Amplicon Architect

A tool for detecting and reconstructing extrachromosomal circular DNAs from NGS data.

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Purpose of this presentation

- Provide a (**very**) brief introduction to eccDNA research and its methods
- Introduce basic concepts and showcase one of the computational problems
- Broadly explain one of the available solutions (*Amplicon Architect*)

Intro to eccDNA biology

Definition

EccDNA (Extrachromosomal circular DNA) is broad class of covalently closed ssDNA and dsDNA of genomic origin, varying in size from 100 bp to 5 Mbp, and is ubiquitous in eukaryotes.

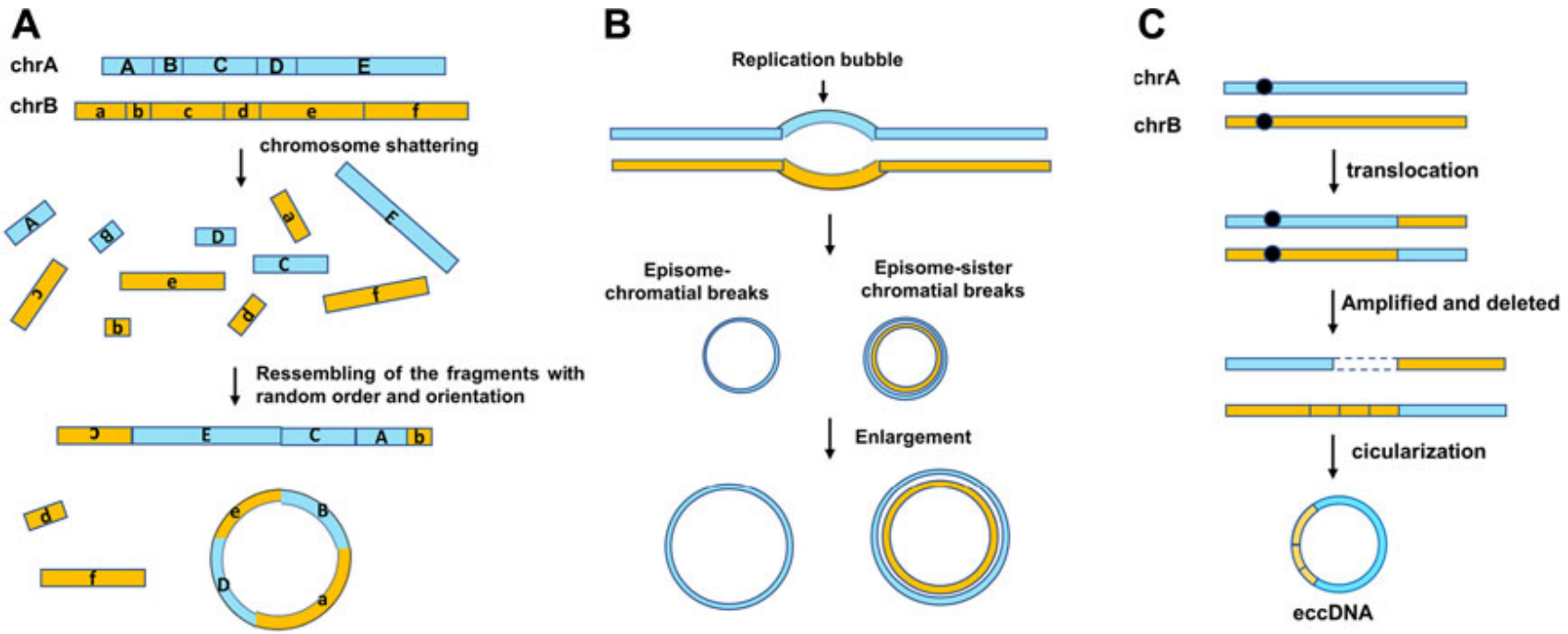
Key traits

- highly accessible chromatin
- independant replication
- uneven segregation during mitosis
- can carry genomic sequences, eg. genes, enhancers
- can be a "collage" of distant genomic sequences
- one of the main causes of focal amplification in cancer

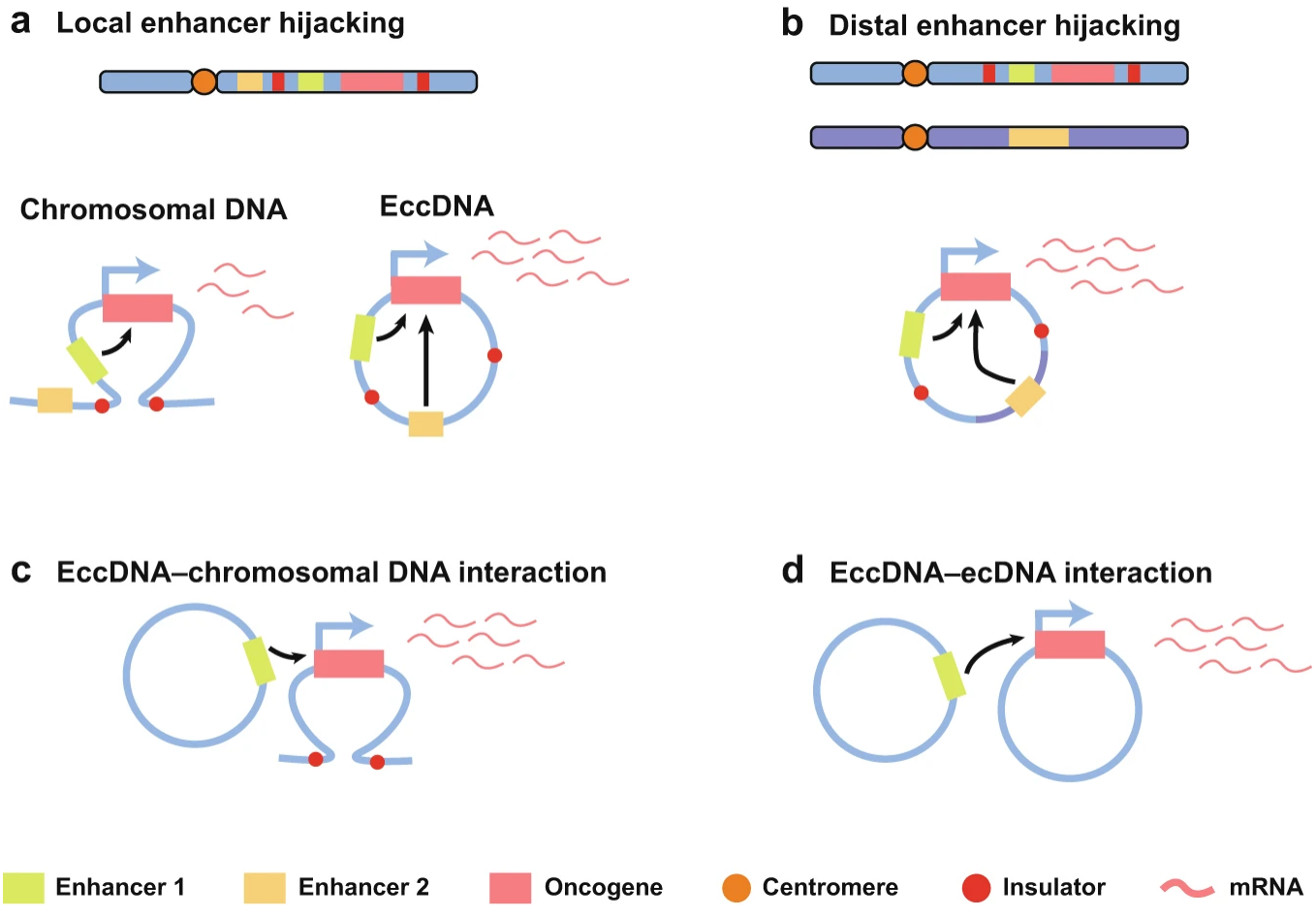
Biogenesis

Known pathways

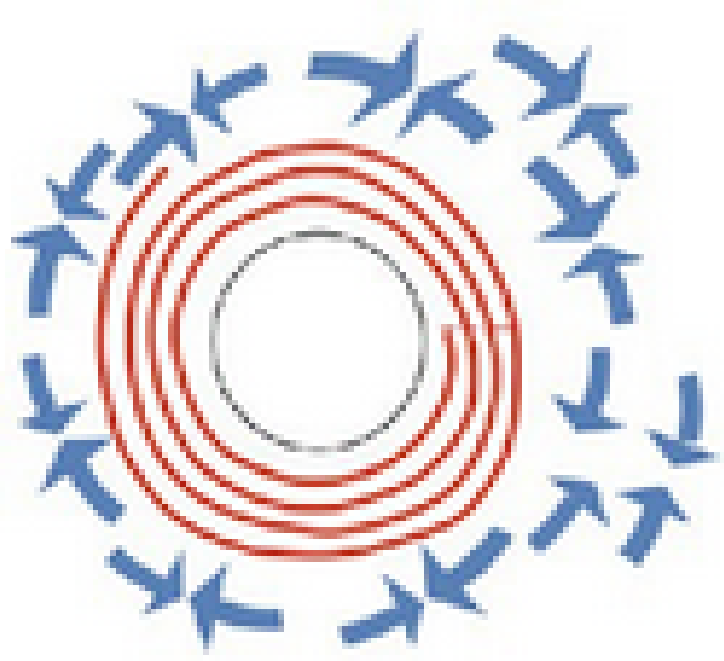
- Chromothripsis (Chromosome shattering)
- Episome model (polymerase slippage)
- Translocation-Deletion-Amplification mechanism
- Breakage-Fusion-Bridge cycle

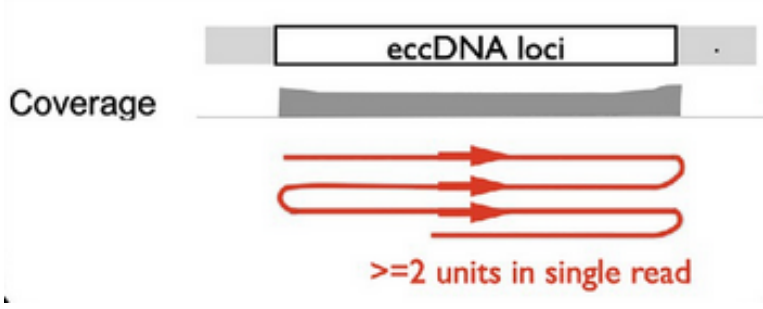


Impact on gene expression



Sequencing strategies — Φ29 polymerase RCA

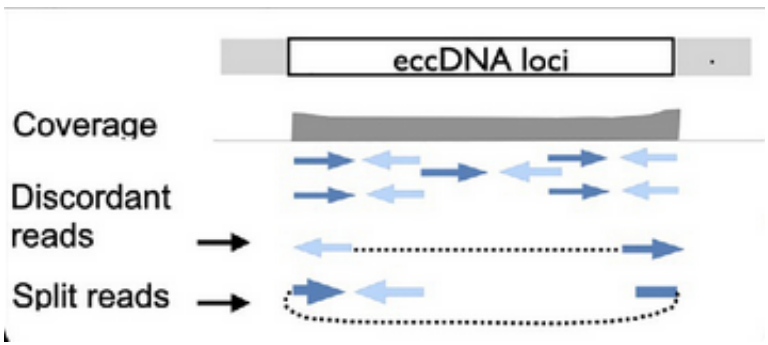


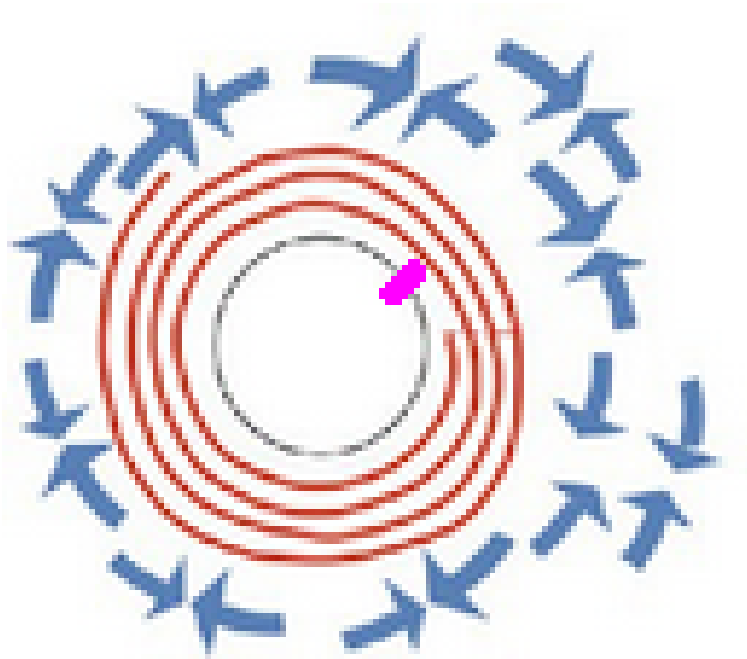


Basic concepts

Simple solution?

- concordant read pairs,
- discordant read pairs (unexpected mapping),
- split reads

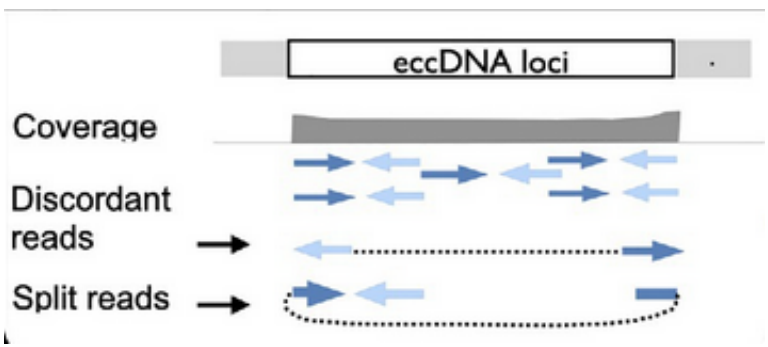


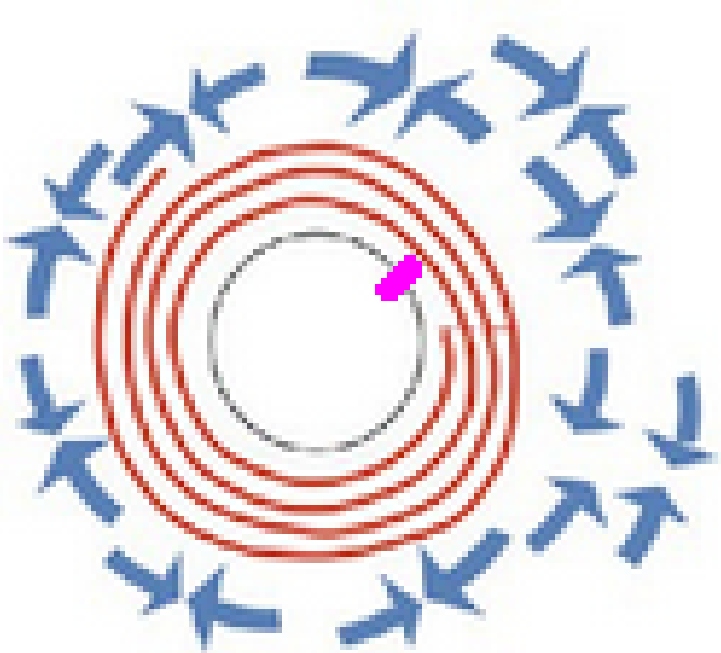


Basic concepts

~~Simple solution?~~ This is not enough!

- structural variants can lead to similar read mappings
- no information about the number of eccDNAs
- no structure reconstruction

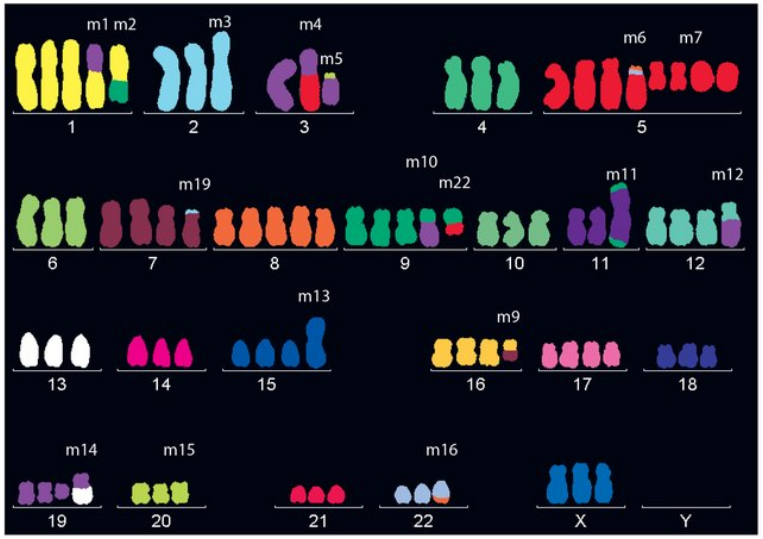




Amplicon Architecht: NGS data from tumor cells

Input

- WGS paired-end reads mapped to reference
- a set of seed intervals



Seed interval selection

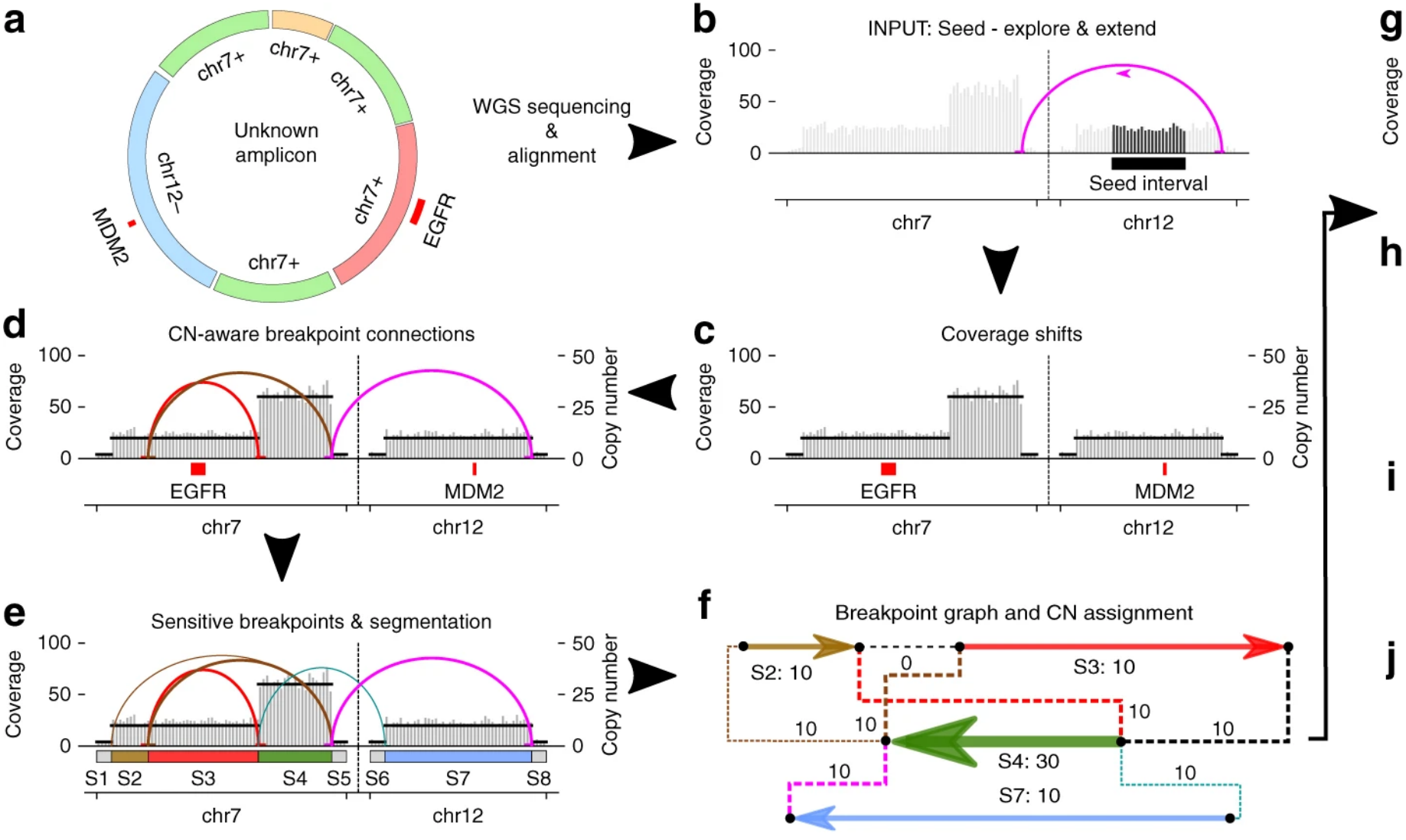
- Find CNV calls (*Read Depth*)
- filter out:
  - blacklisted regions (CNV calls from non-cancerous cells)
  - regions with high average repetitiveness (*Duke Mapability Uniqueness*)
  - known segmental duplications
- correct for aneuploidy

Main pipeline

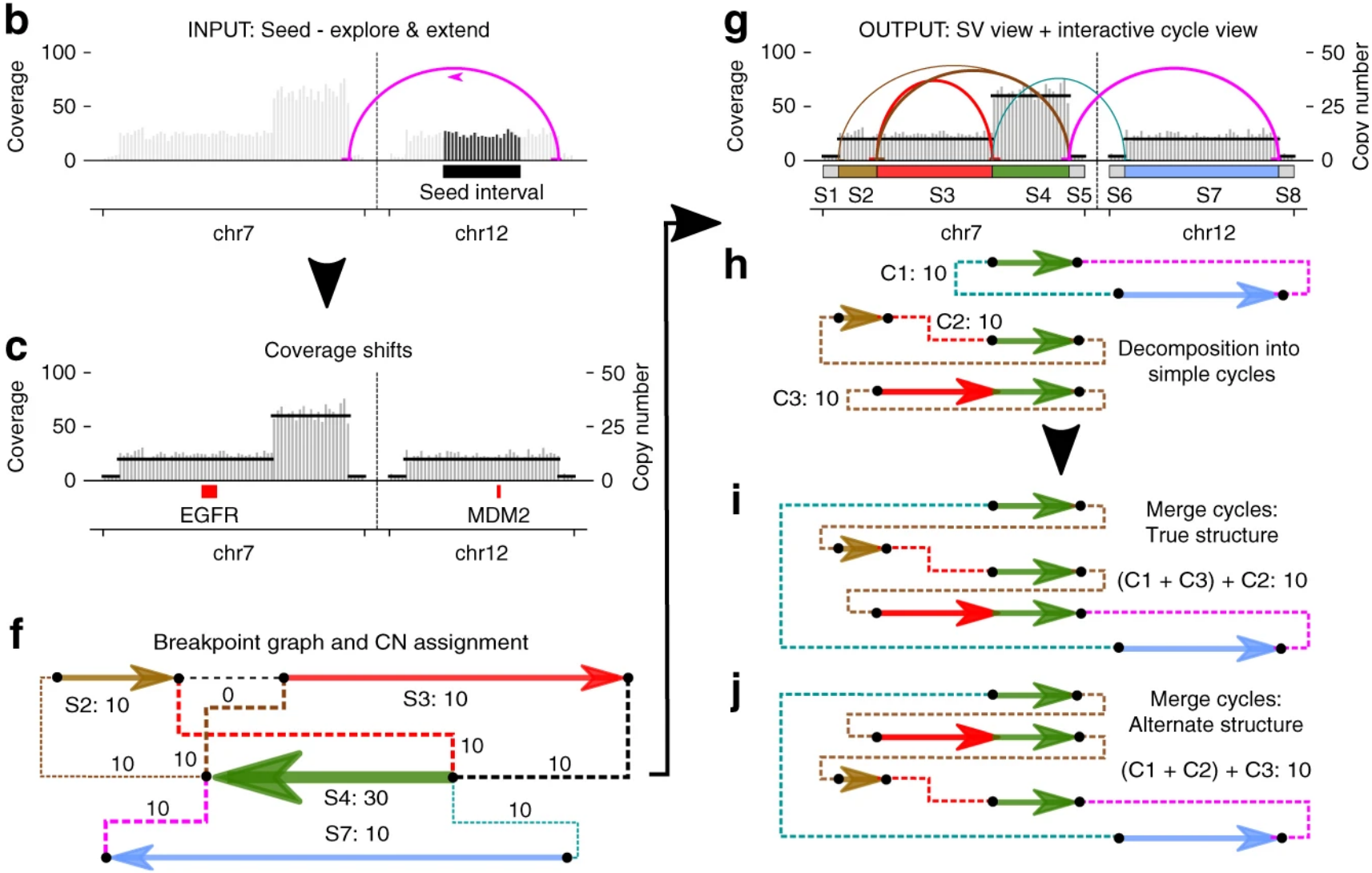
AA pipeline

- Iteratively identify intervals belonging to the same amplicon (discordant read pairs)
- Identify rearangements within the interval (based on discordant read pairs and CNV boundary detection)
- create a breakpoint graph (a graph showing how segments of the amplicon are connected) and estimate copy numbers for each segment
- Breakpoint graph decomposition into simple cycles (based on CN using a heuristic method)
- Merge simple cycles into possible amplicon structures

Schematic of Amplicon Architect pipeline



Schematic of Amplicon Architect pipeline, cont.



Additional information

Other available tools

NGS data

- Circle-Map,
- Circle_finder,
- ecc_finder,
- ECCsplorer

Long-read sequencing

- NanoCircle,
- eccDNA_RCA_nanopore,
- CReSIL,
- FLED

Better diagram of eccDNA formation pathways that was too big for the slide.

doi: 10.3389/fonc.2022.814504. PMID: 35494014; PMCID: PMC9046939.

Literature

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