Next Generation Sequencing (NGS)

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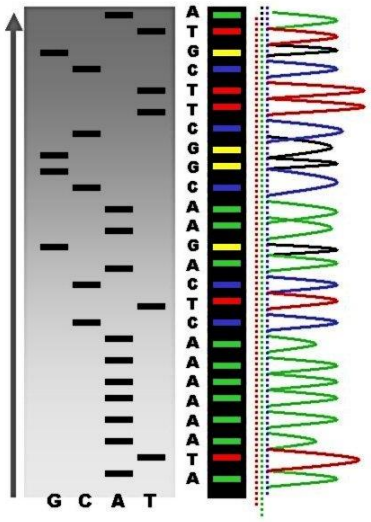
Aims

1. What is NGS?
2. NGS work-flow
3. Some applications of NGS in cancer research
    1. Cancer driver discovery
    2. Genome-wide profiling of all mutation types in individual cancers
    3. Cancer evolution

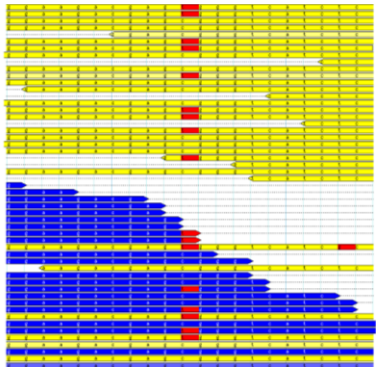
Genomic Sequencing

Capillary (Sanger) Sequencing



- Candidate Genes
- Long read lengths
- Small scale projects
- Sensitivity?

Next Generation Sequencing



- High throughput
  - All/ selected genes in many samples
  - Whole genome
- High Sensitivity
- Quantification
- All mutation types

Various platforms used for NGS

Illumina Hiseq/Miseq
Sequencing by synthesis

SOLiD
Sequencing by ligation

Ion Torrent
Semiconductor sequencing

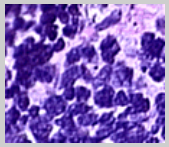
Pacific Bioscience (PacBio)
Single molecule sequencing

454 Pyrosequencing

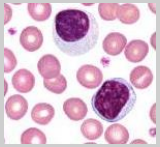
mini ion
Nanopore sequencing

Overview of NGS Workflow (1)

(A) Sample collection



Tumor

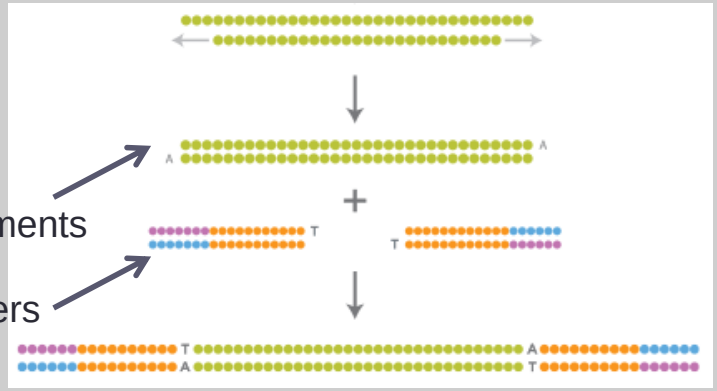


Normal

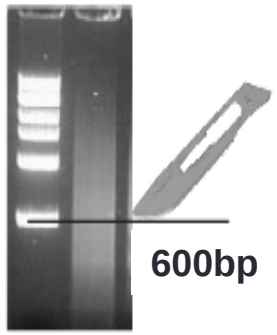
DNA extraction

(B) Library Preparation

1. Fragment DNA
2. Purify
3. Ligate adapters



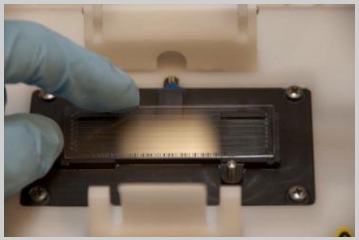
4. Size select



Overview of NGS Workflow (2)

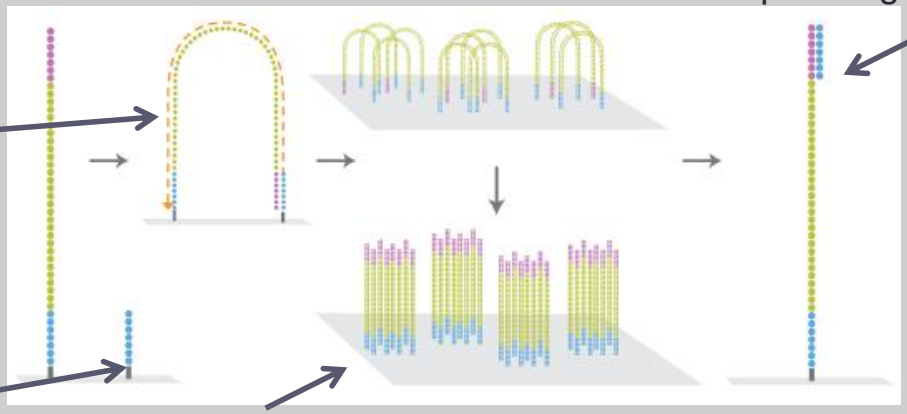
(C) Cluster Generation

Flow Cell



Polymerase based extension

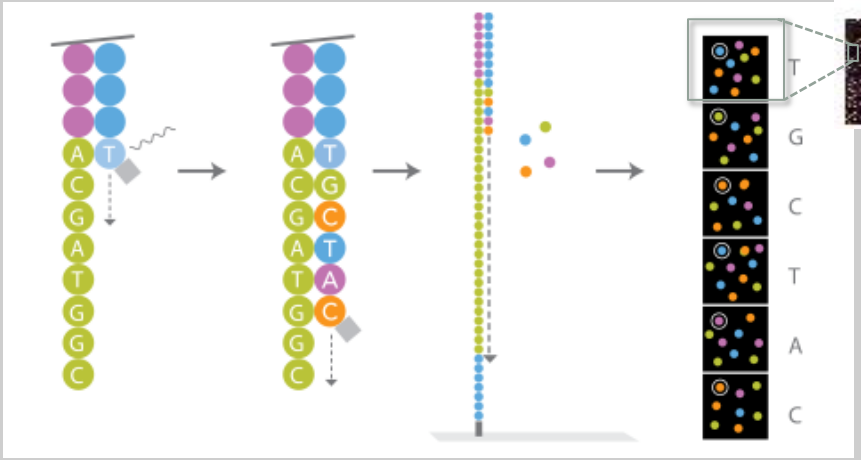
Universal sequencing primer



Surface oligos

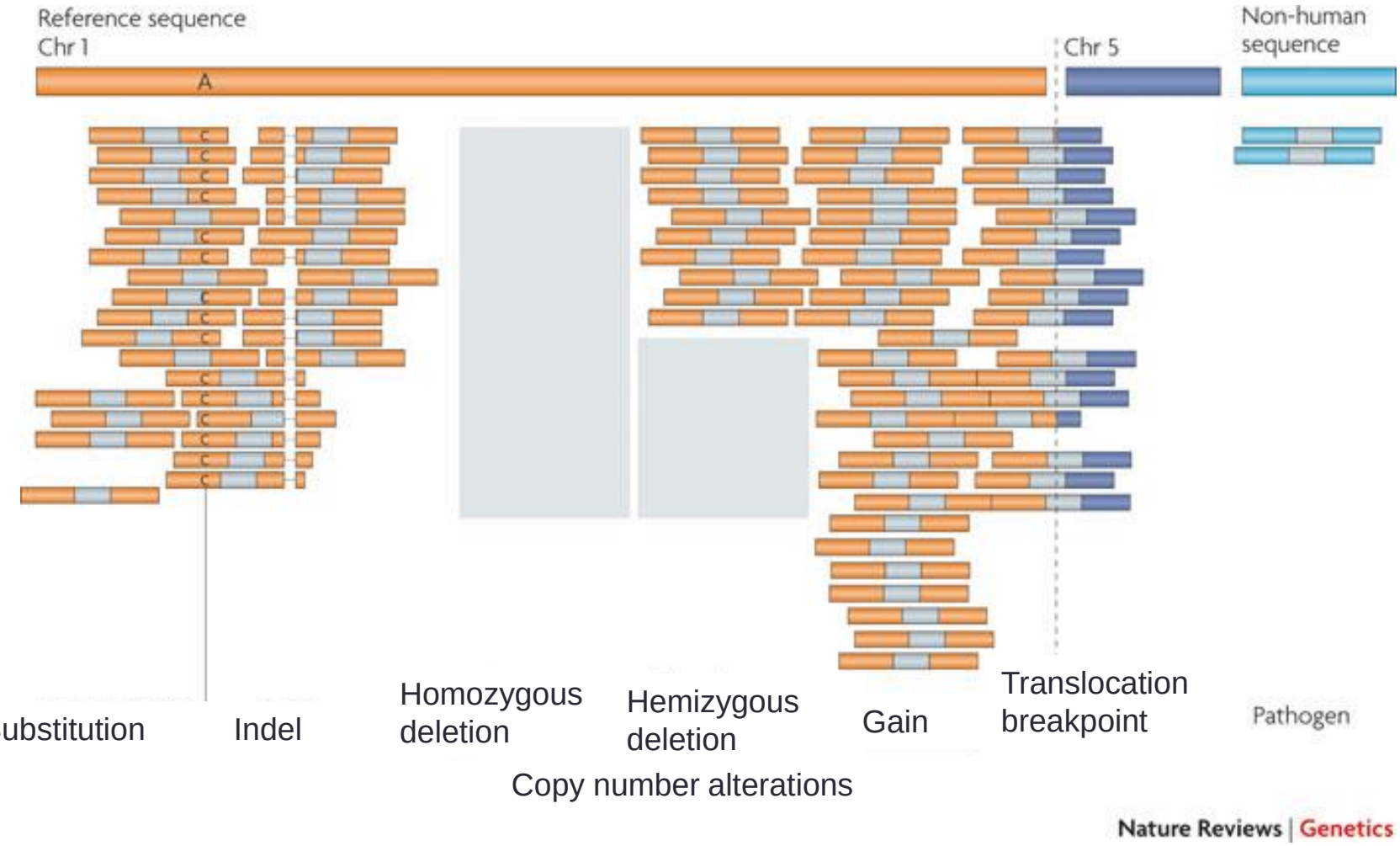
Localised amplification of DNA fragments

(D) Sequencing by synthesis



~150 million clusters/ flow cell

Alignment & Mutation Calling



Some Applications of NGS

1. **Discovering cancer genes**

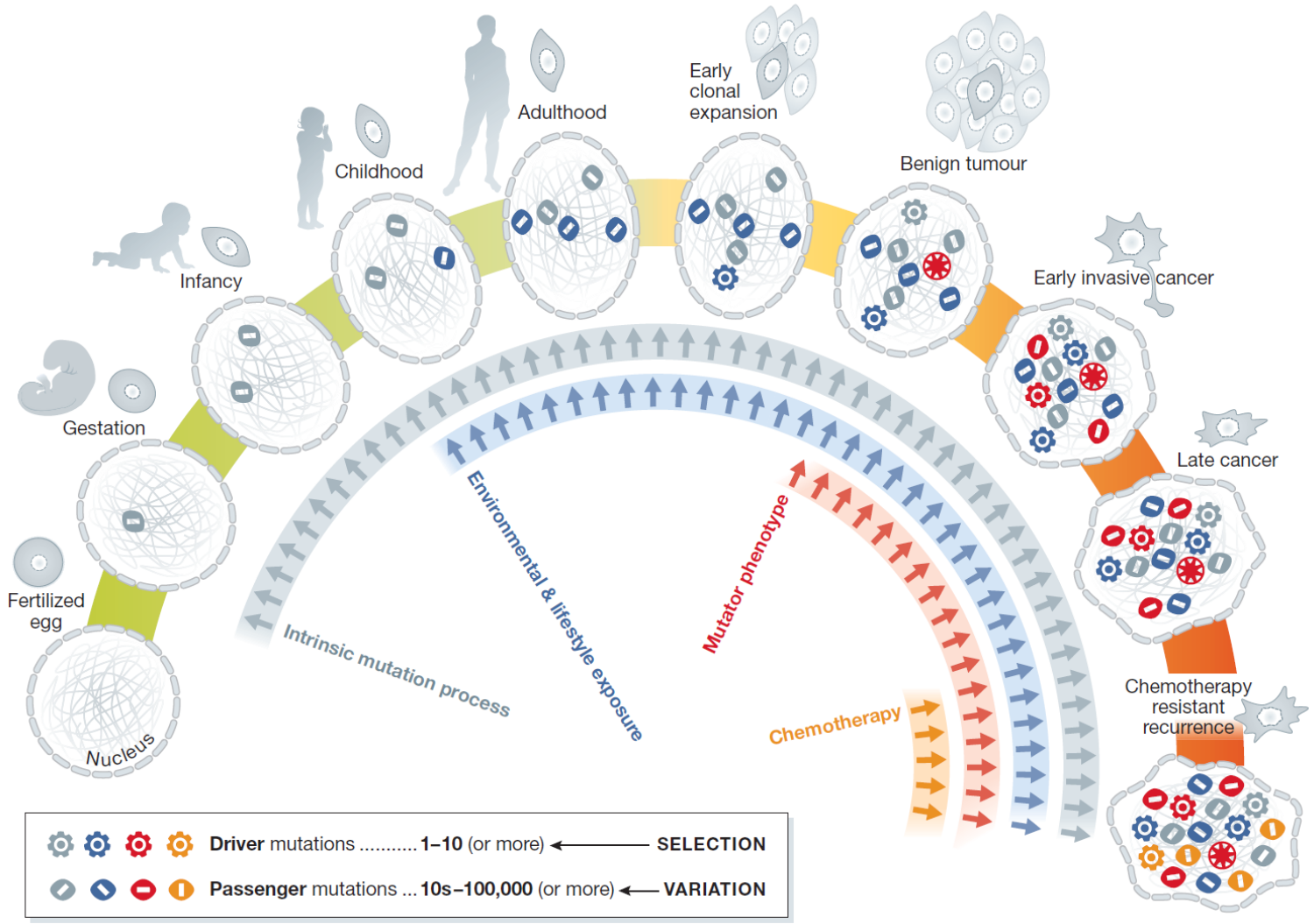
1. **Understanding the complexity of cancer genomes**

1. **Studying cancer evolution**

Genomics in the Clinic: Genome-Drug Combinations

Genomic scenario	Targeted drug
ERBB2 Amplification (Breast Cancer)	Herceptin & Lapatinib
BCR-ABL (CML)	Imatinib (and others)
EGFR *(NSCLC)*	Erlotinib, Gefitinib
EML4-ALK *(NSCLC)*	Crizotinib
KRAS-negative (colorectal cancer)	Cetuximab
BRAF(V600E) *(Metastatic Melanoma)*	Vemurafenib

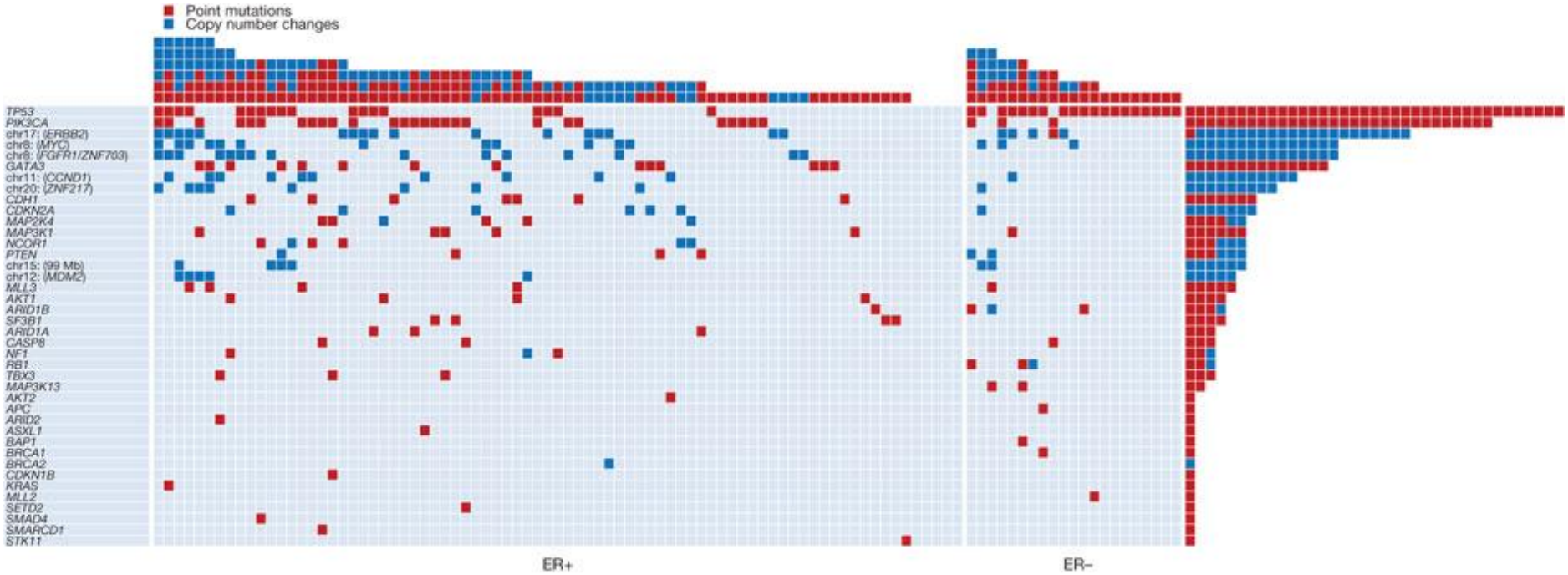
Tumorigenesis: Passengers & Drivers



Stratton (2013), EMBO Mol Med 5:169-172

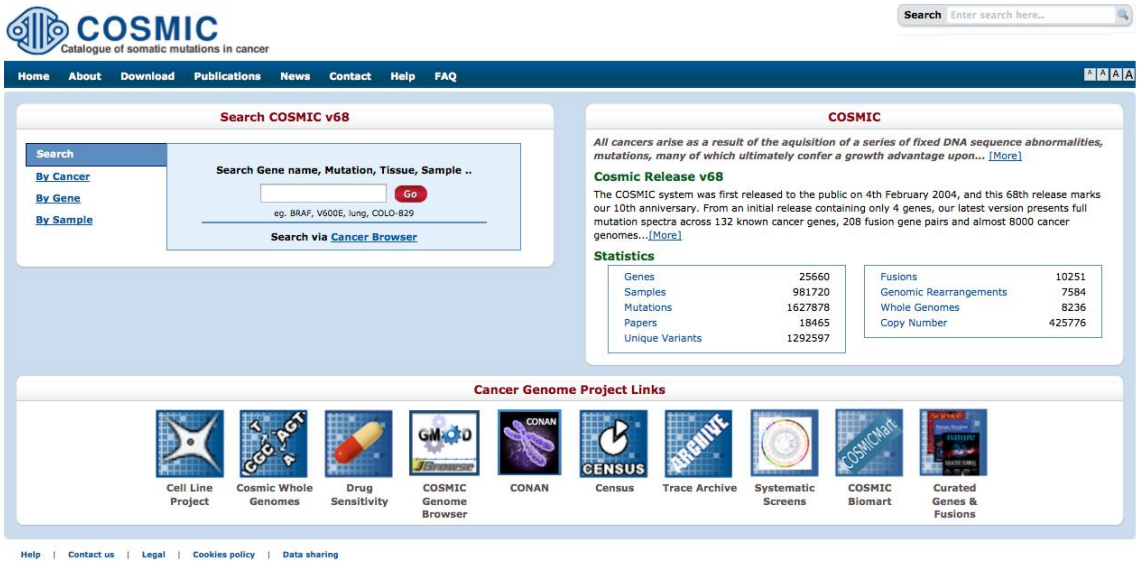
Cancer Driver Discovery

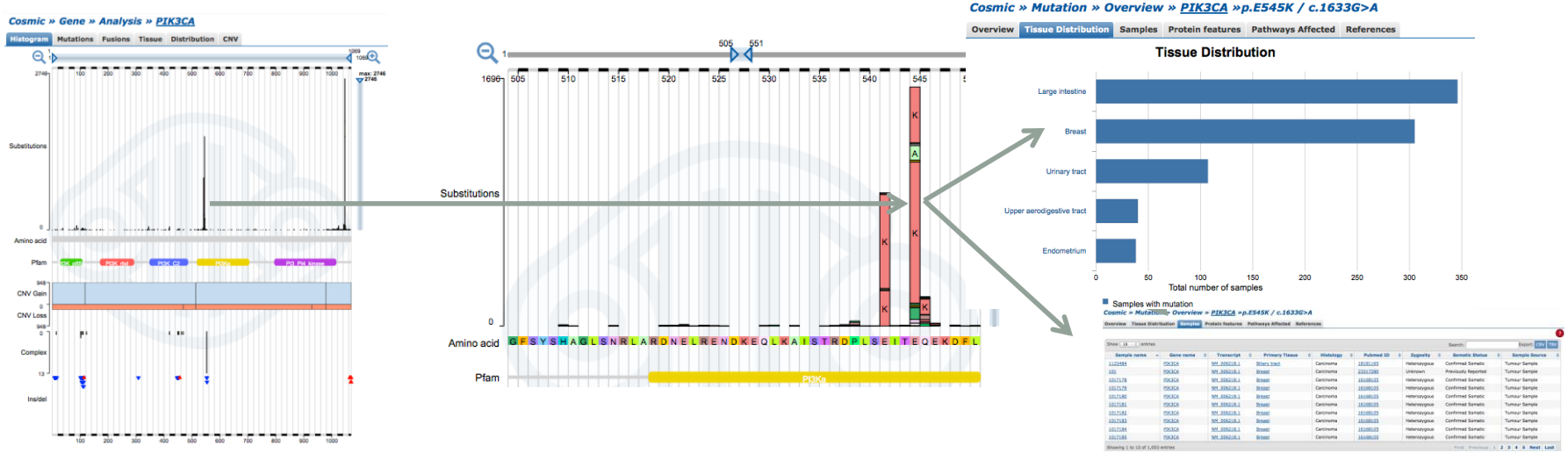
- **100 breast cancer exomes** reveal heterogeneity of breast cancer ‘driver’ mutations



Stephens et al. *Nature* 2012

Mutation Catalogues: ‘COSMIC’





cancer.sanger.ac.uk/

Some Applications of NGS

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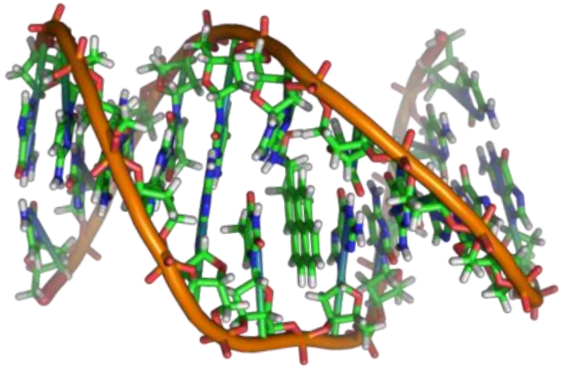
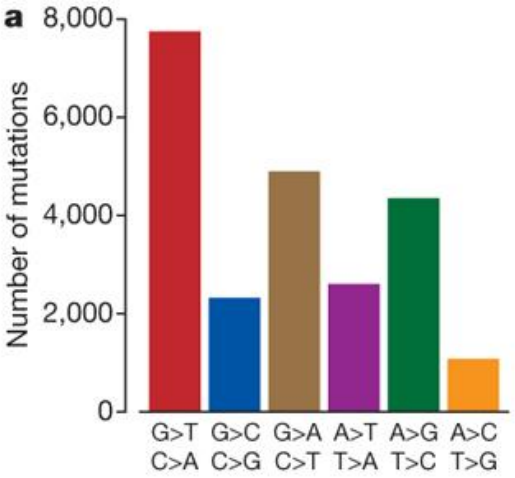
Mutation Signatures

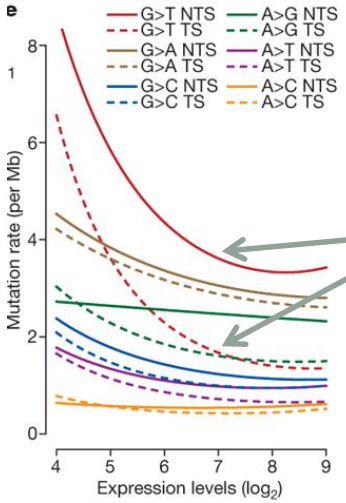
Thousands of '*passenger mutations'* in every cancer

PAH Form Bulky Adducts at Guanines



Tobacco smoke signature

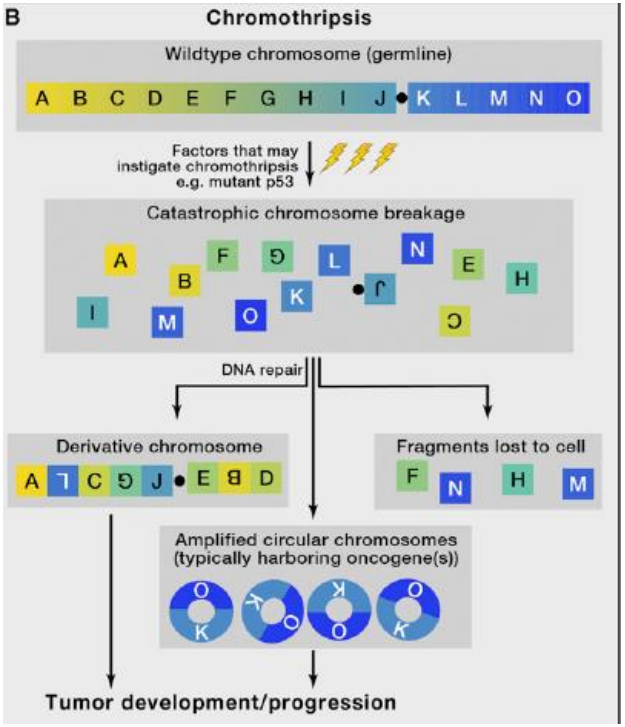


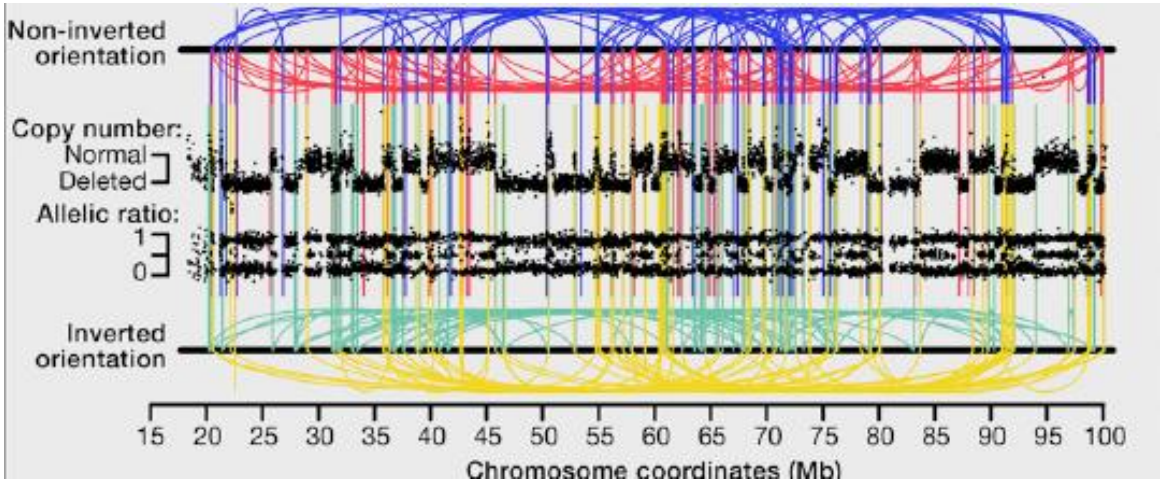


'Strand bias'

ED Pleasance *et al. Nature* **000**, 1-7 (2009) doi:10.1038/nature08629

Chromothripsis: ‘Chromosome shattering’





Korbel & Campbell, Cell, 2013

Whole genome profiling

Coding Mutations
- Silent: Grey
- Misssense: Purple
- Nonsense:Red
- Splice: Black

Heterozygous & homozygous Point mutations/10Mbp

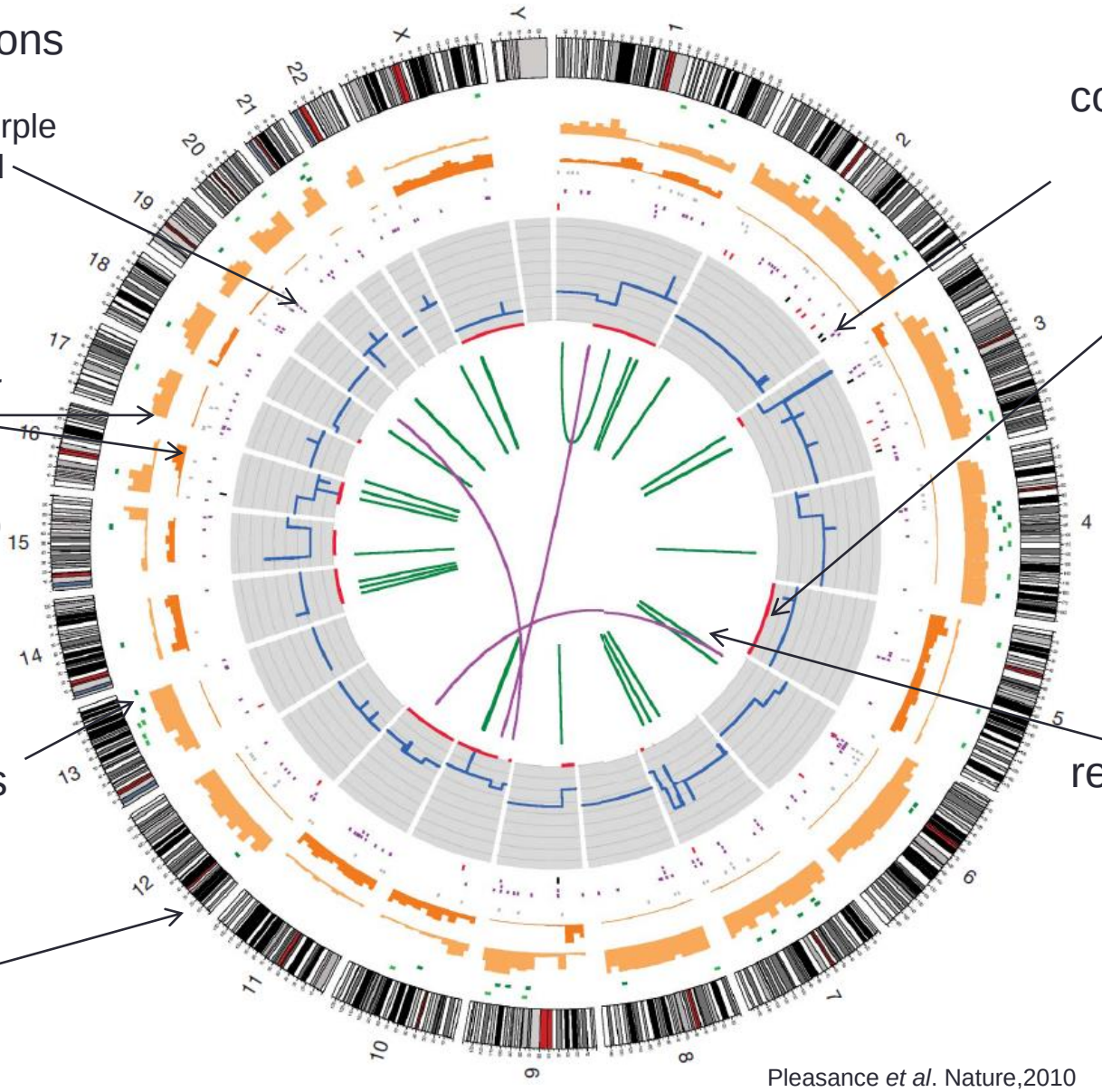
Small insertions and deletions

Chromosomal ideograms

copy number changes

Regions of LOH

genomic rearrangements



Pleasance *et al*. Nature,2010

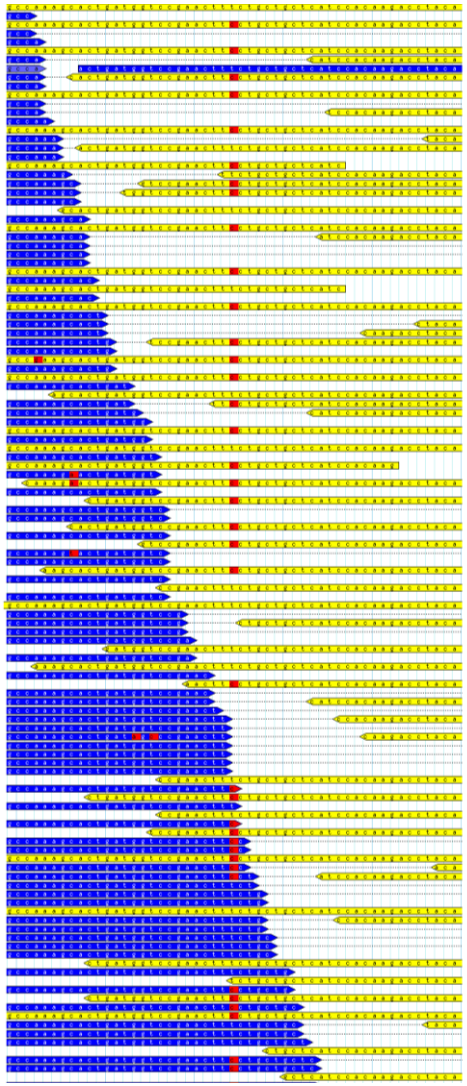
Some Applications of NGS

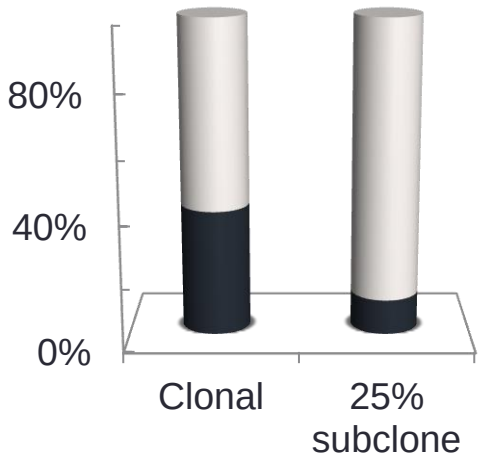
1. Discovering cancer genes

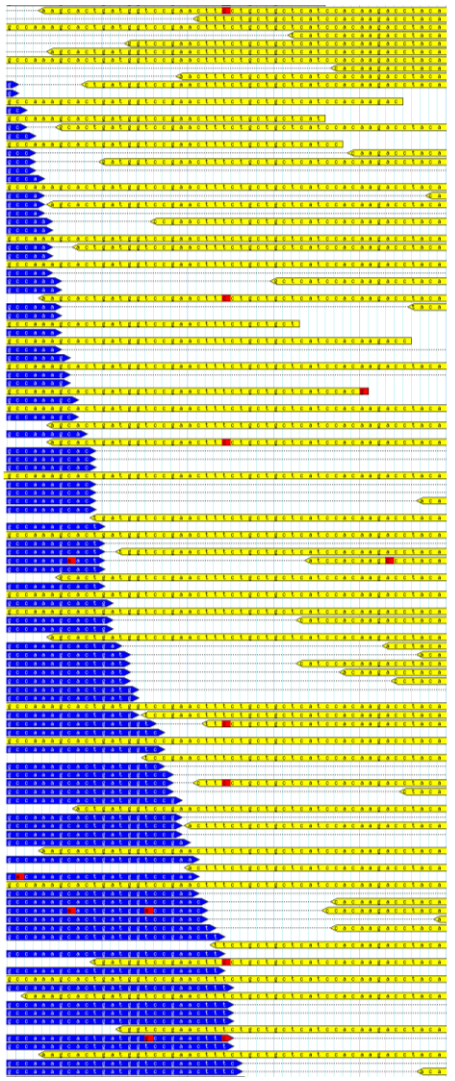
1. Understanding the complexity of cancer genomes

1. Studying cancer evolution

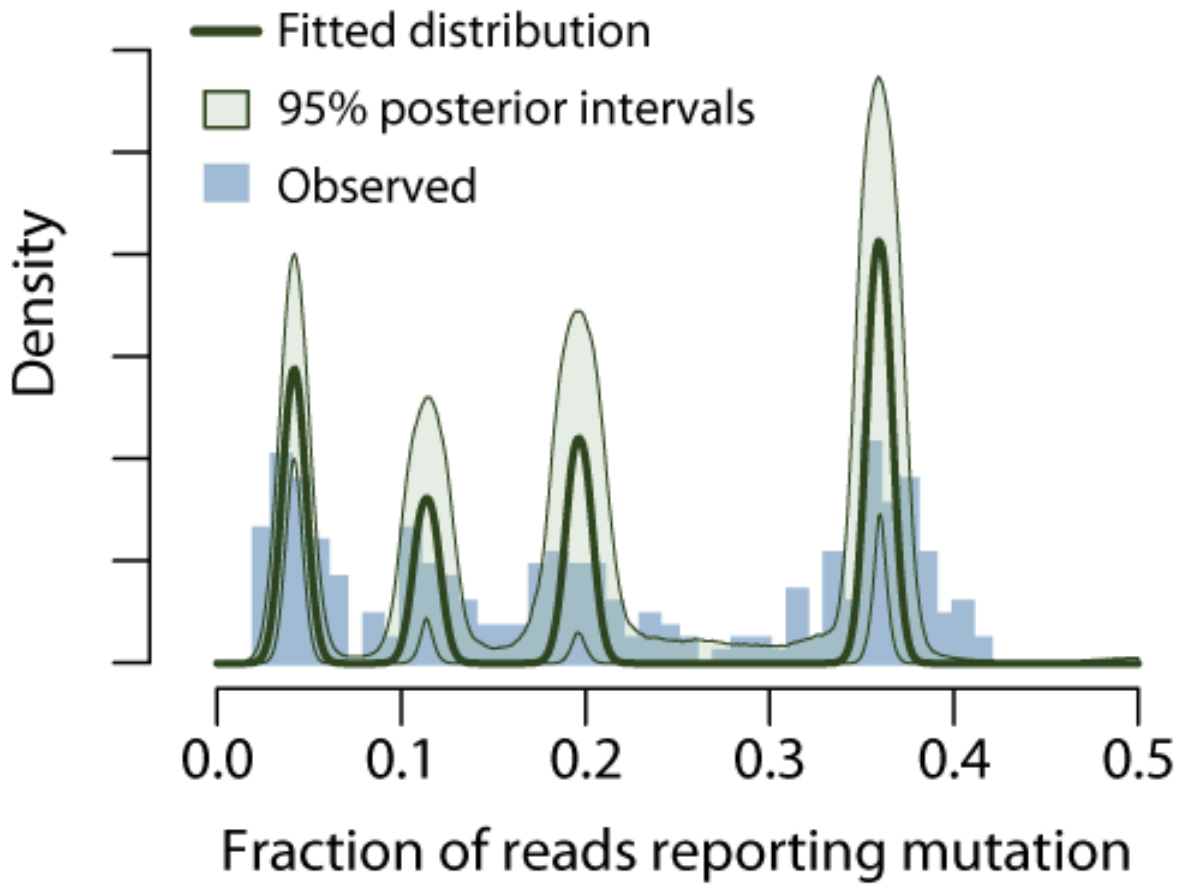
Variant allele fraction



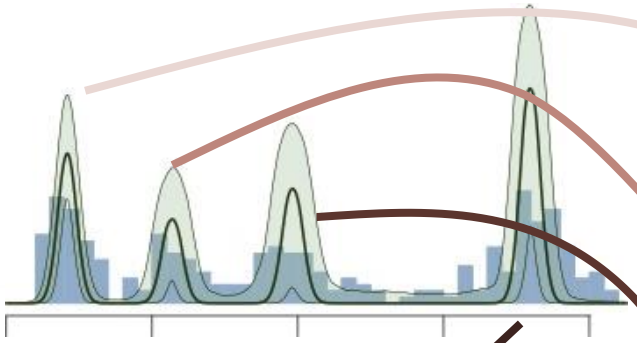




Clusters of point mutations



Phylogenetic tree



14% cells
Tetraploid with
- Loss of 2 copies of 7^{M}
- Loss of 2^{P} and 2^{M}
- Loss of 6, 8, 9, 11, 12, 14, 15, 18 & 21

18% cells
11,000 muts

Most recent common ancestor

14% cells

Fertilised egg

100% cells
26,700 mutations
10 CN changes
PIK3CA, TP53, GATA3, SMAD4, NCOR1 muts

68% cells
Del 13 & few mutations

14% cells

65% cells
Del 13 & extra
15,600 muts

Summary

1. **What is NGS?**

Technology that permits the parallel sequencing of up to hundreds of thousands of nucleotide sequences

2. **NGS work-flow**

DNA fragmentation, cluster amplification, light detection, read alignment to reference genome

1. **Some applications of NGS in cancer research?**
    1. **Cancer driver discovery**

    Catalogues of cancer driver mutations & clinical trials

    1. **Genome-wide profiling of all mutation types in individual cancers**

    Point mutations, indels, structural variants and copy number changes

    1. **Cancer evolution**

    Quantitative nature of NGS permits reconstruction of evolutionary histories

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Useful references: Overviews

- Michael Metzker, Sequencing technologies — the next generation, *Nature Reviews Genetics*, 2010
- Meyerson, Stacey & Getz. Advances in understanding cancers through second-generation sequencing*, Nature Reviews Genetics*, 2010
- Yates & Campbell, Evolution of the Cancer genome*, Nature Reviews Genetics*, 2013