WORKING WITH SV CALLS

Lots of noise!

Sources of noise – what filter should we use?

- Repeat regions
- High-depth regions
- Poor quality mapping
- Mobile elements
- Bacterial genome insertion
- Viral genome insertion
- Poor quality reference (telomere and centromere)

Other common filters

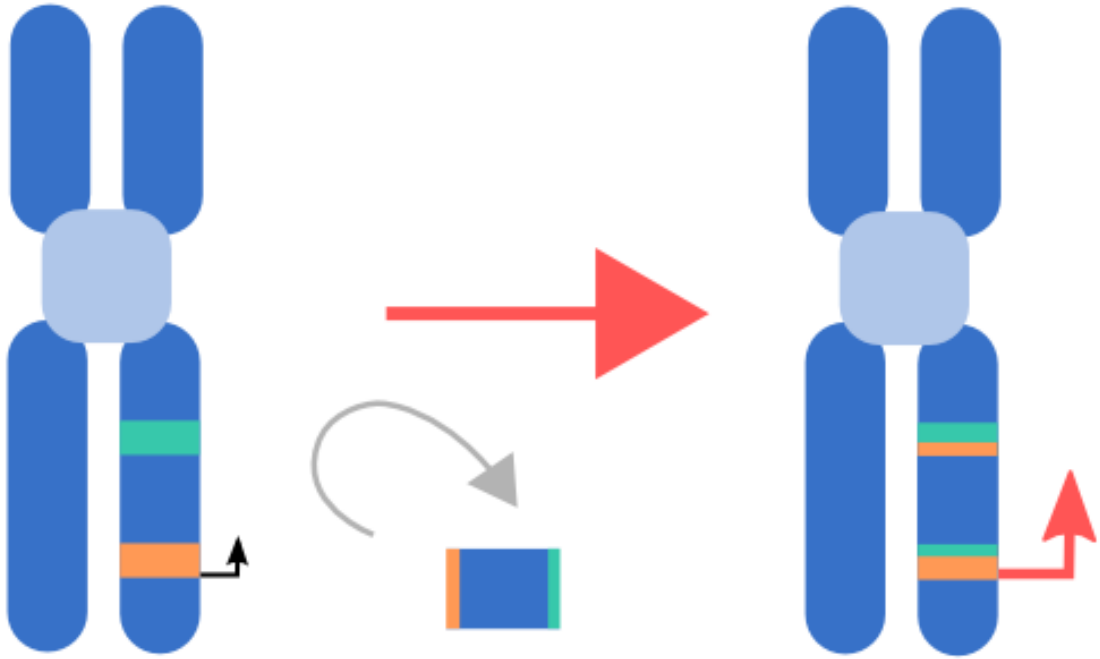
- Read depth
- Reads supporting both sides of the break
- Concomitant copy-number change

BRASS – Breakpoint by assembly

- Supporting read > 4
- Remove read groups overlapping:
  - repeats
  - high GC content
  - high-depth regions
  - known viral insertion sites
  - known bacterial insertion sites
  - telomeric and centromeric regions
- Require events to have
  - Concomitant copy-number change
  - Assembly support

THE FUNCTIONAL CONSEQUENCES OF STRUCTURAL VARIATION

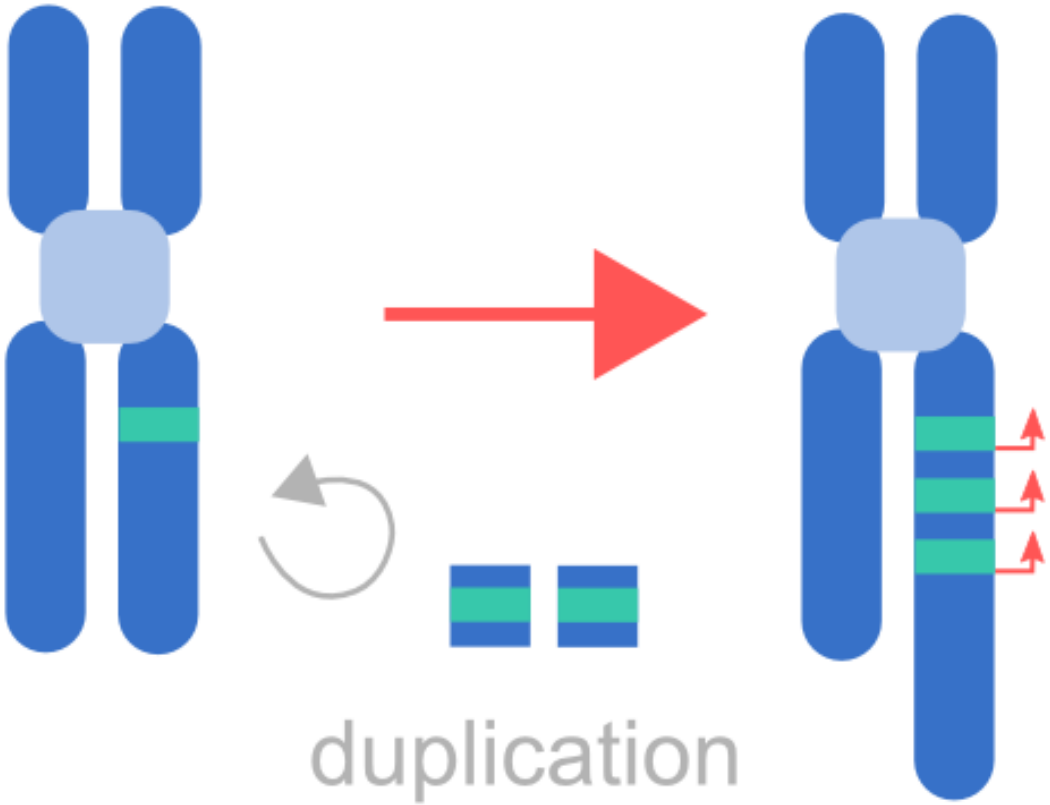
Oncogenic fusion



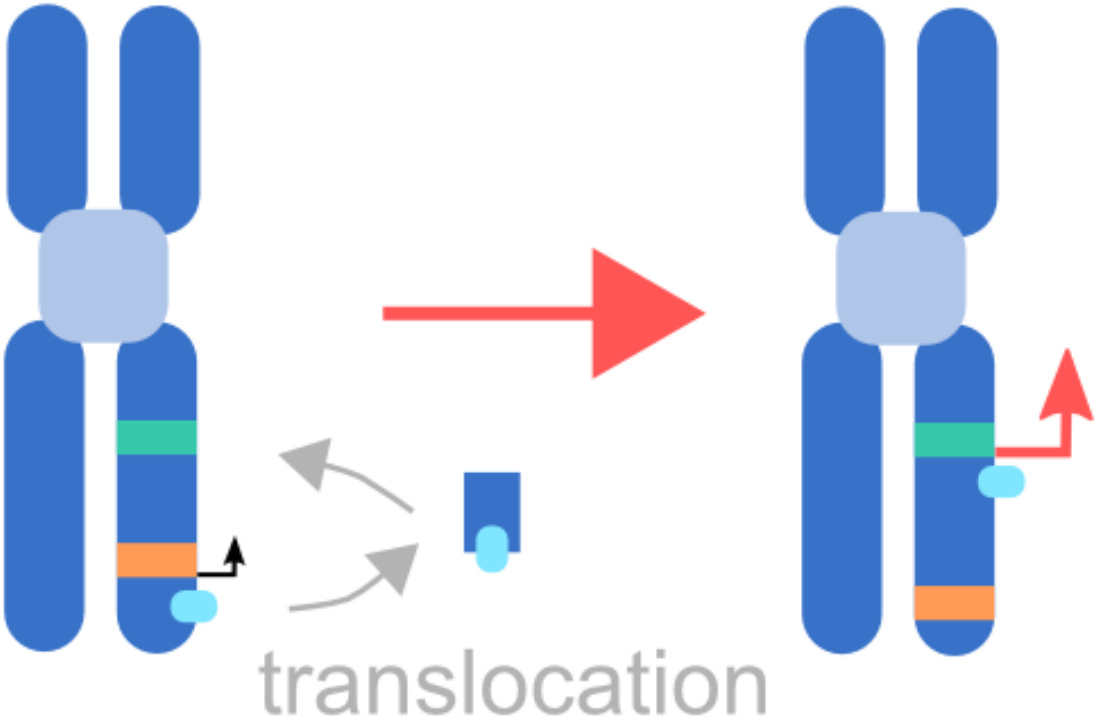
inversion

(also deletion, translocation)

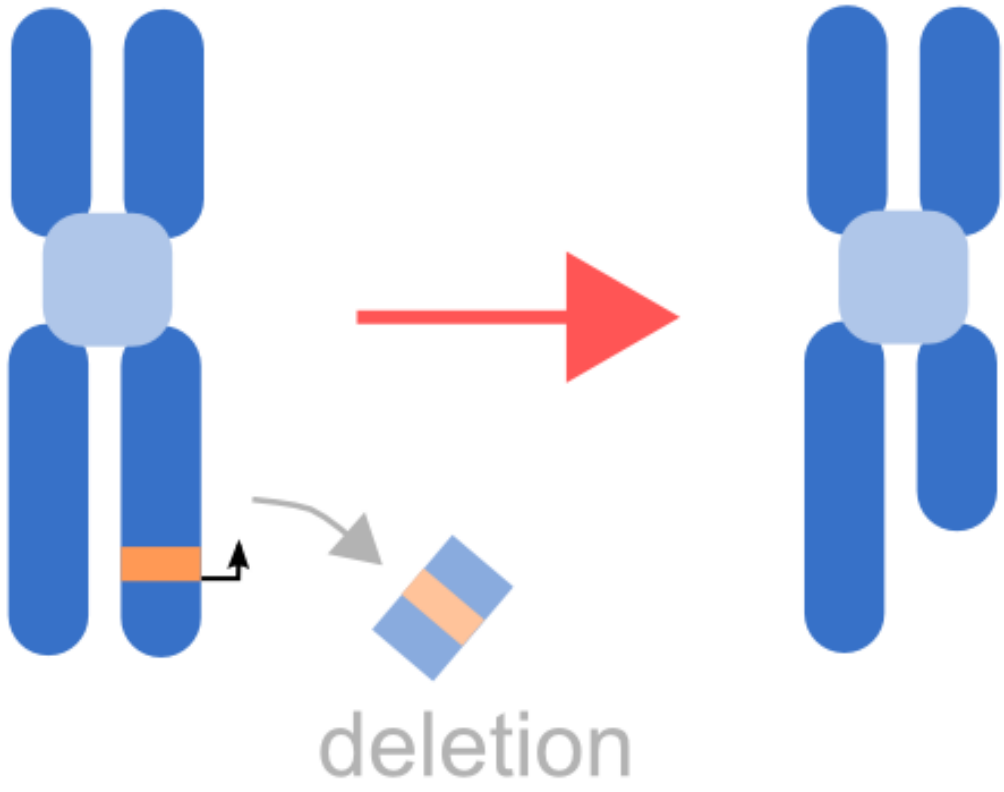
Oncogene amplification



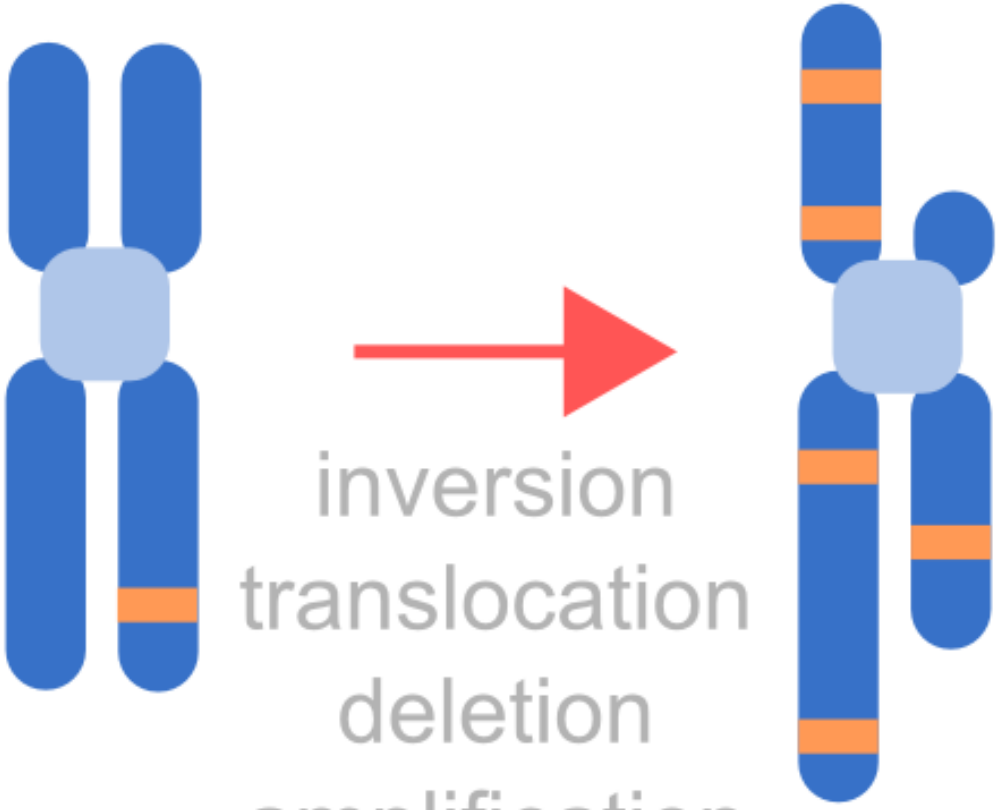
Enhancer hijacking



Tumour suppressor deletion



Genomic instability

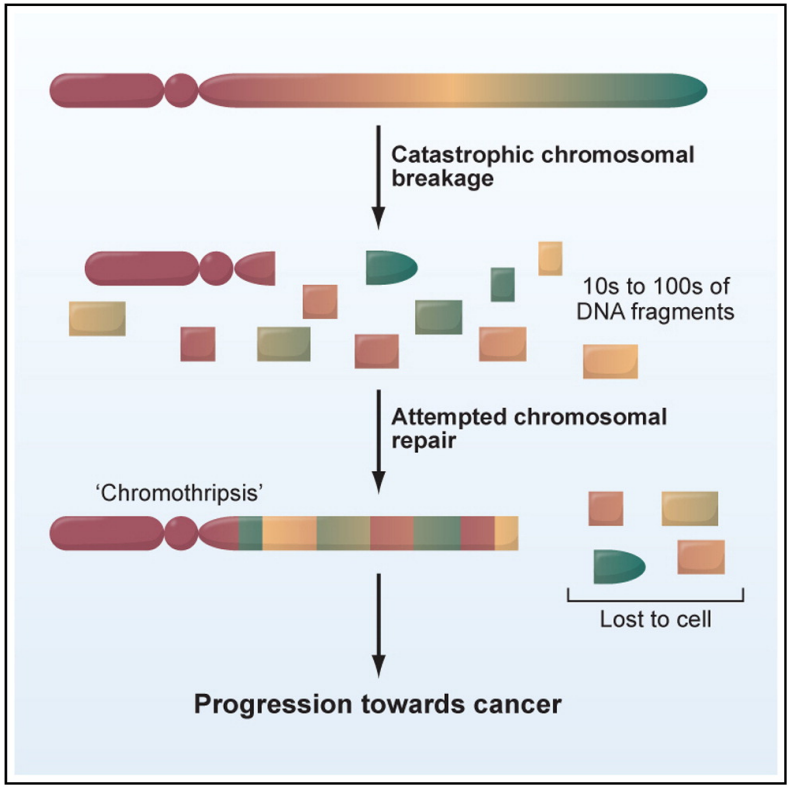


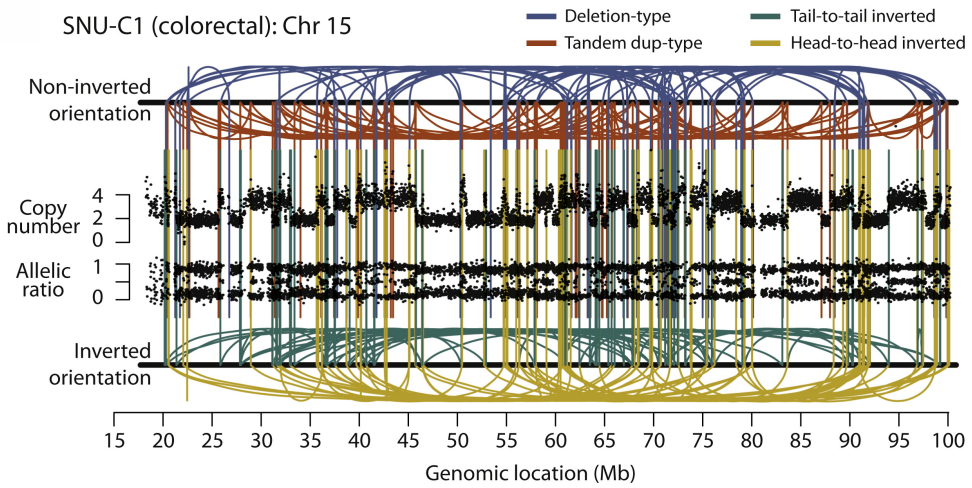
Examples of methods for predicting function

- Oncogenic fusions: GRASS
- Amplification/deletion: GISTIC (multisample)
- Enhancer hijacking: ?
- Genomic instability: Complex Arm Aberration Index (CAAI) and Genomic Index (GI)

COMPLEX REARRANGEMENTS

Chromothripsis

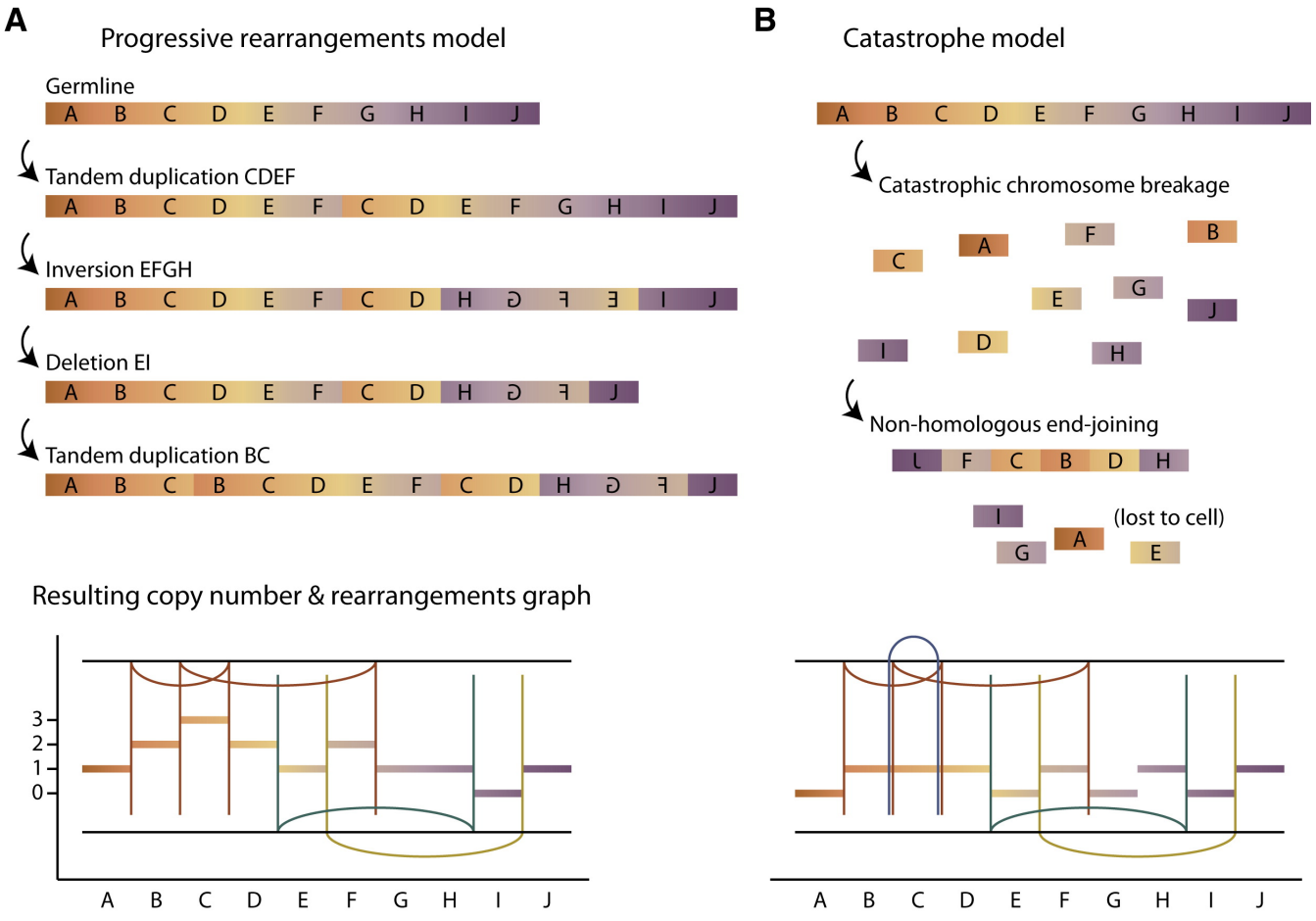




Stephens et al., Cell 2011

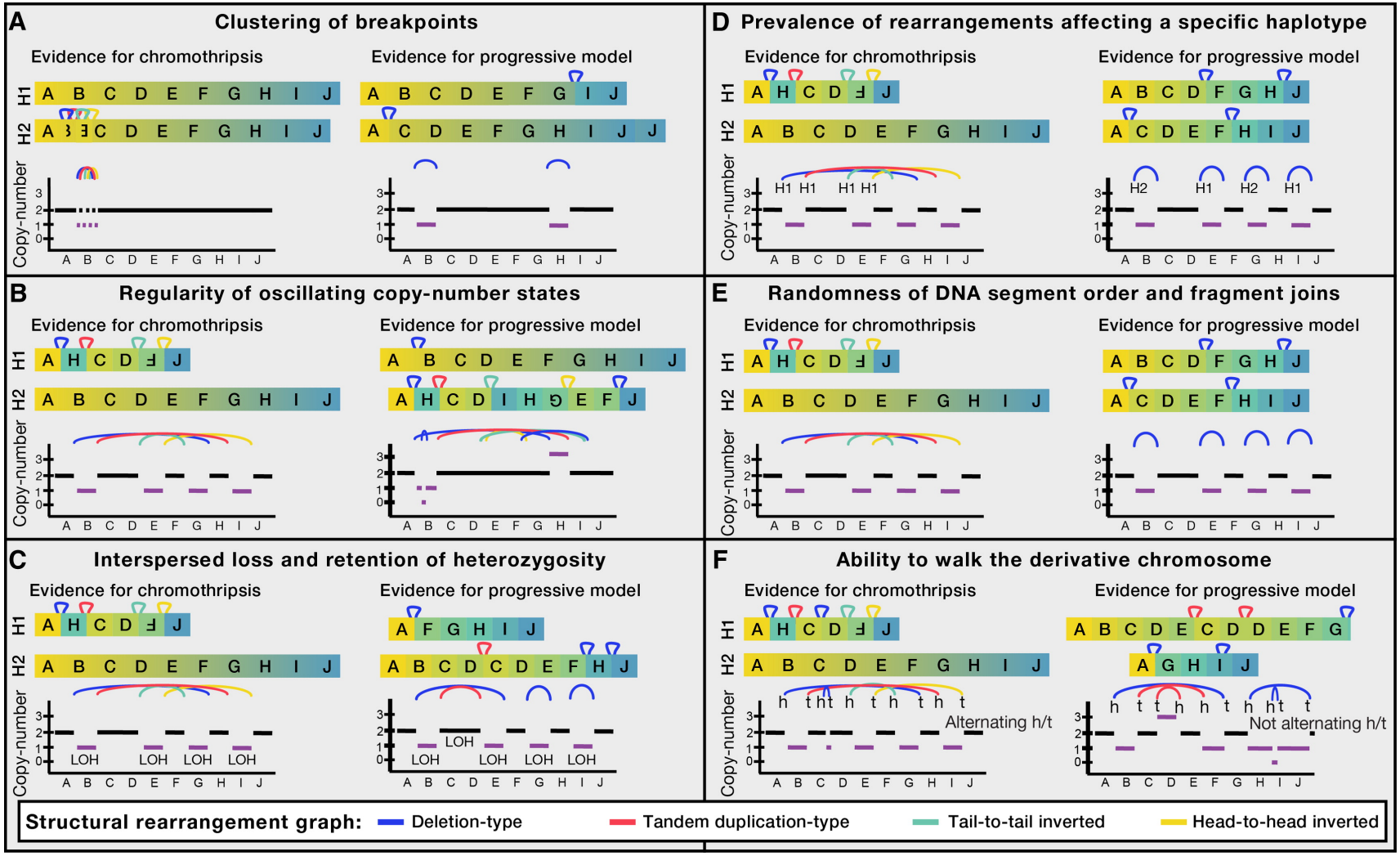
- prevalence in cancer varies (2-3% - 70% of cases in different cancers)

Chromothripsis



Stephens et al., Cell 2011

Criteria for inferring chromothripsis



Korbel and Campbell, Cell 2013

Criteria for inferring chromothripsis: computational application

1. **Clustering of breakpoints**

   Kolmogorov-Smirnov test for exponential distribution of breakpoint distances

2. **Regularity of oscillating copy number states:**

   Calculated as percentage of consecutive 2-1/1-2 copy number steps in a chromosome

3. **Interspersed loss and retention of heterozygosity**

   Calculated as percentage of consecutive retention/loss of fragments in a chromosome

4. **Randomness of DNA segment order**

   Compare breakpoint distances with Monte Carlo simulations (t-test)

5. **Randomness of DNA fragment joins**

   DEL, TanDUP, H2H, T2T-type rearrangement counts should follow a multinomial distribution (p=1/4)

6. **Ability to walk the derivative chromosome**

   Alternating heads and tails (Wald-Wolfowitz test)

7. **Prevalence of rearrangements affecting a specific haplotype**

   Chromosome-wide phasing data can be obtained when germline whole-genomic sequencing data from both parents or somatic genome sequencing data from aneuploid secondary tumors (which are common in the context of hereditary disorders such as Li-Fraumeni syndrome; Li and Fraumeni, 1969) are available for a patient sample in question. (Korbel and Campbell, 2013)

Criteria for inferring chromothripsis: in practice

No clear-cut rules:

> Stephens et al., 2011:

1. massive number of rearrangements on 1 or a few chromosomes (>10)

2. alternate copy number between 2 states only and alternate loss/retention of heterozygosity

3. clustering of breakpoints

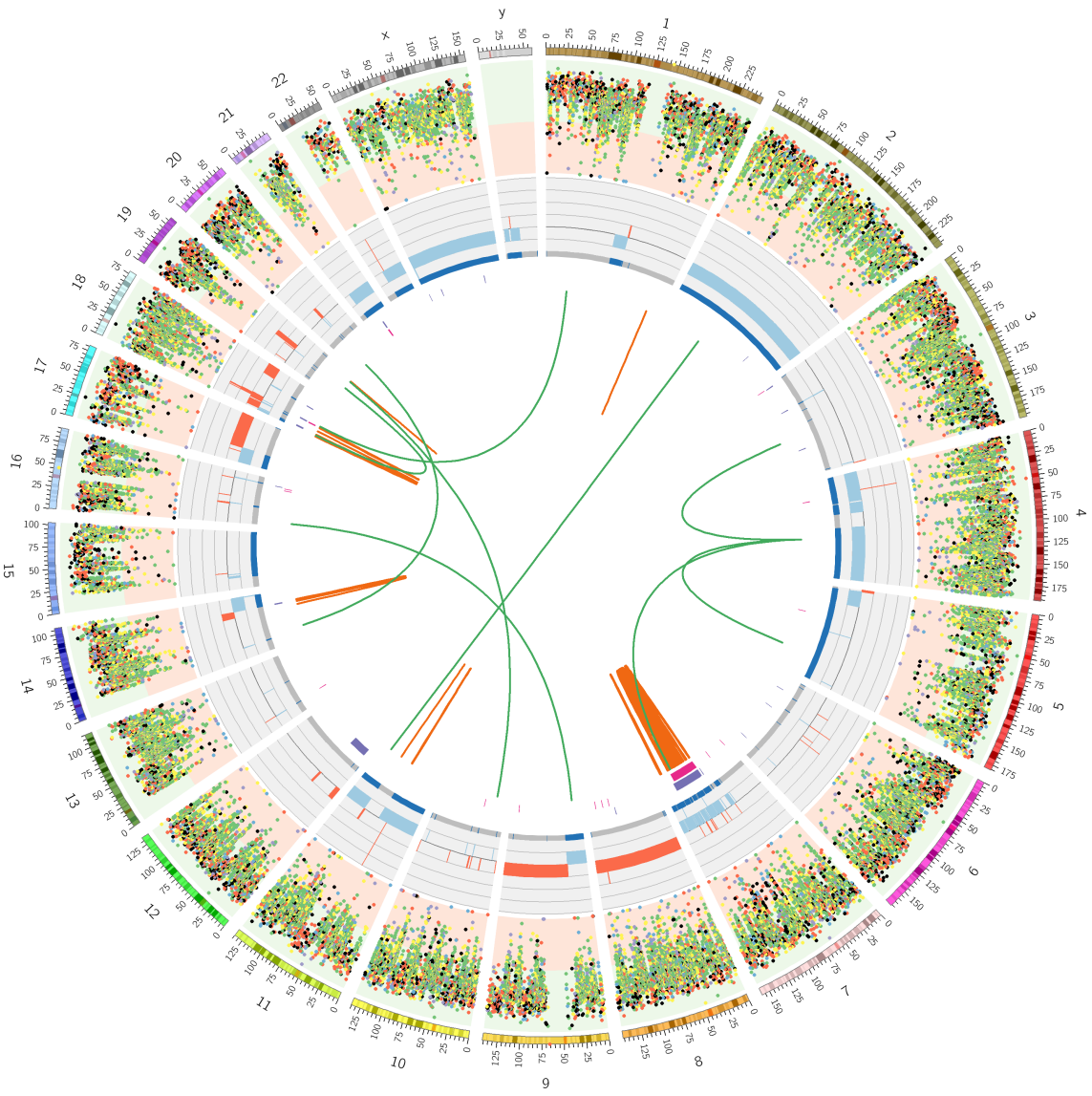
> Rausch et al., 2012:

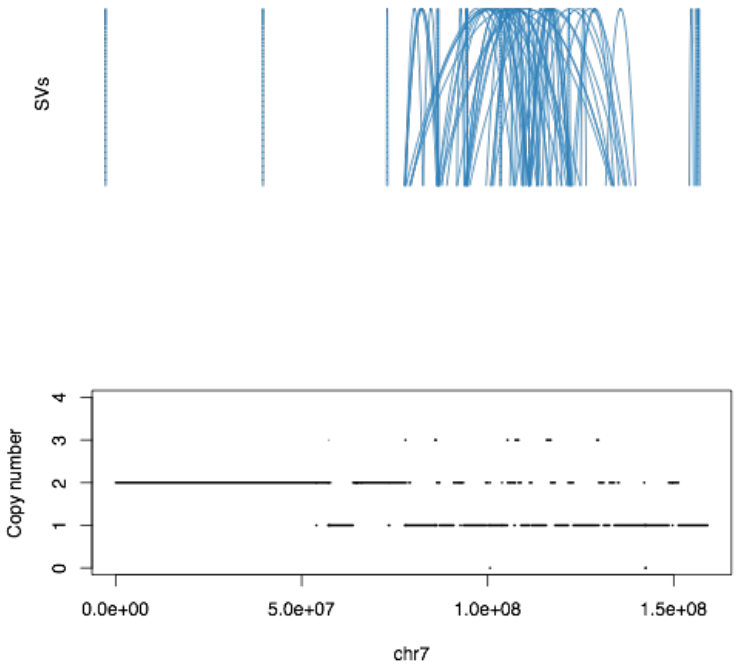
10 changes in segmental copy number involving 2-3 distinct copy number states on a single chromosome

> Nones et al., 2014:

Evidence of clustering of breakpoints was estimated as proposed by Korbel and Campbell36. Chromosomes with evidence of clustering of breakpoints ($P<0.001$, Kolmogorov–Smirnov test—goodness of fit test) were reviewed for: (1) evidence of chromothripsis which included oscillation of copy number, random joins and retention of heterozygosity […] A larger cohort of EACs (n=101) was screened for evidence of chromothripsis using SNP arrays (Illumina), chromothripsis was inferred in cases where one or few chromosomes showed at least 10 switches in copy number states, with retention of heterozygosity.

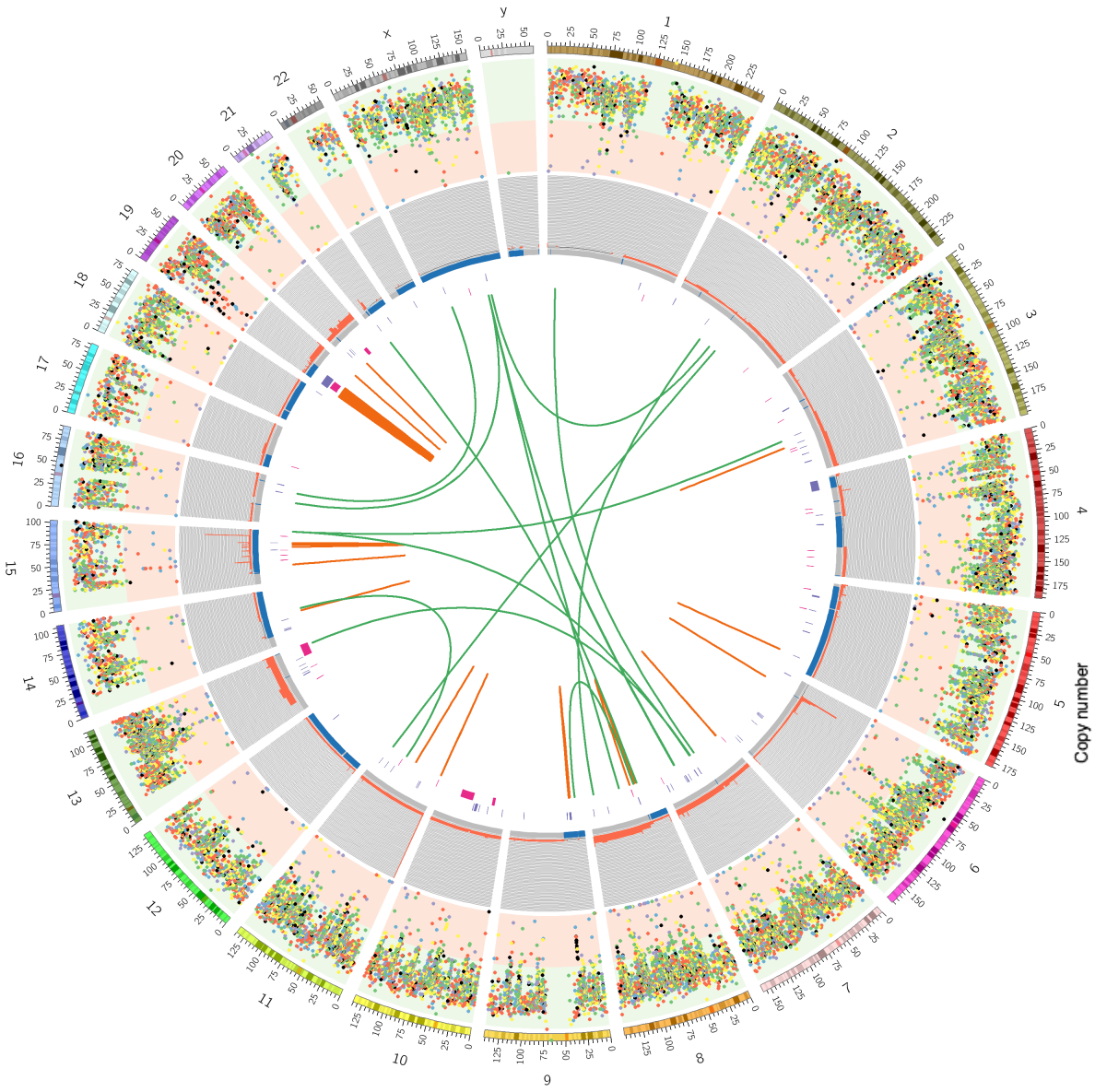
Chromothripsis example 1

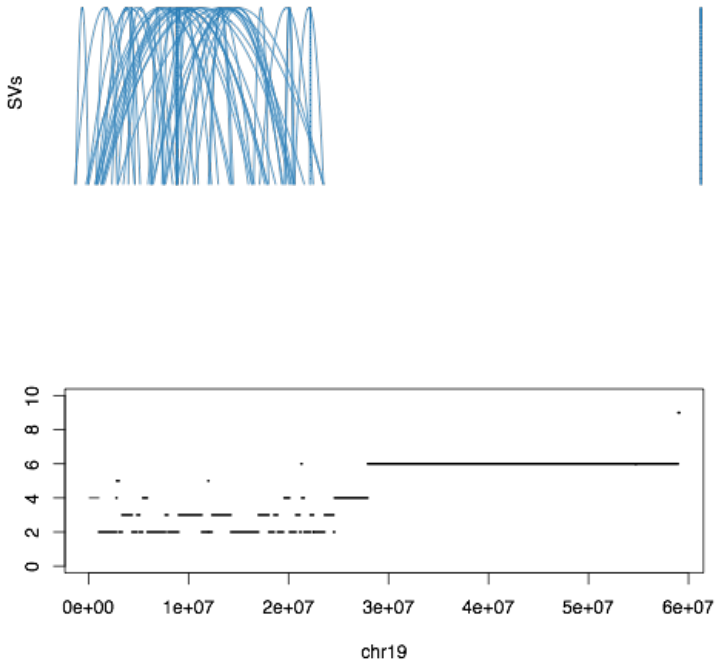




cellularity: 32%

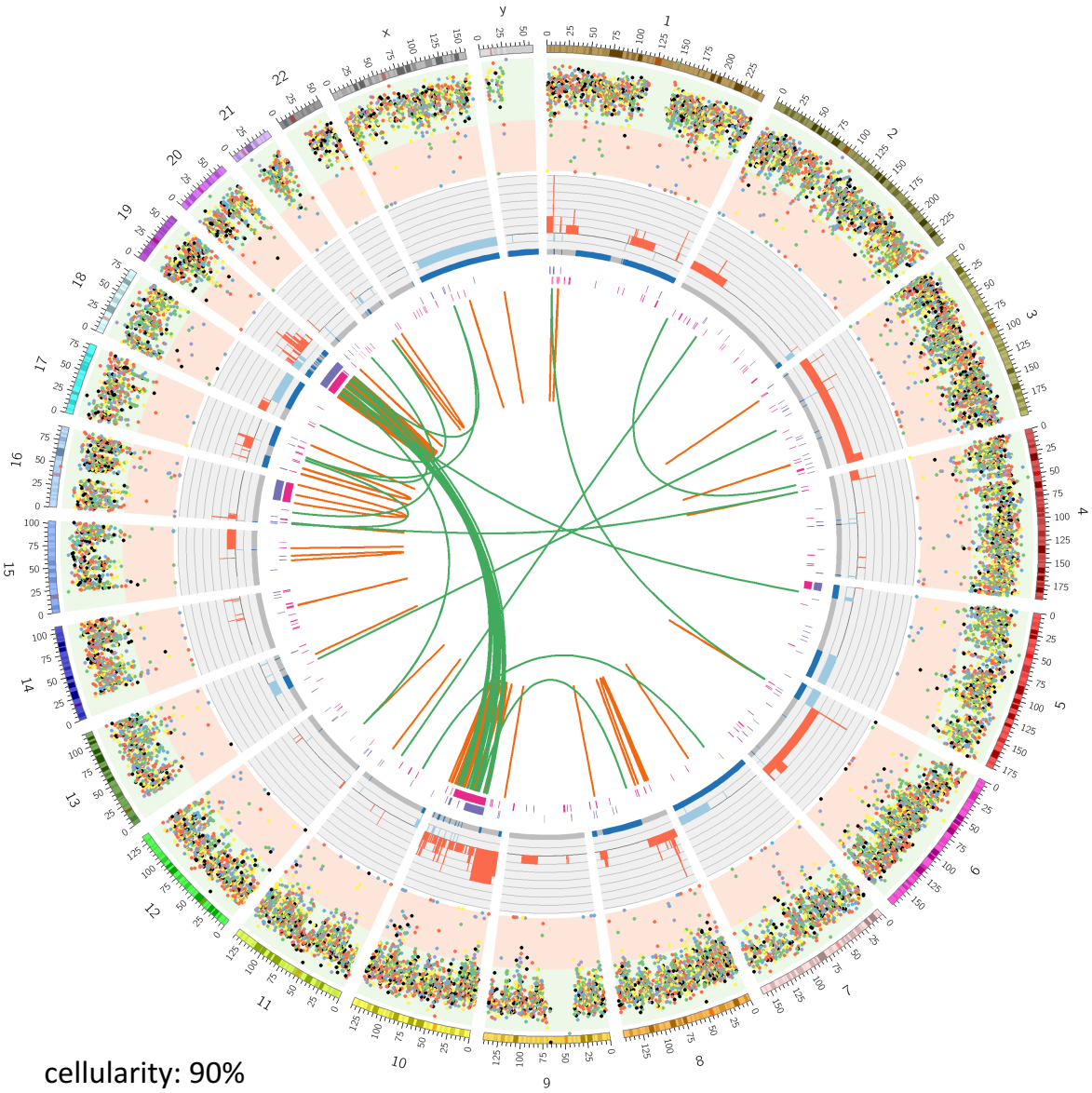
Chromothripsis example 2



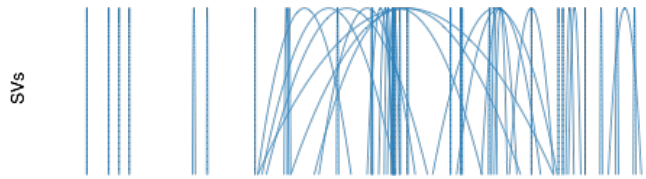


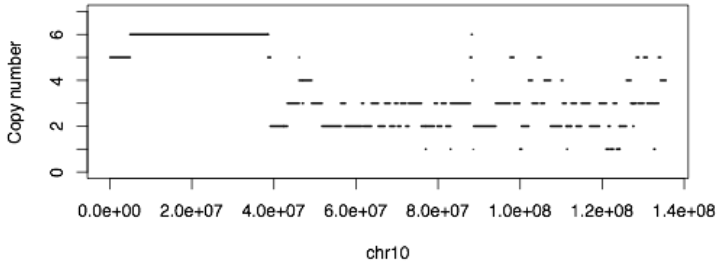
cellularity: 41%

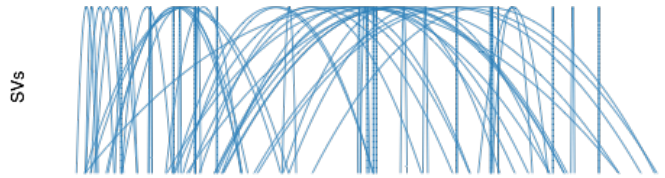
Chromothripsis example 3

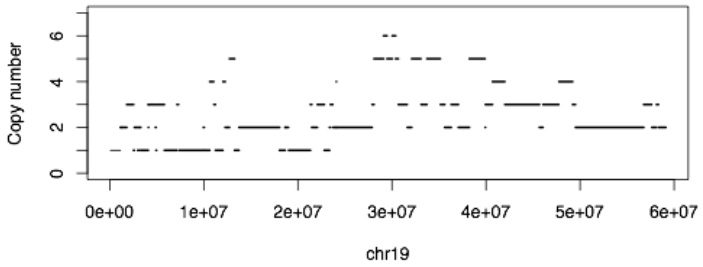


cellularity: 90%

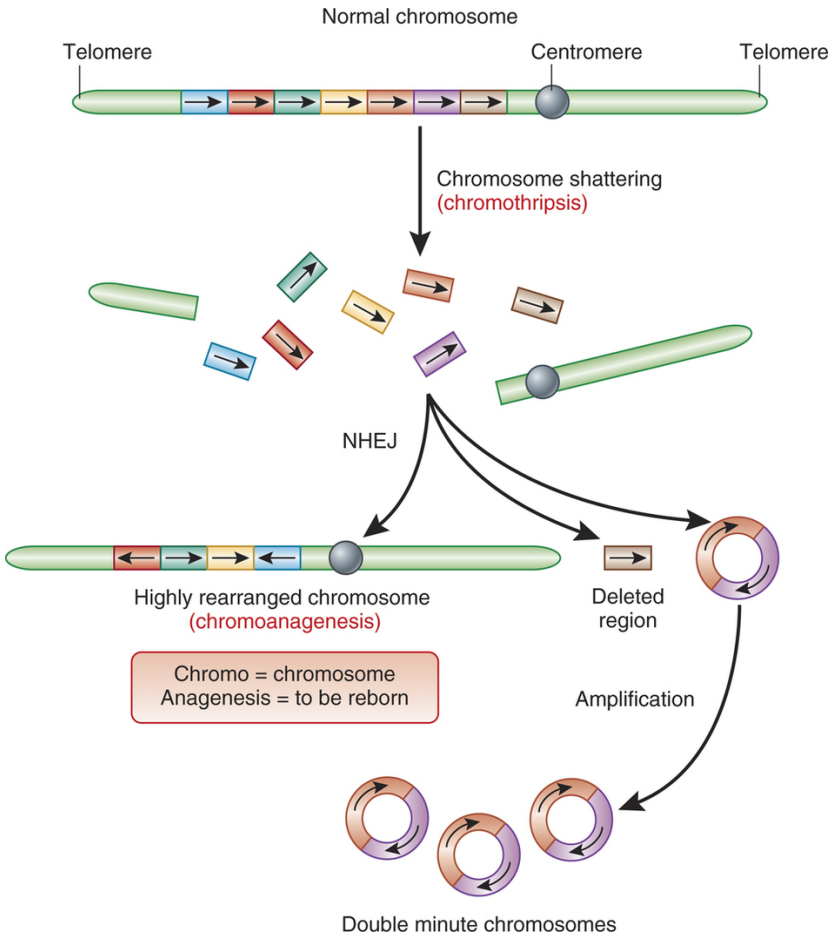






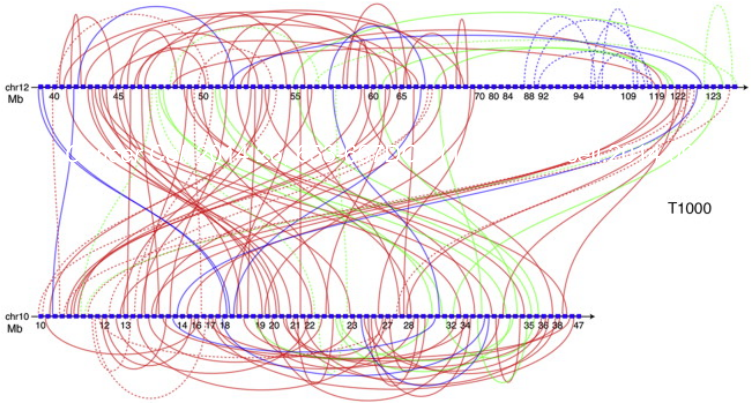


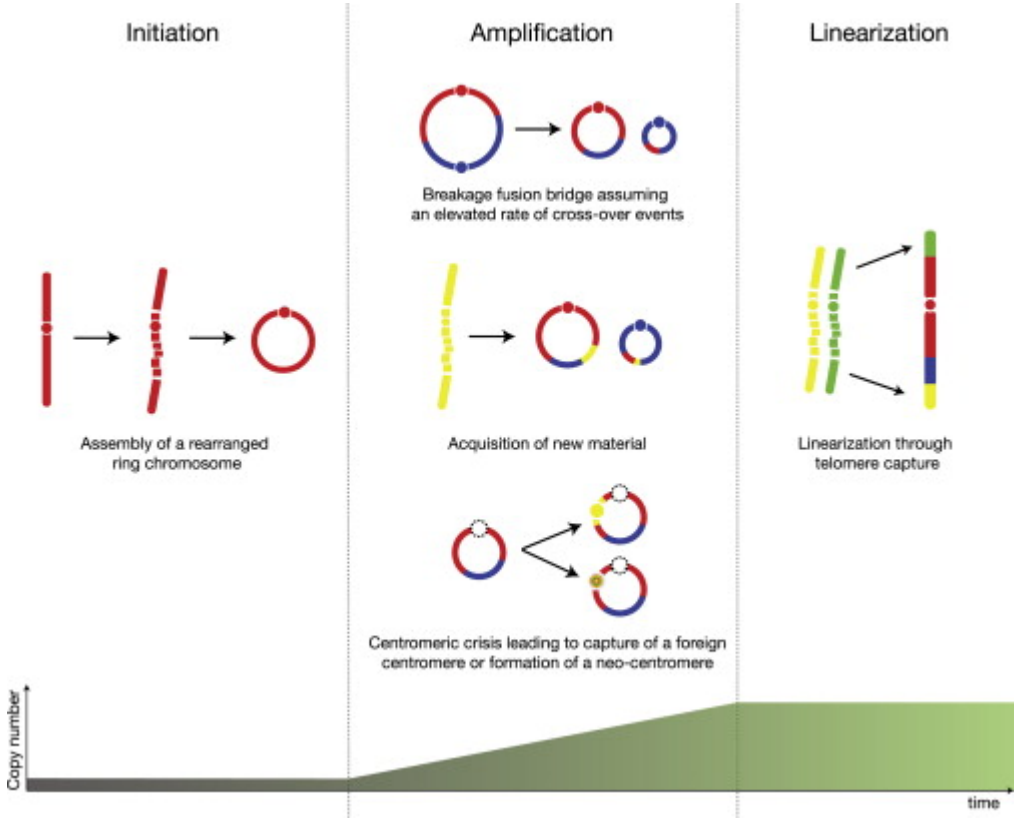
Double minute chromosomes



FromChromoanagenesis and cancer: mechanisms and consequences of localized, complex chromosomal rearrangements
Nature Medicine 18, 1630–1638 (2012) doi:10.1038/nm.2988

Neochromosome characterisation





Cancer Cell 2014 26, 653-667DOI: (10.1016/j.ccell.2014.09.010)

EXERCISE 3