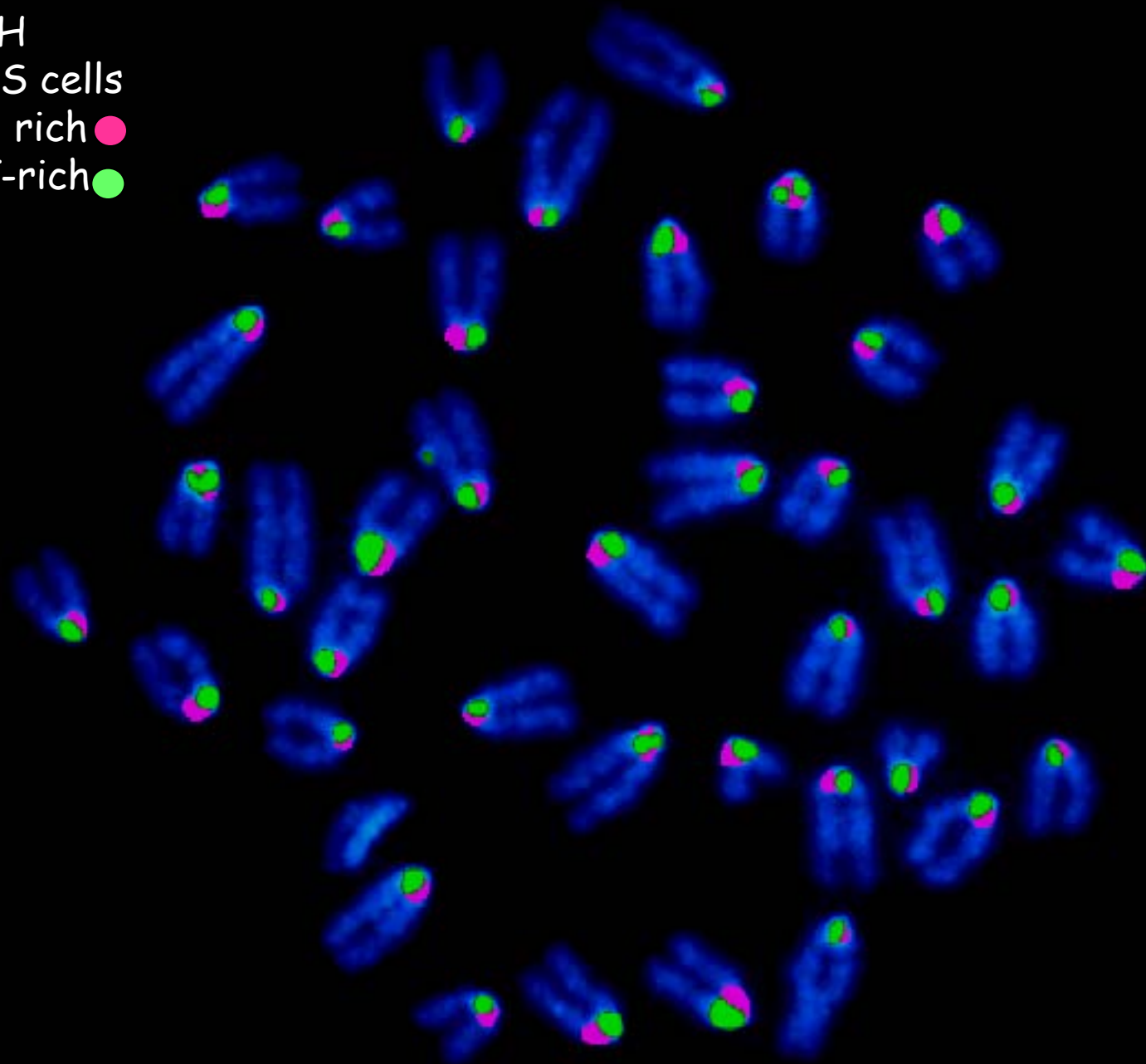


CO-FISH

mouse ES cells

Major A rich ●

Major T-rich ●



Conclusion

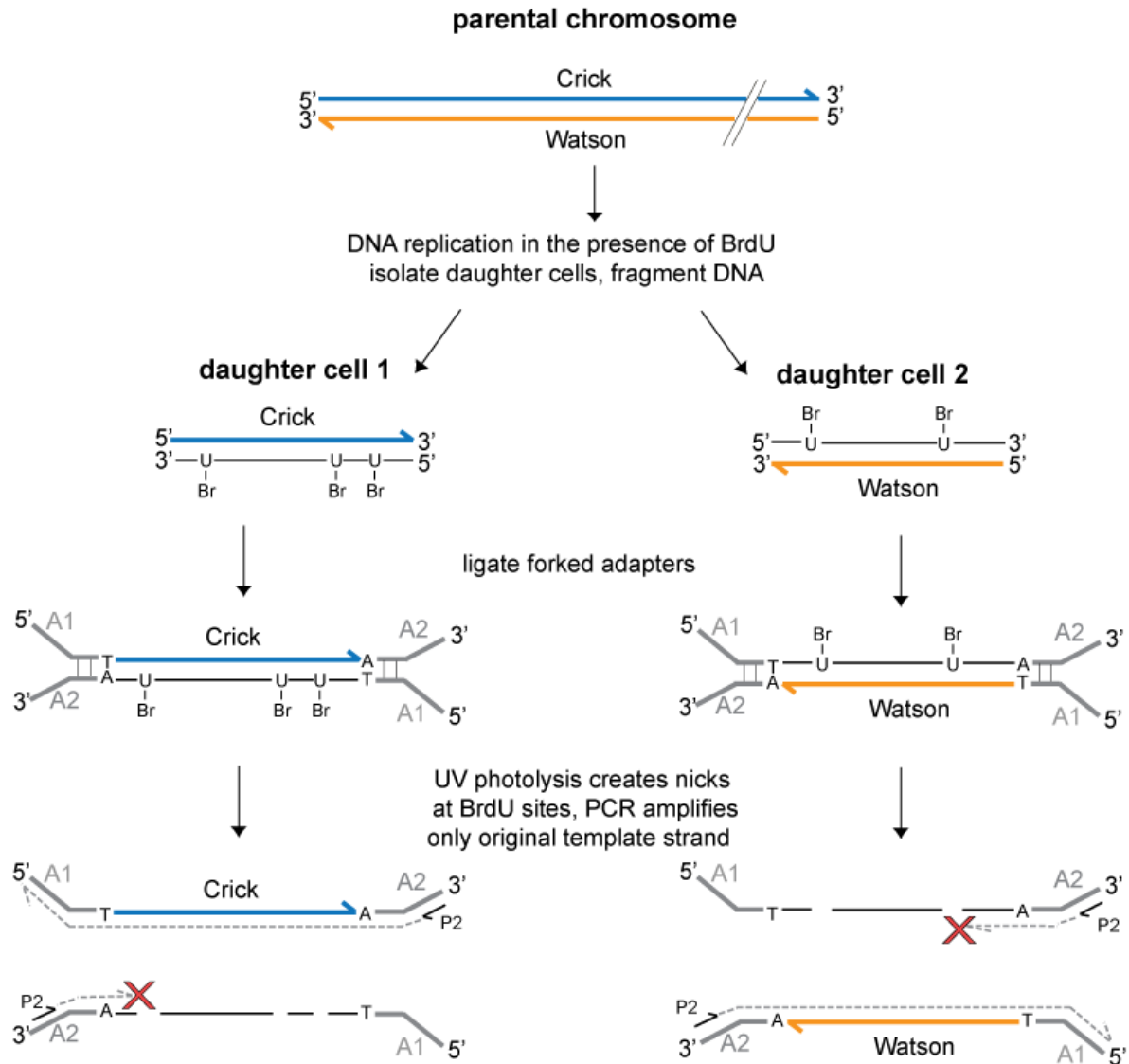
Sister chromatid segregation is not random in all dividing colon cells

Questions

Is non-random segregation of sister chromatids unique to specific chromosomes? Specific cells?

Is it possible to define all the sister chromatids present in a single cell by sequencing of DNA template strands?

Principle of single cell template strand sequencing (Strand Seq)



Cells synchronized in G1



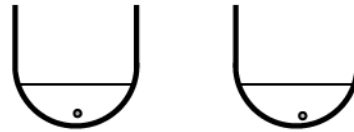
↓ BrdU



isolate/sort single cells in G1 after one round of DNA replication with BrdU



sort directly into lysis buffer



spin

harvest supernatant for analysis transcripts

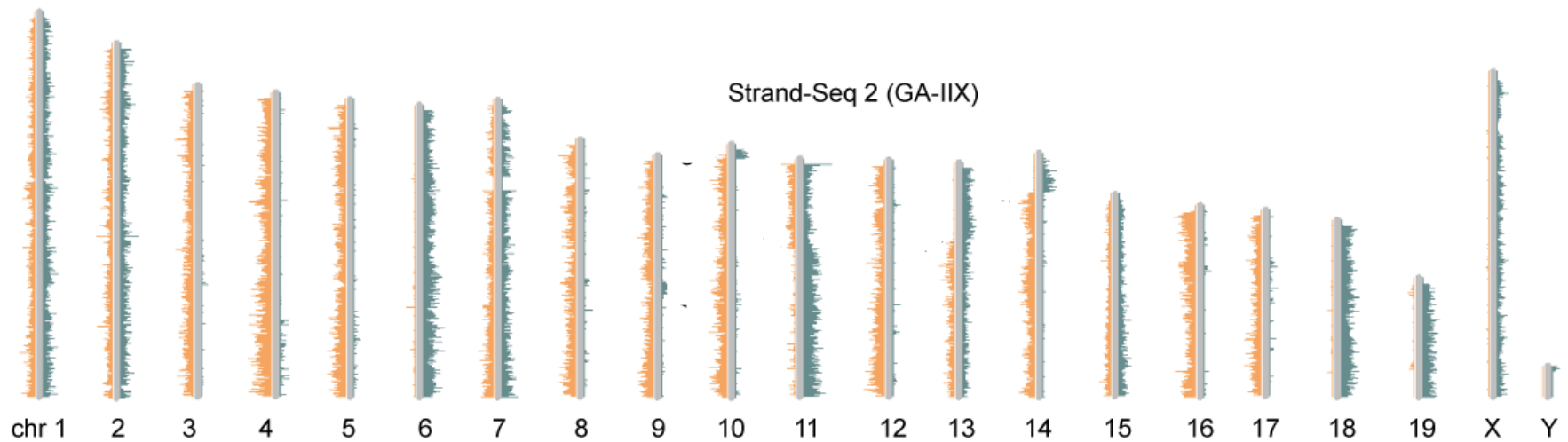
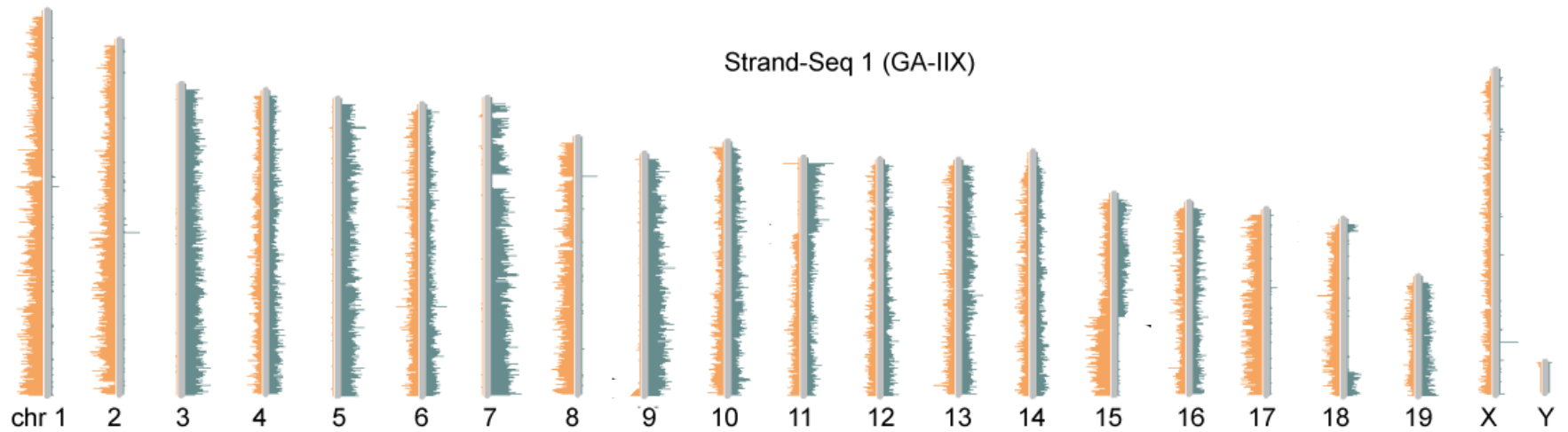
pellet nucleus, treat with MNase
make directional library

Solexa sequencing

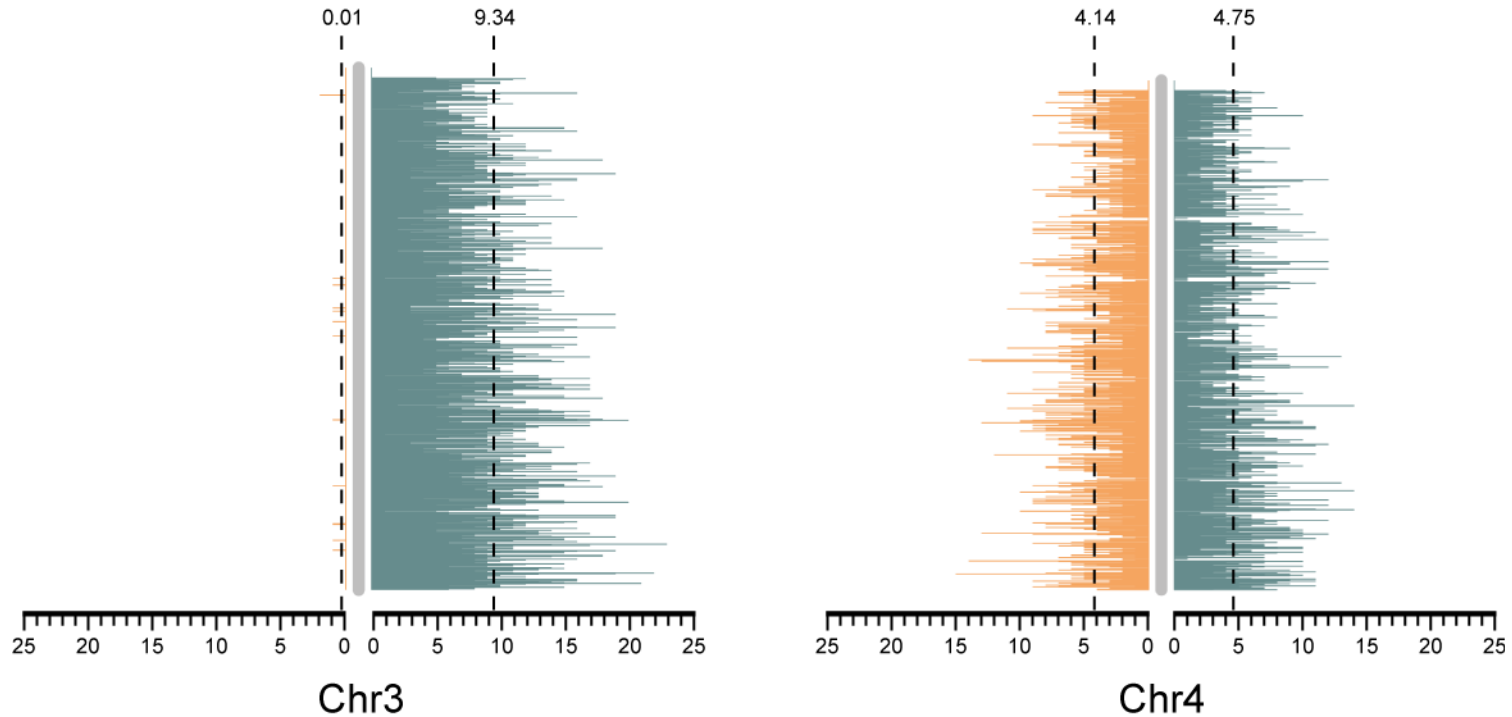
identify DNA
template strands

chr 1 WW
chr 2 CW
chr 3 CC
chr 4 CW
chr 5 CW
etc.

Results of single cell template strand sequencing (Strand Seq)



High resolution analysis of read depth demonstrates reads are proportional to template strand inheritance



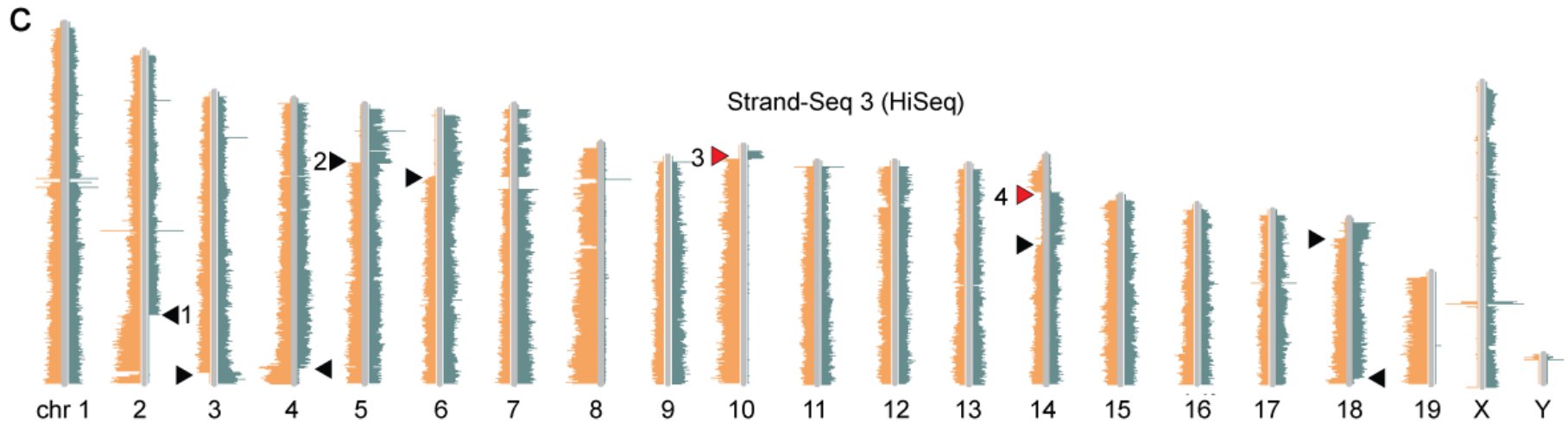
Number of reads mapping to 200 kb segment of the mm9 reference genome

Summary sequencing results of indexed single cell genomic libraries

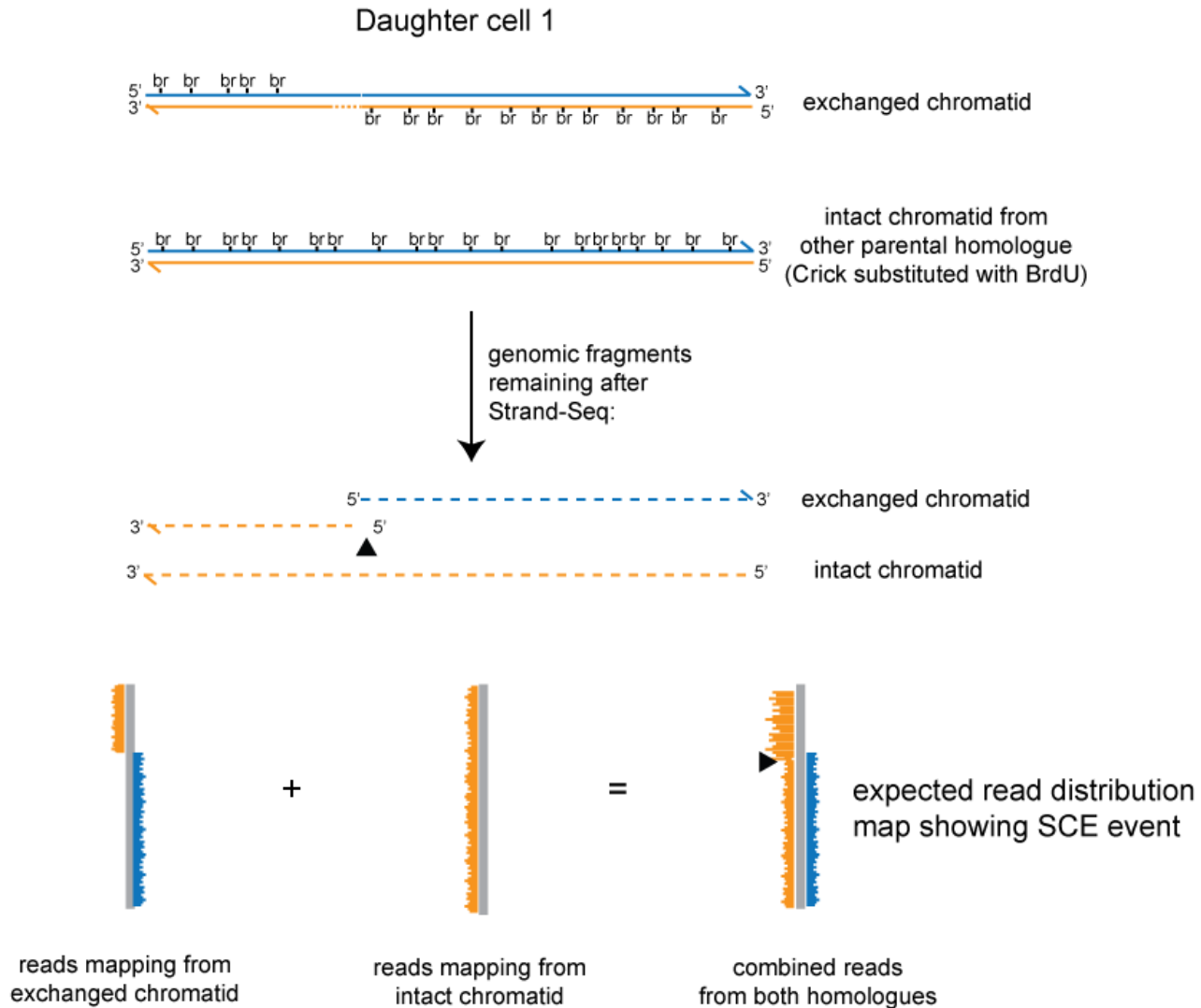
Library ID	Cell input	Platform	Number of Reads				Genomic Coverage	
			Total	Mapped (q>20)	% duplicates	% unique	% Coverage	Reads per Mb
Strand-Seq3	0.5	GA-IIX	137,919	93,485	4.8	95.2	0.24	35.2
		HiSeq	7,662,698	1,086,967	54.2	45.8	2.51	409.4
Strand-Seq4	0.5	GA-IIX	142,826	97,394	4.8	95.2	0.21	36.7
		HiSeq	7,743,034	1,159,148	49.2	50.8	2.10	436.6
Genomic4	1	GA-IIX	590,252	395,943	14.6	85.3	1.04	149.1
		HiSeq	22,045,372	2,033,926	23.2	76.8	4.85	766.1

Capture of MNase digested DNA into sequencing libraries is remarkably efficient

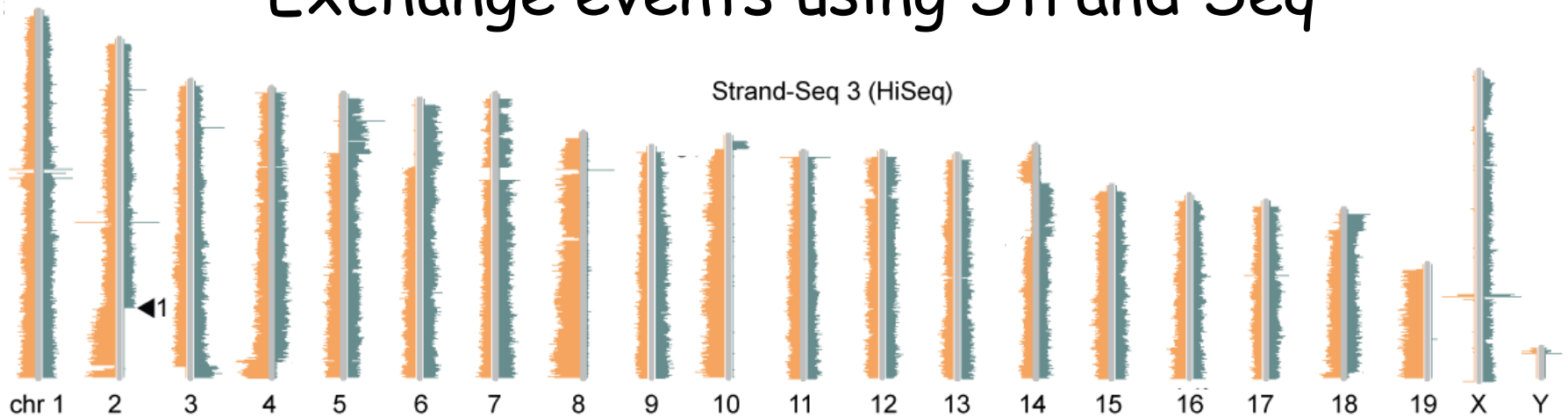
Results of single cell template strand sequencing (Strand Seq)



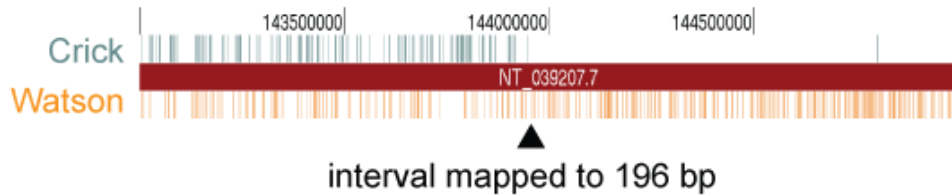
Strand-Seq can be used to enumerate and map sister chromatid exchange events at high resolution



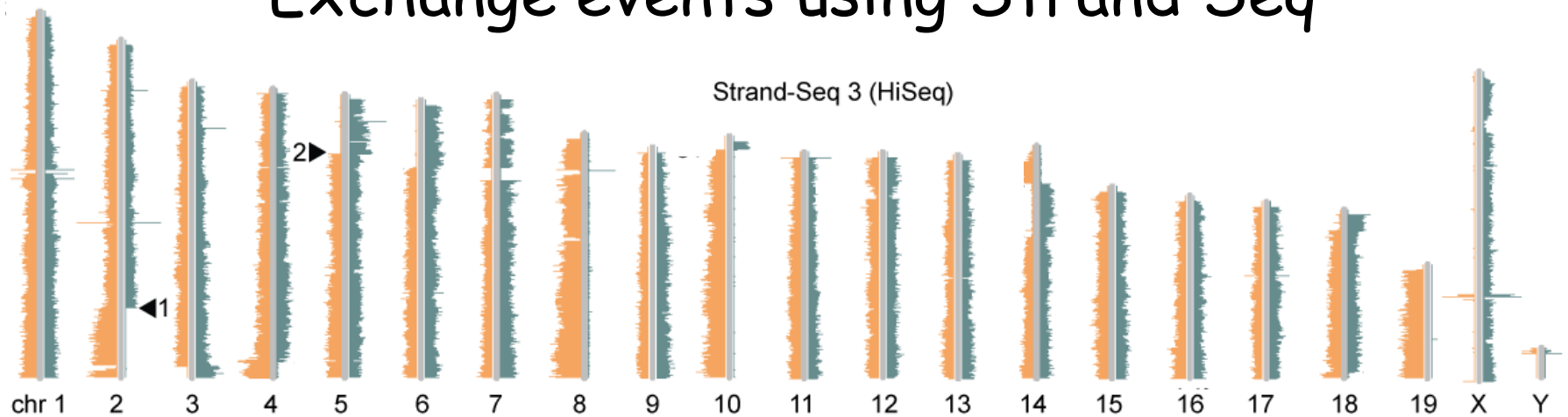
High resolution mapping of Sister Chromatid Exchange events using Strand Seq



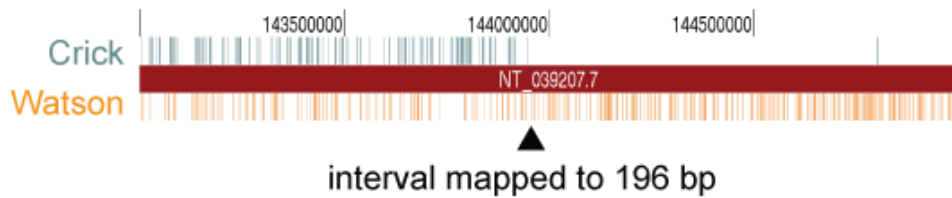
1. SCE Chr 2:143,916,759-143,916,955



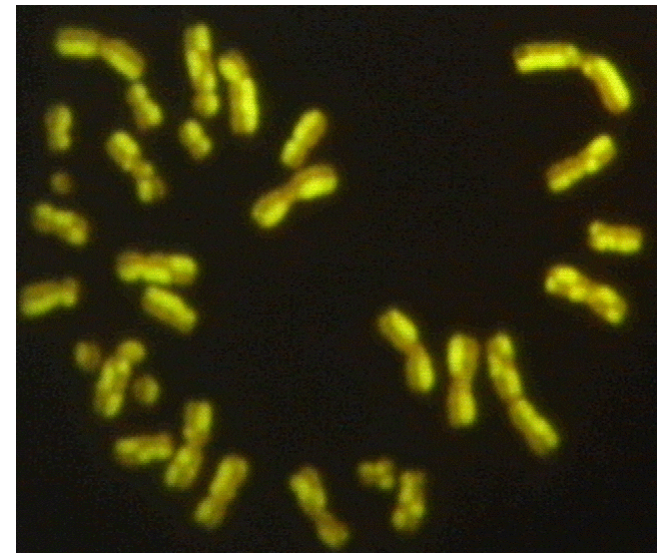
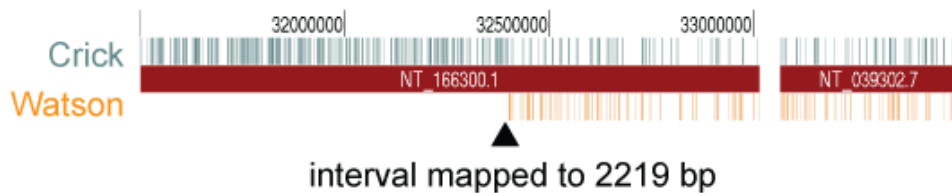
High resolution mapping of Sister Chromatid Exchange events using Strand Seq



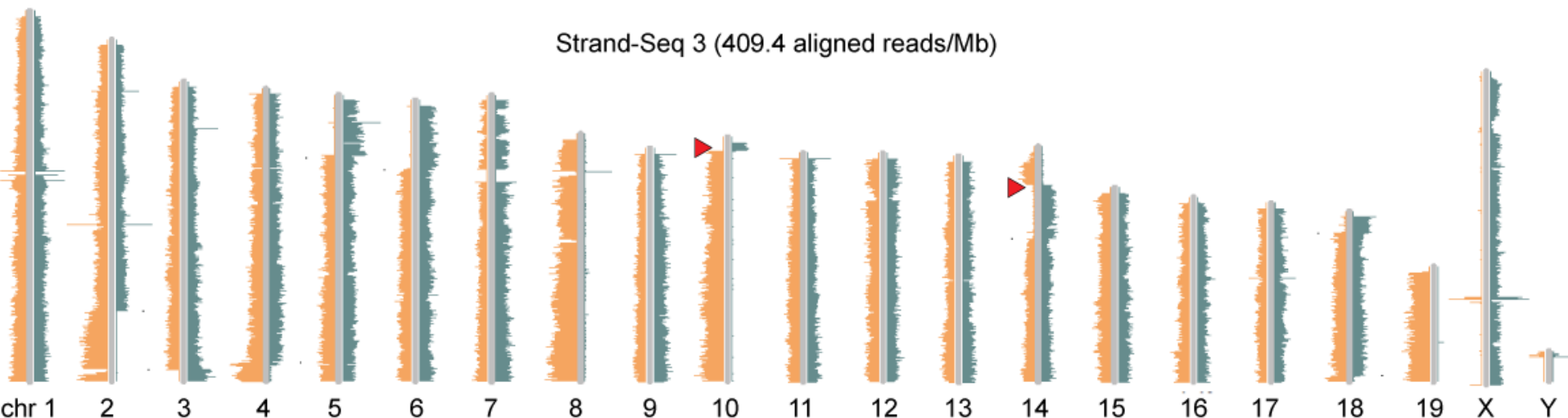
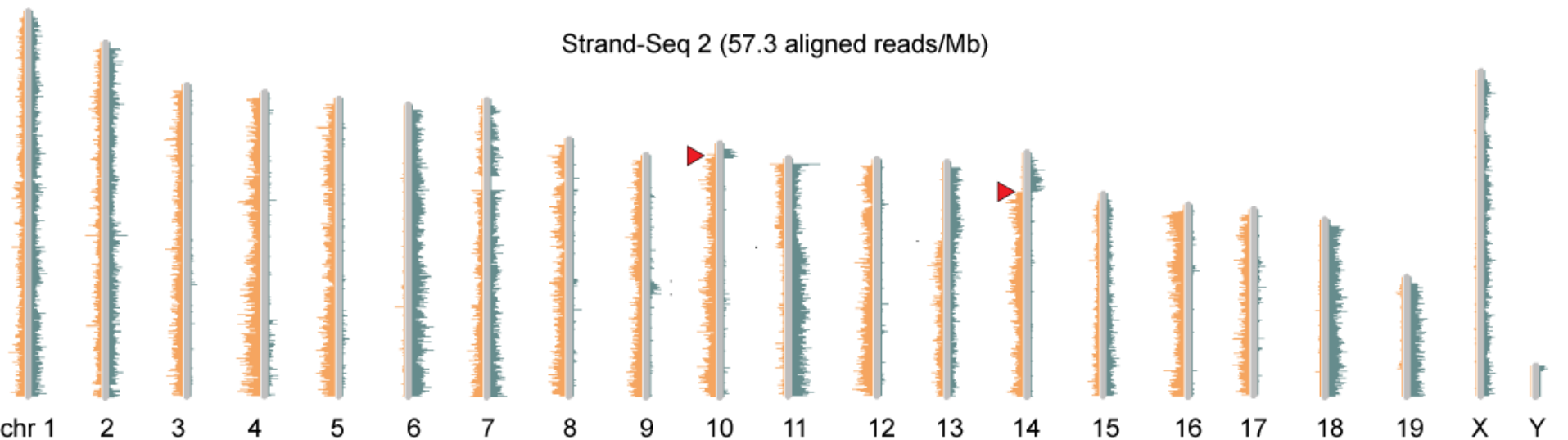
1. SCE Chr 2:143,916,759-143,916,955



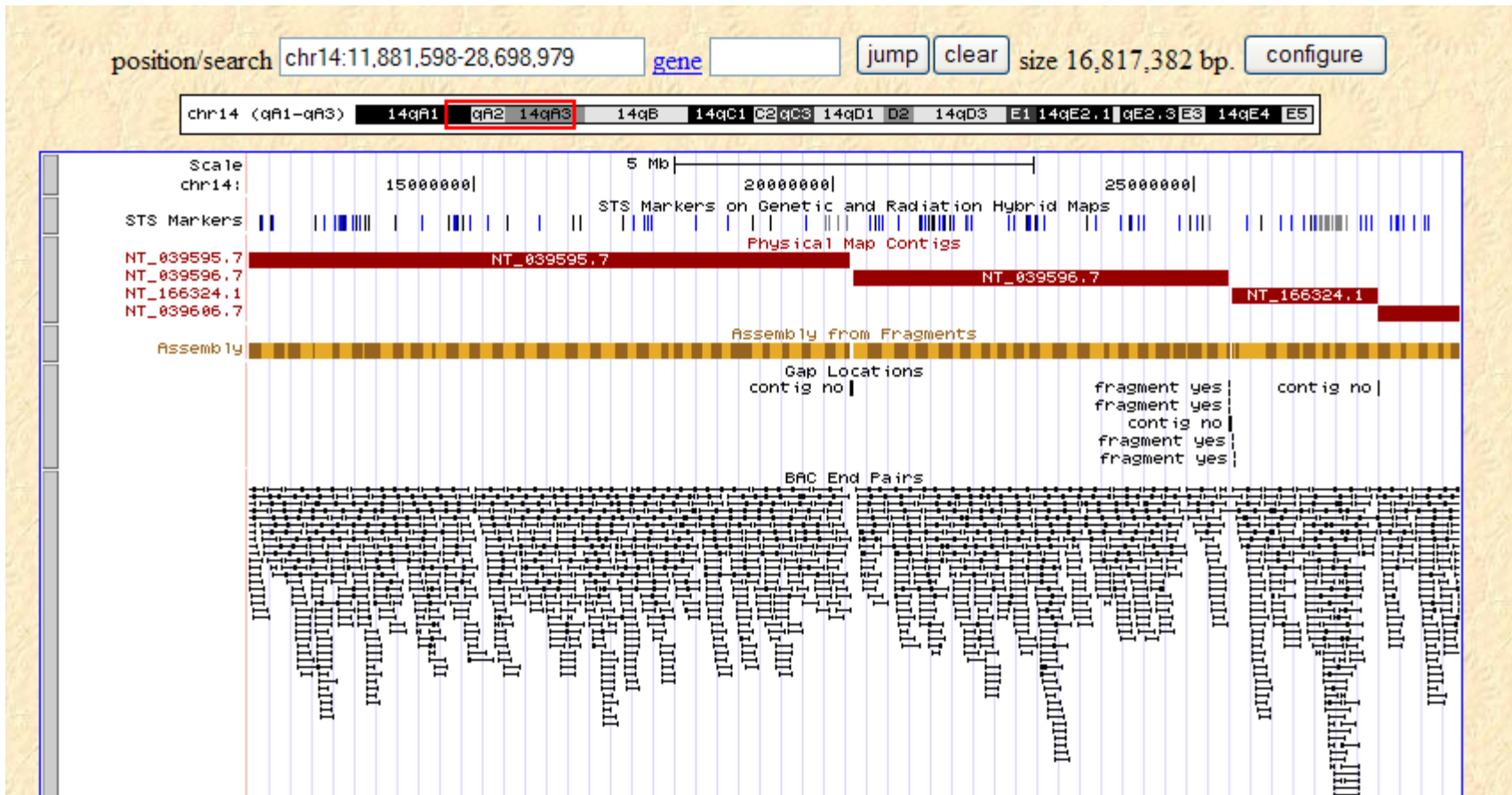
2. SCE Chr 5:32,399,300-32,401,519



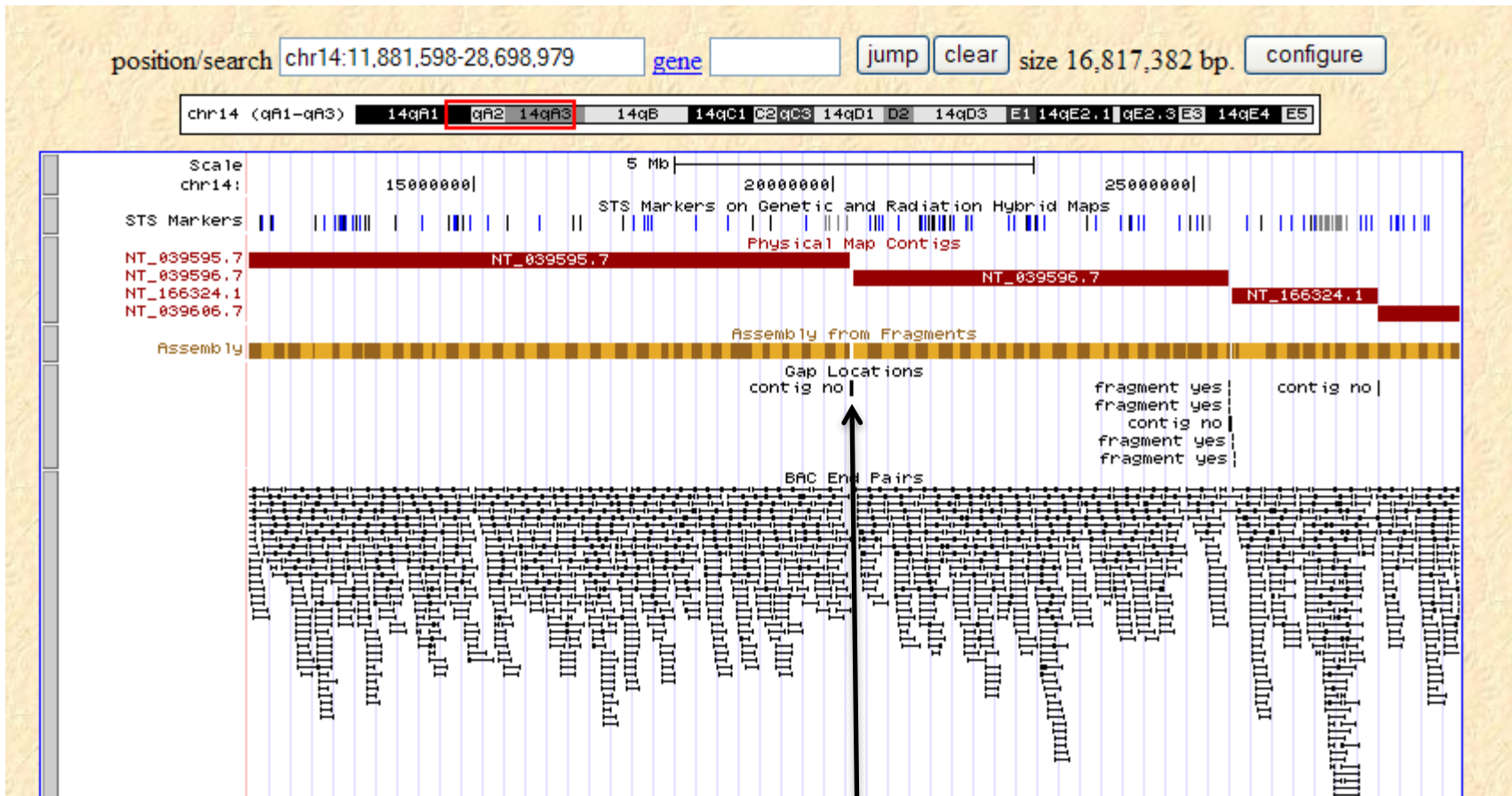
Complete strand exchanges in chromosomes 10 and 14?



Complete strand exchanges in chromosome 14?

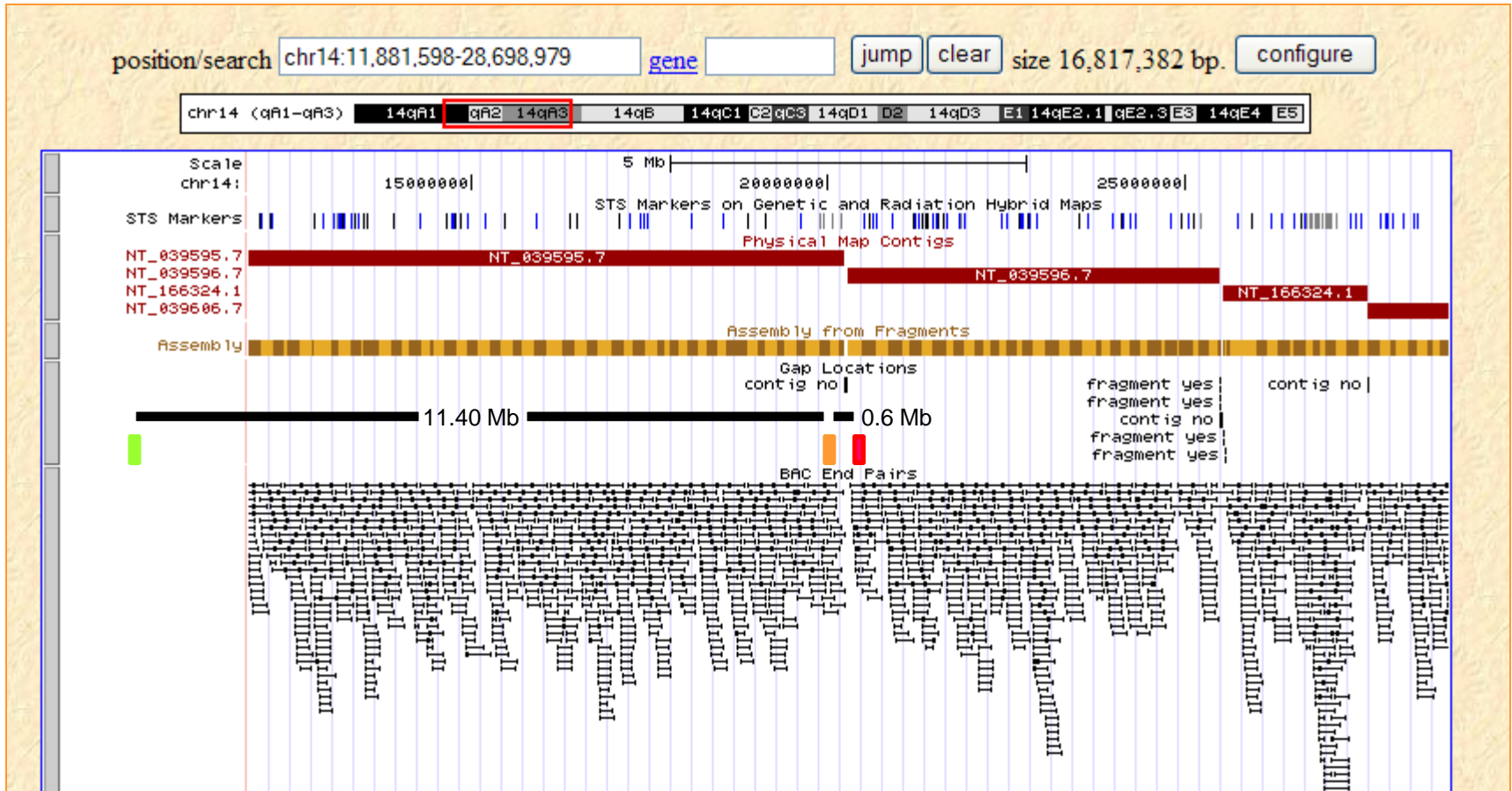


Complete strand exchanges in chromosome 14?

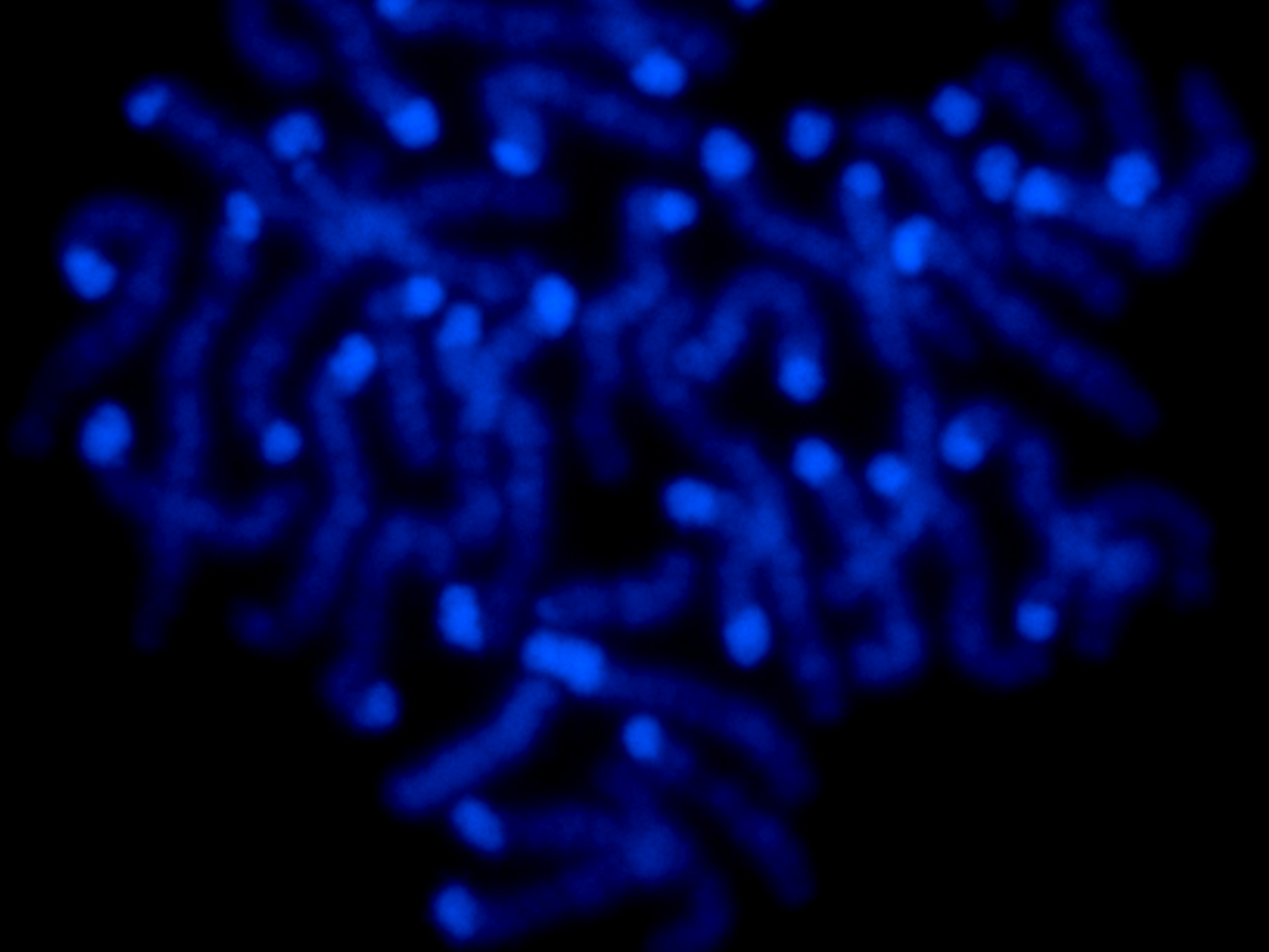


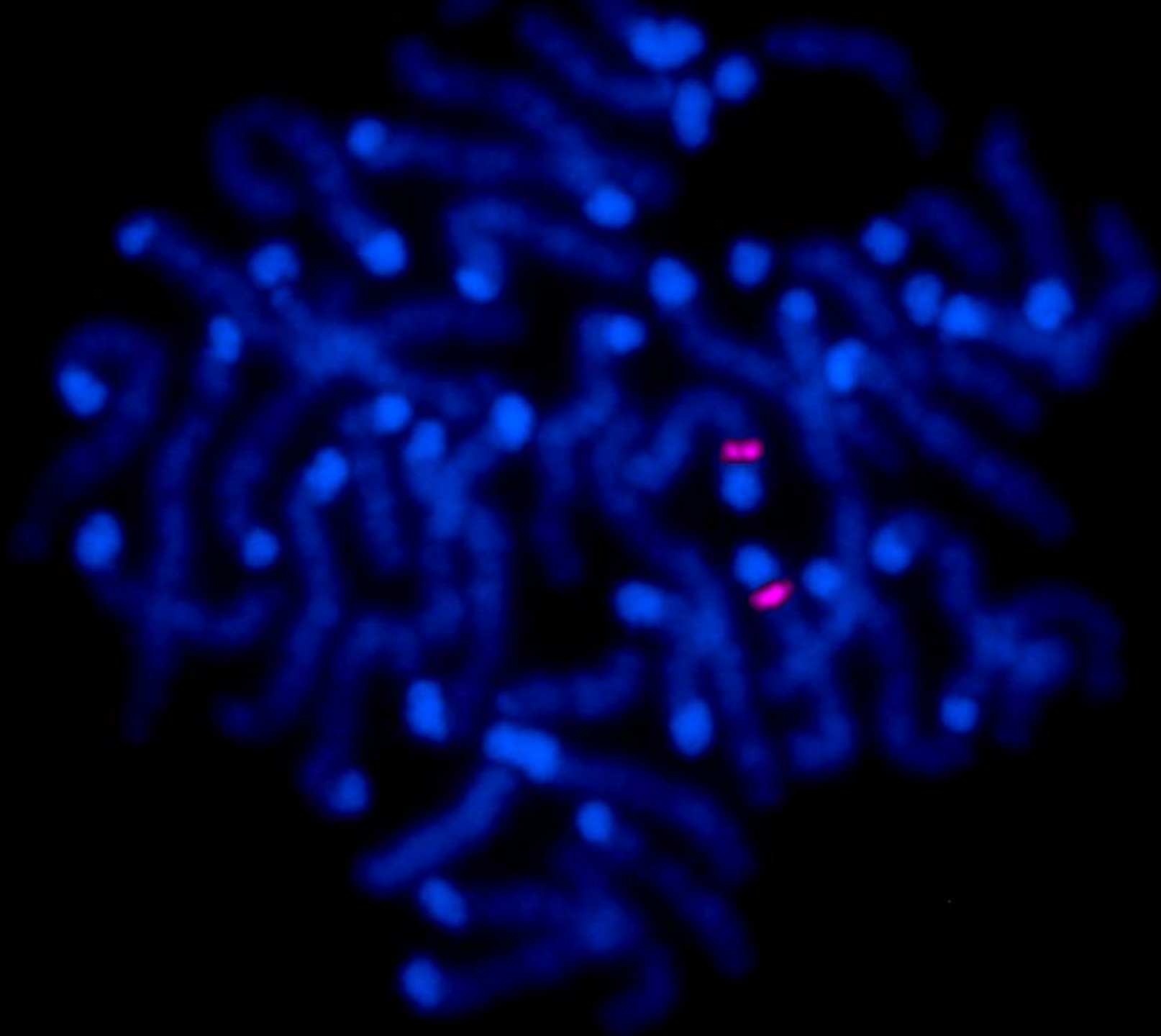
Location switch maps to a gap in the genome

Complete strand exchanges in chromosome 14?



FISH analysis of reference genome map

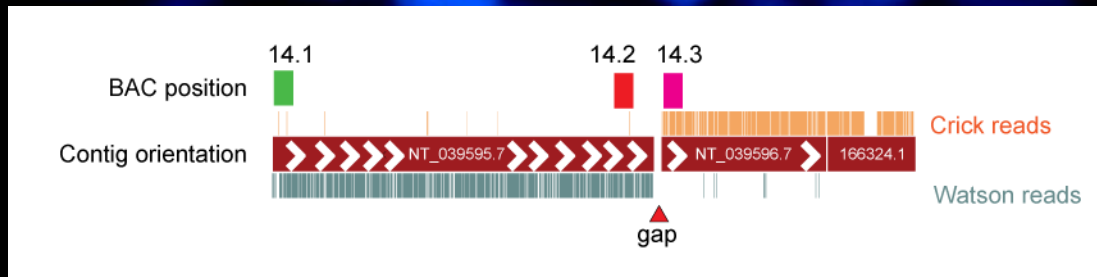




14.3

14.3

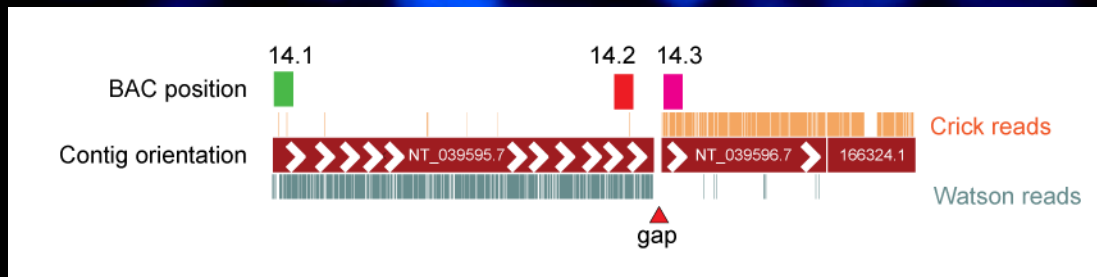
Orientation of Contig in mm9 reference genome



14.1

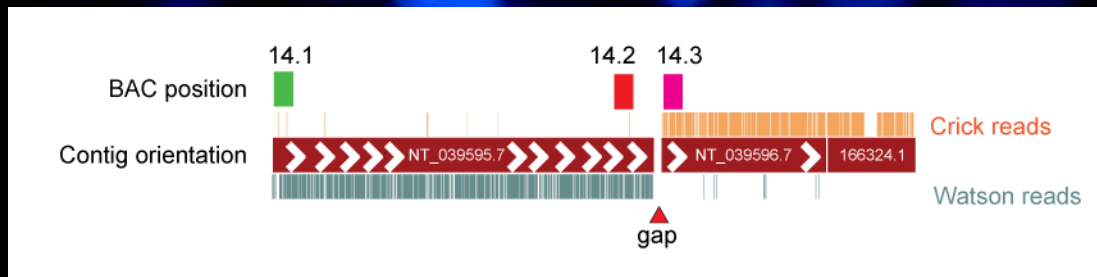
14.1

Orientation of Contig in mm9 reference genome

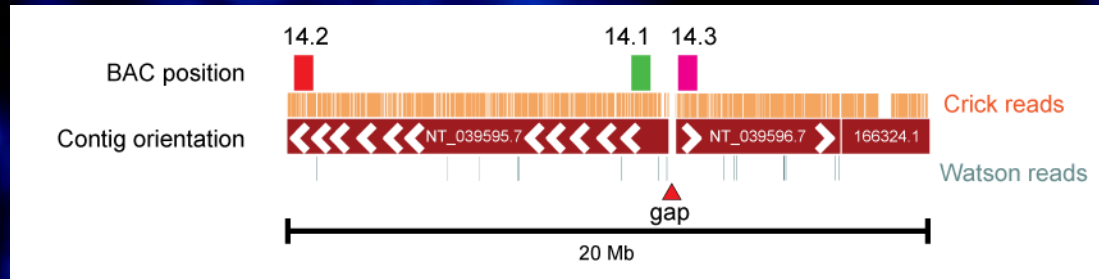


14.3
14.2
14.2
14.3

Orientation of Contig in mm9 reference genome



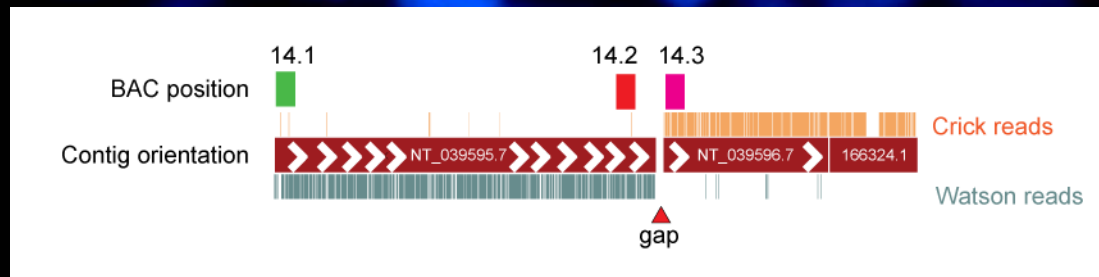
Orientation deduced from Strand-Seq and FISH



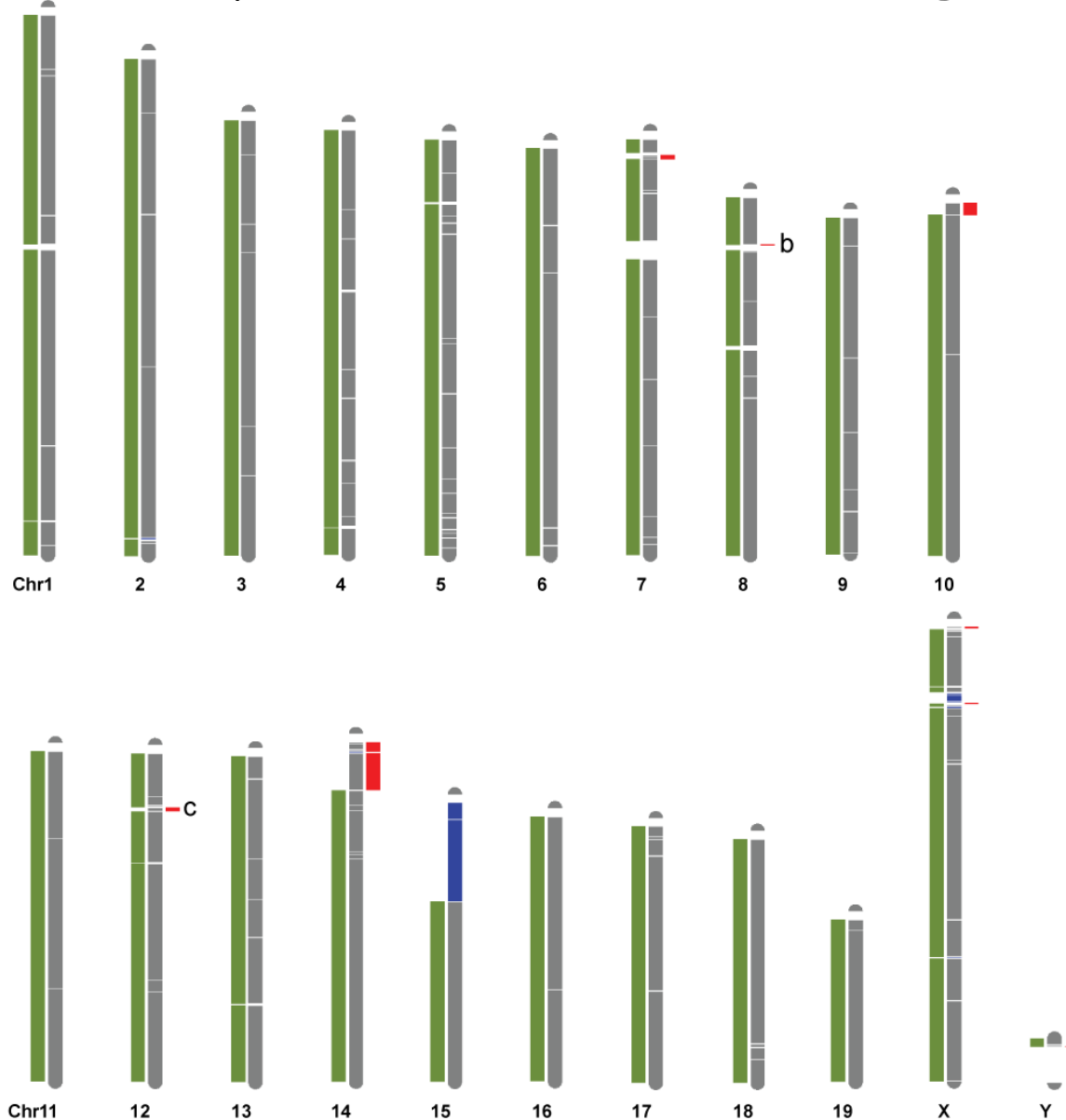
14.2

14.2

Orientation of Contig in mm9 reference genome

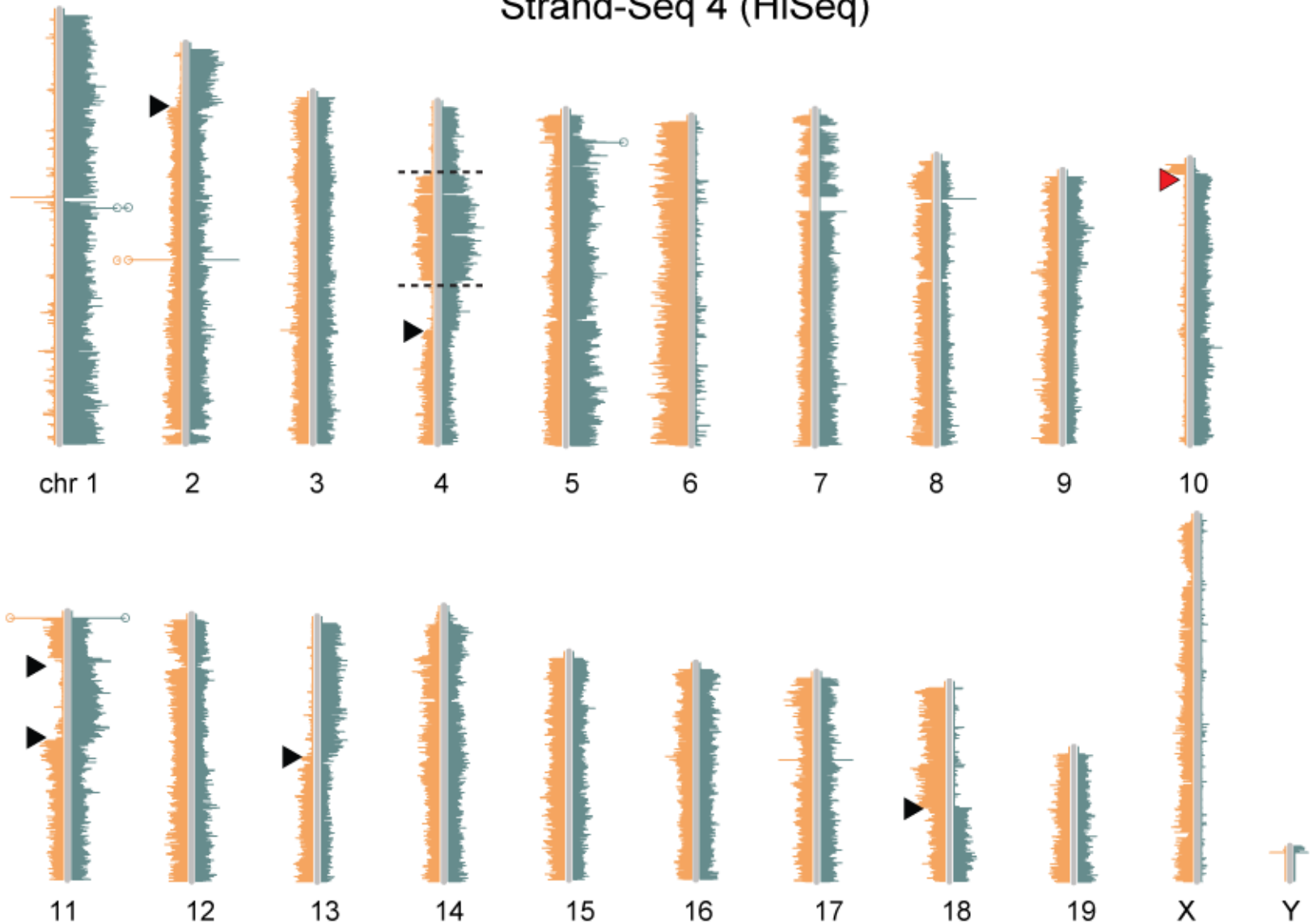


Strand-Seq can be used to identify errors in genomic maps and help build better reference genomes

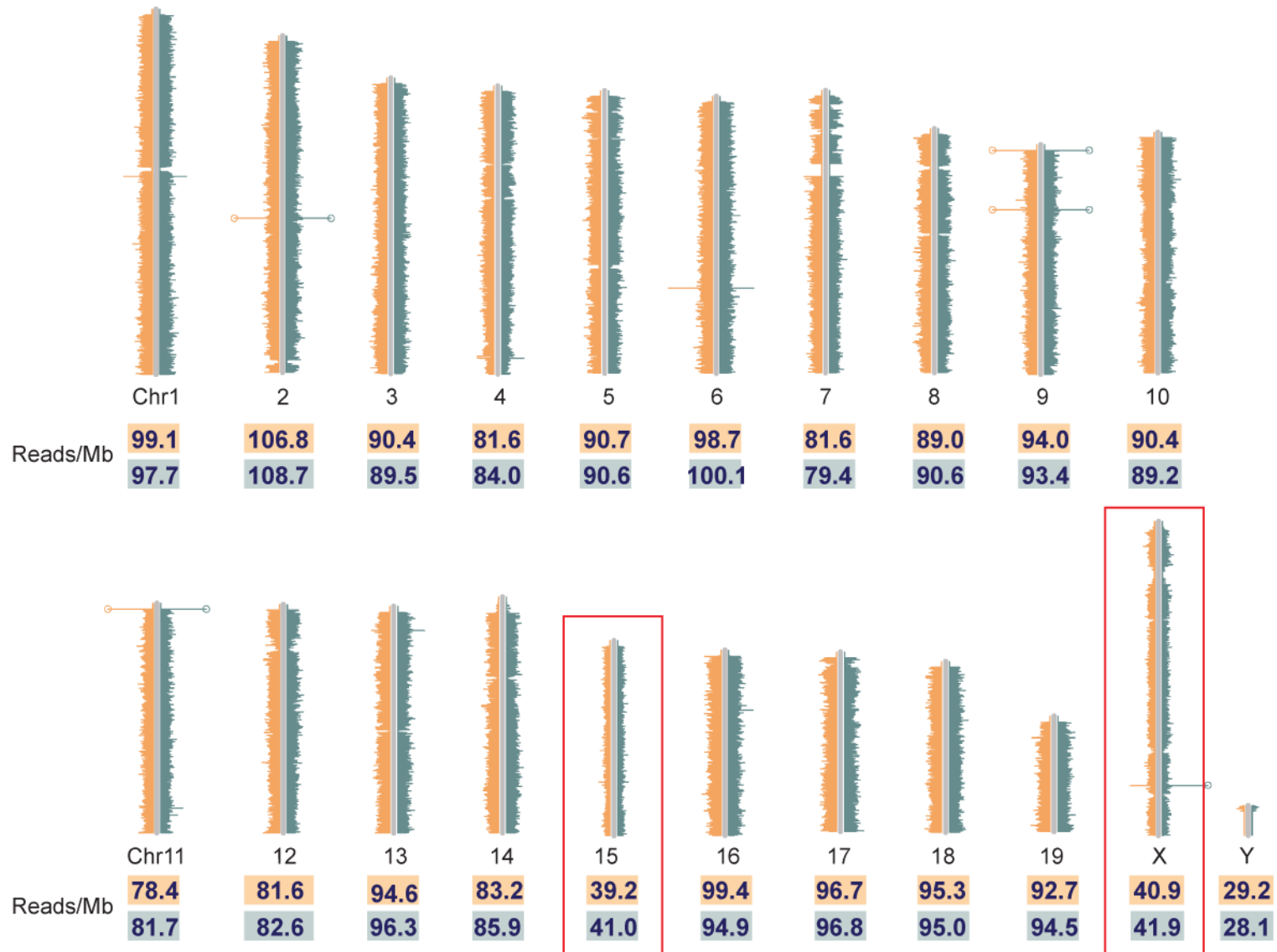


Strand-Seq libraries reveal numerical chromosomal abnormalities

Strand-Seq 4 (HiSeq)



Strand-Seq libraries reveal numerical chromosomal abnormalities



Sister chromatid asymmetry provides a paradigm for studies of asymmetric cell divisions and cell fate

Single cell DNA template strand sequencing (Strand-Seq) allows:

- identification of all parental sister chromatids in a cell
- direct identification of sister chromatid exchange events
- assign correct orientation contigs in reference genomes
- map chromosomal copy number variations
- study linkage SNP's, assemble haplotypes?
- study complex chromosomal abnormalities?
- test the silent sister hypothesis?

Acknowledgements

Ester Falconer

Mark Hills

Uli Neumann

Liz Chavez

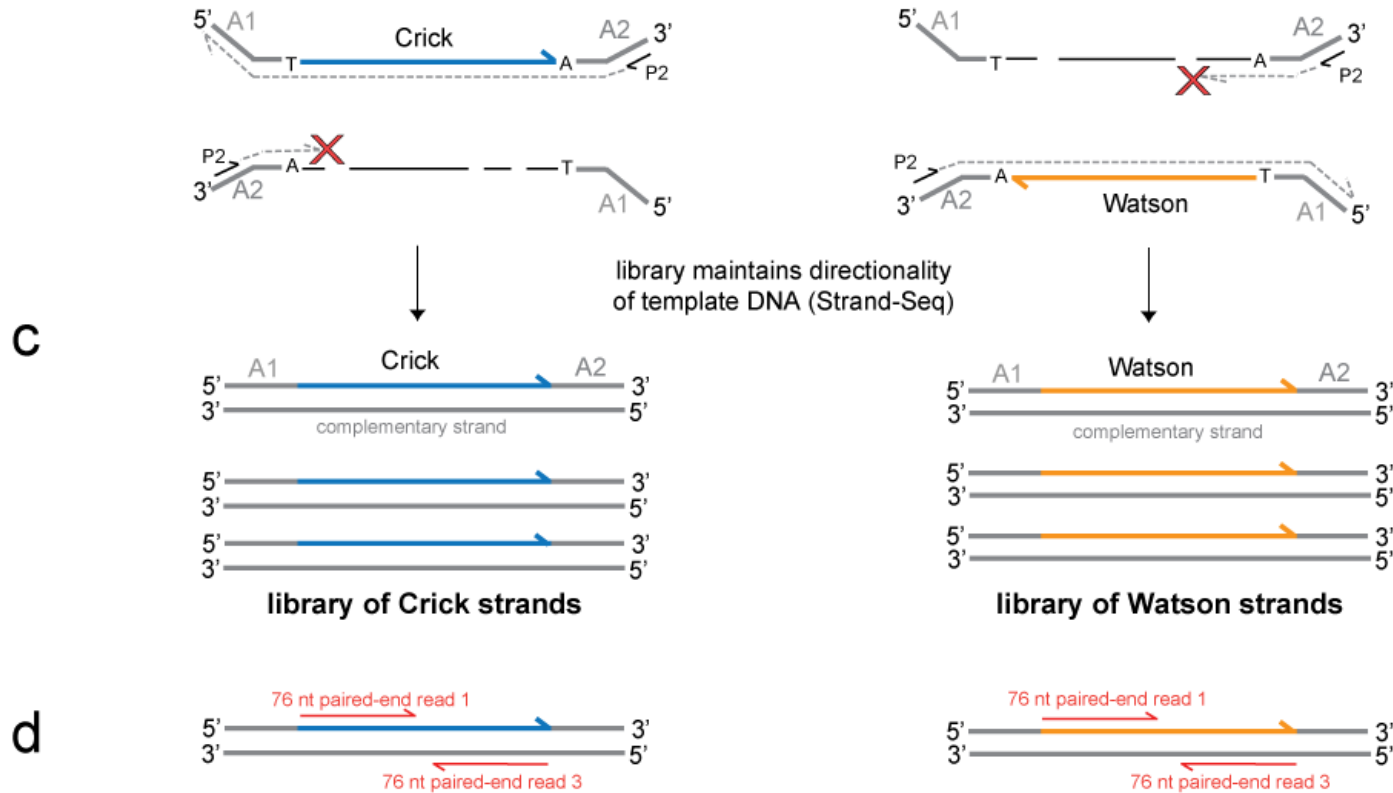
Steven Poon

Martin Hirst

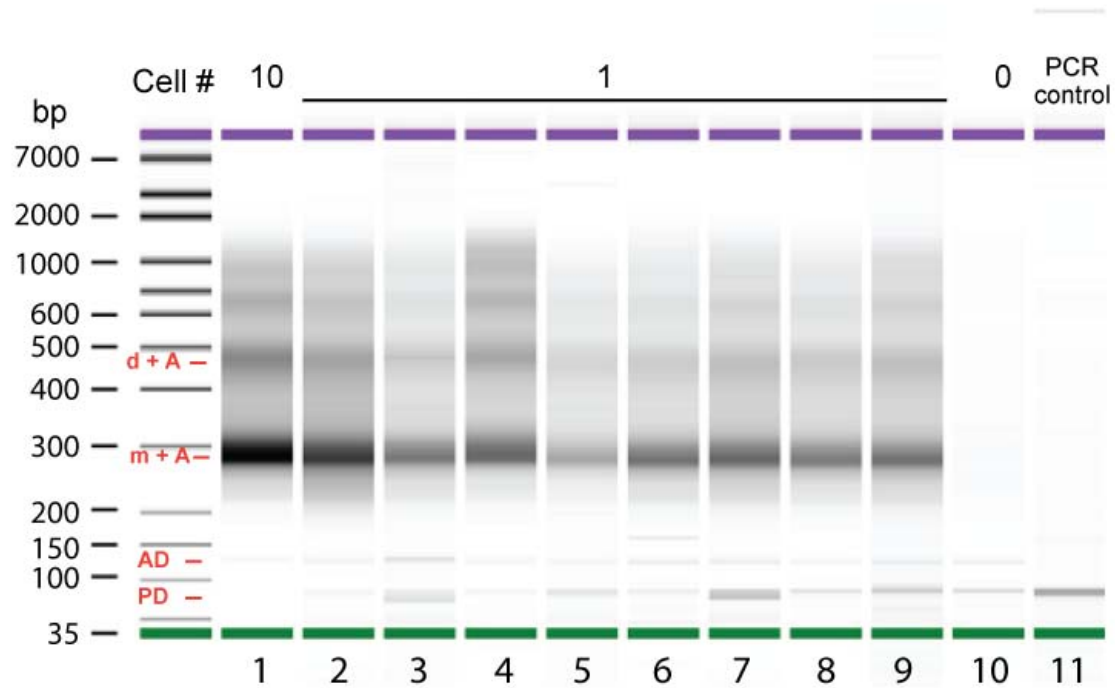
Yongjun Zhao

Funding CIHR, NIH

Principle of single cell template strand sequencing (Strand Seq)



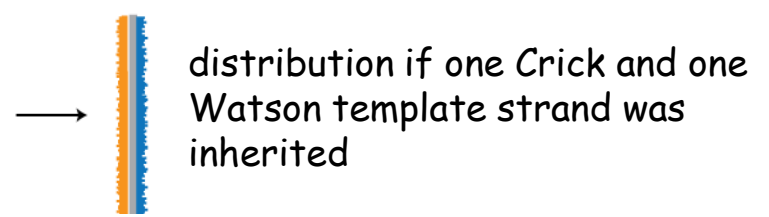
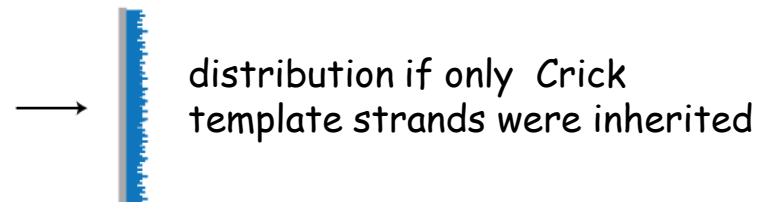
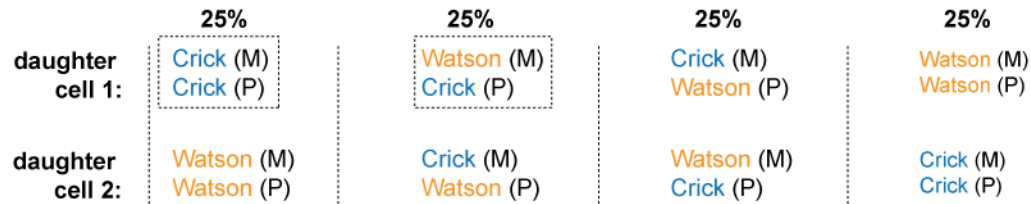
Size distribution of DNA in typical single cell genomic libraries



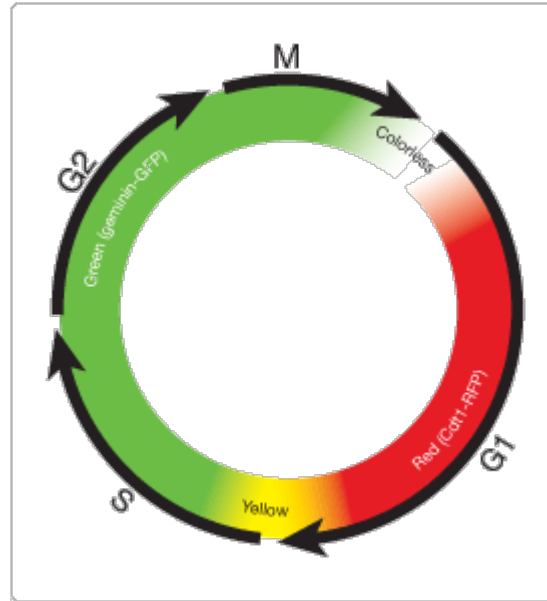
Strand-Seq read distribution reflects template strands inherited from both parental homologues



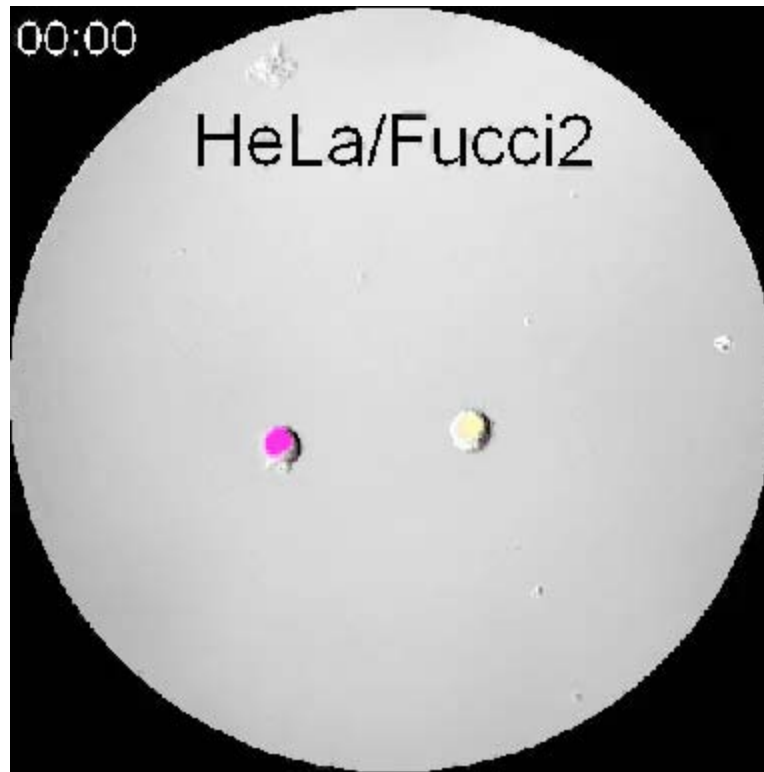
Each daughter cell inherits one template strand from each homologue



Selection of cells in G1 stage of the cell cycle



Selection of cells in G1 stage of the cell cycle



Sakaue-Sawano A, Kobayashi T, Ohtawa K, Miyawaki A. BMC Cell Biol. 12:2, 2011.