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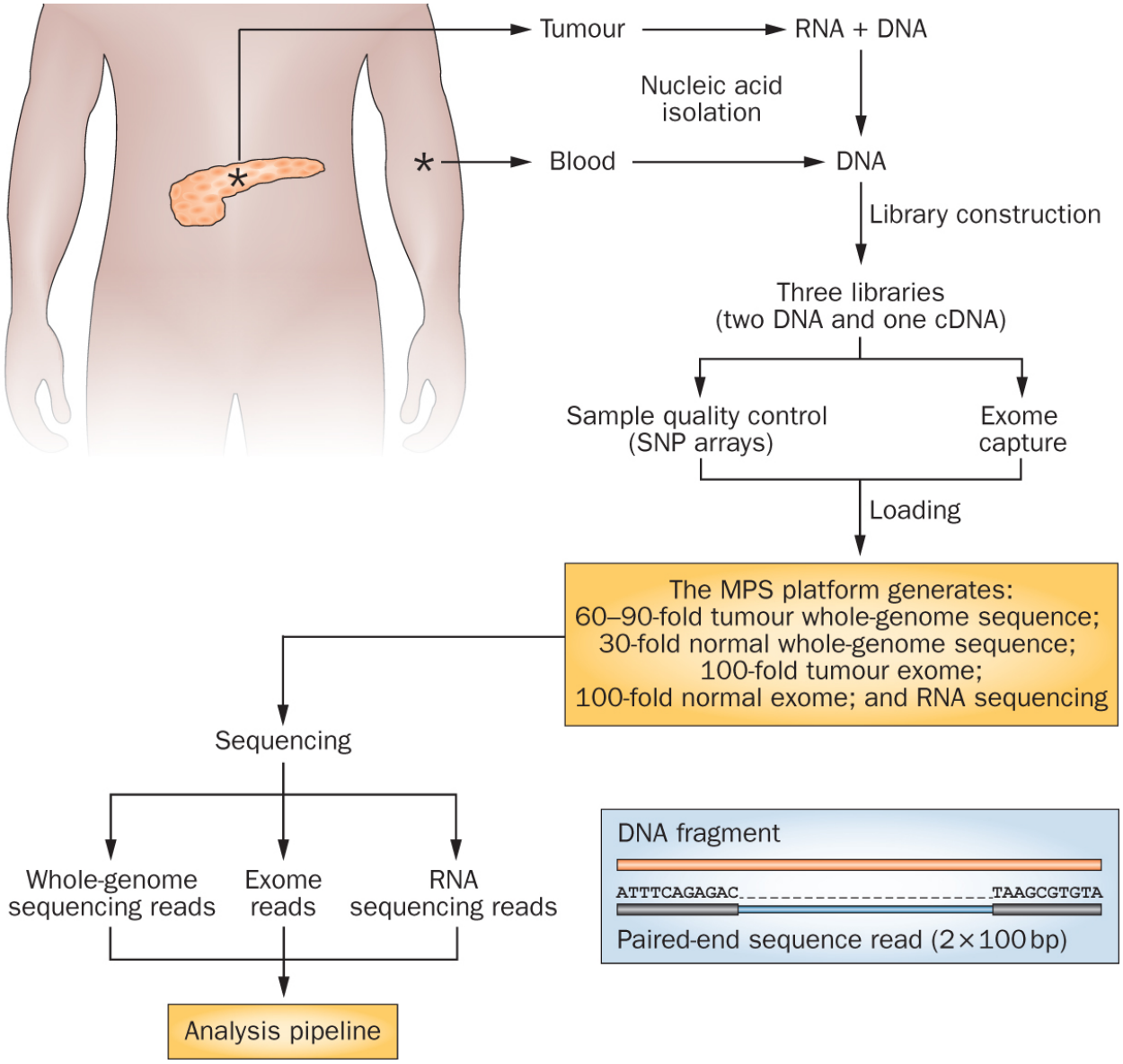
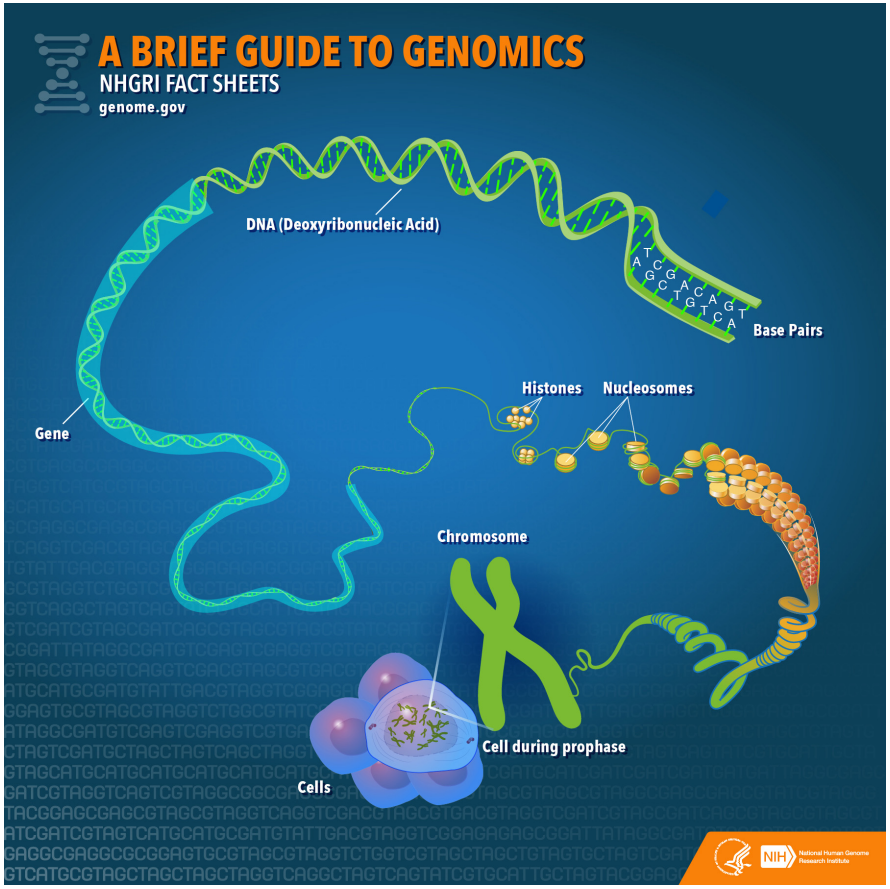
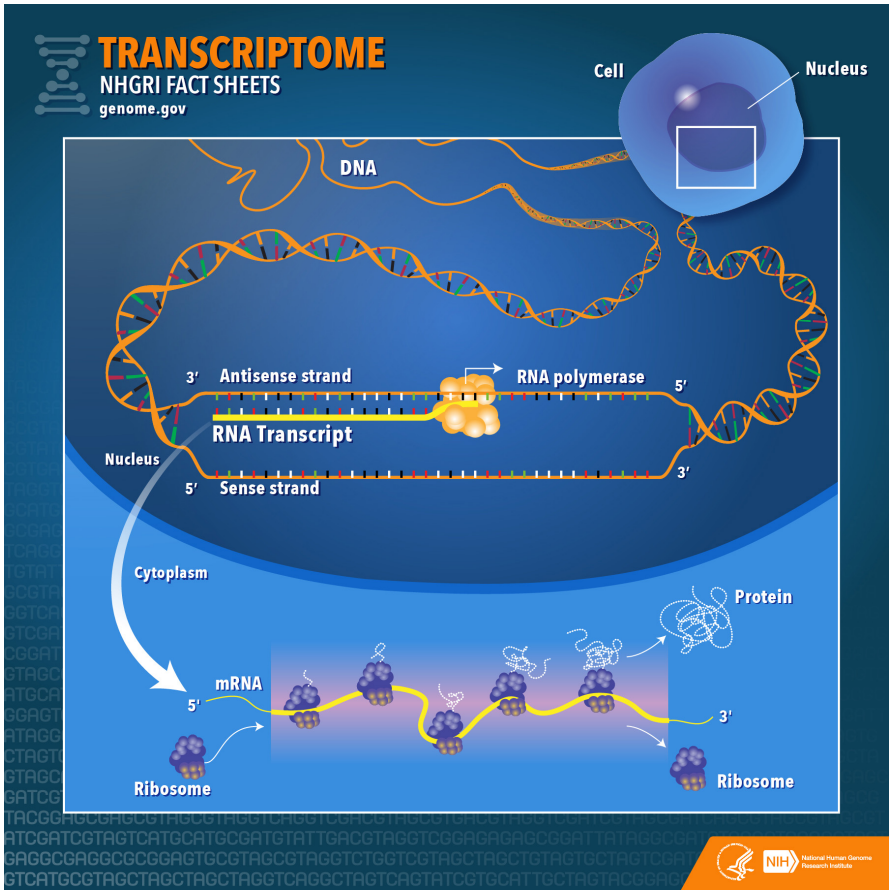
Cancer genomics data has exploded with rapid advances in sequencing technologies

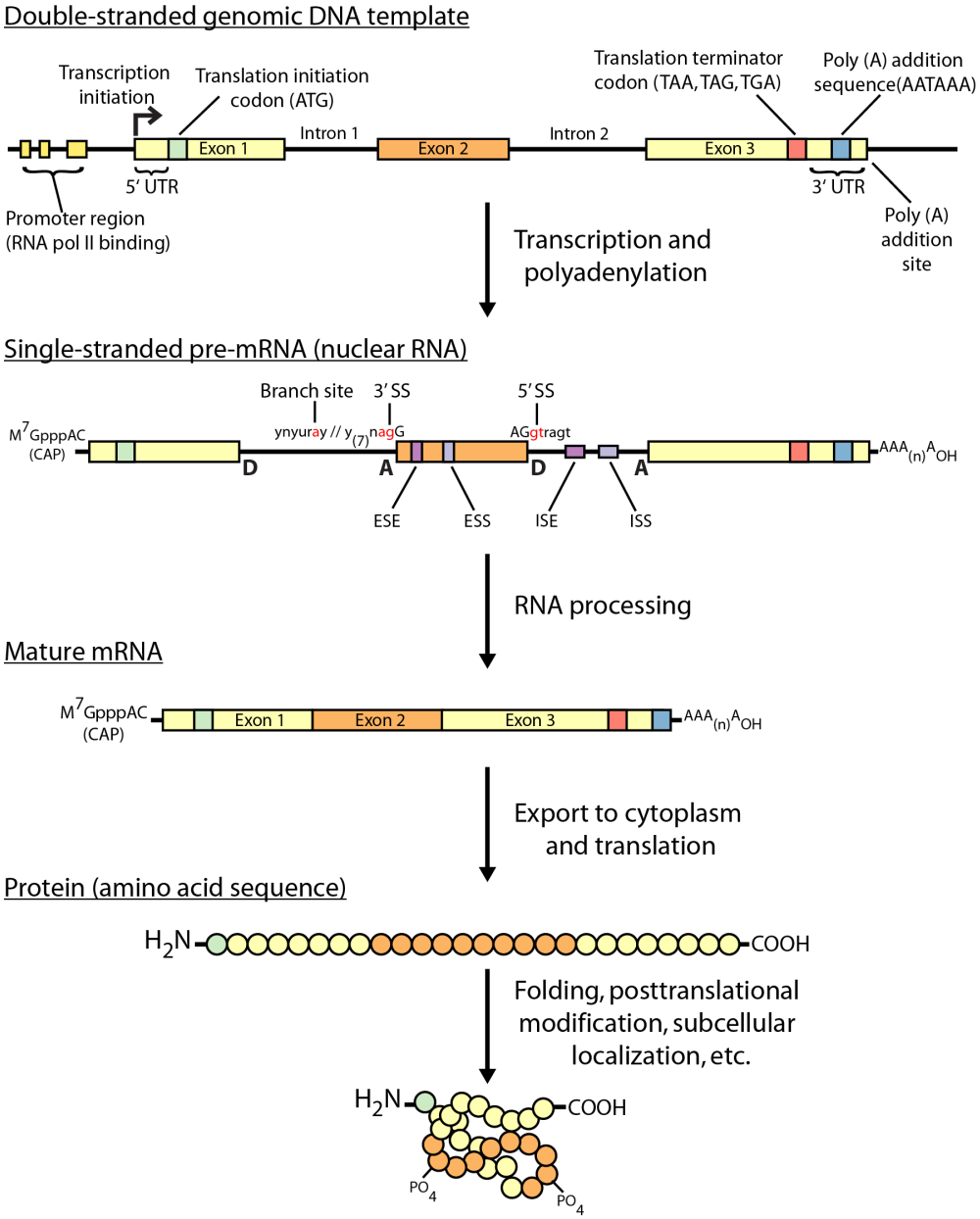


Genomes and transcriptomes

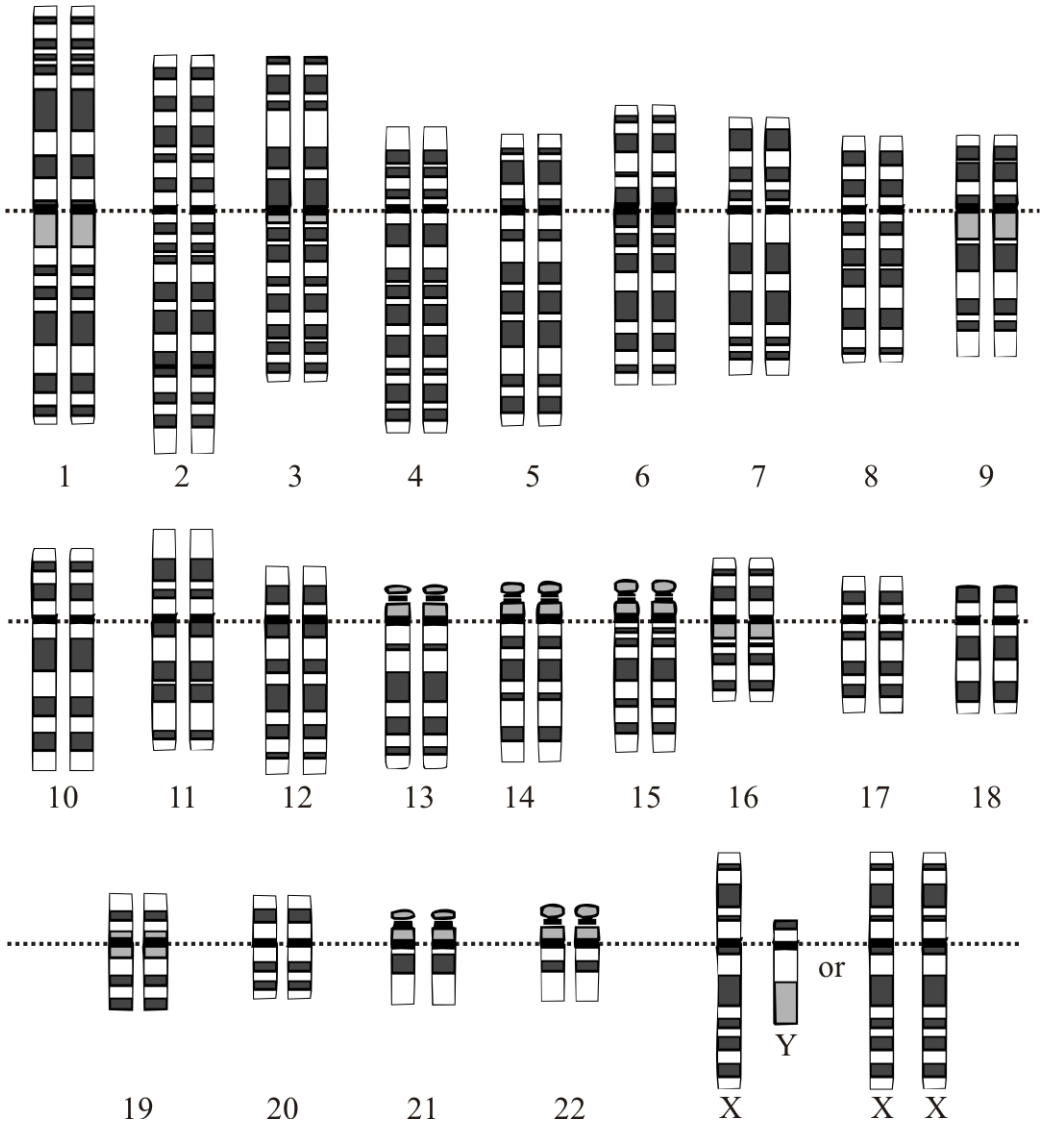




The Central Dogma

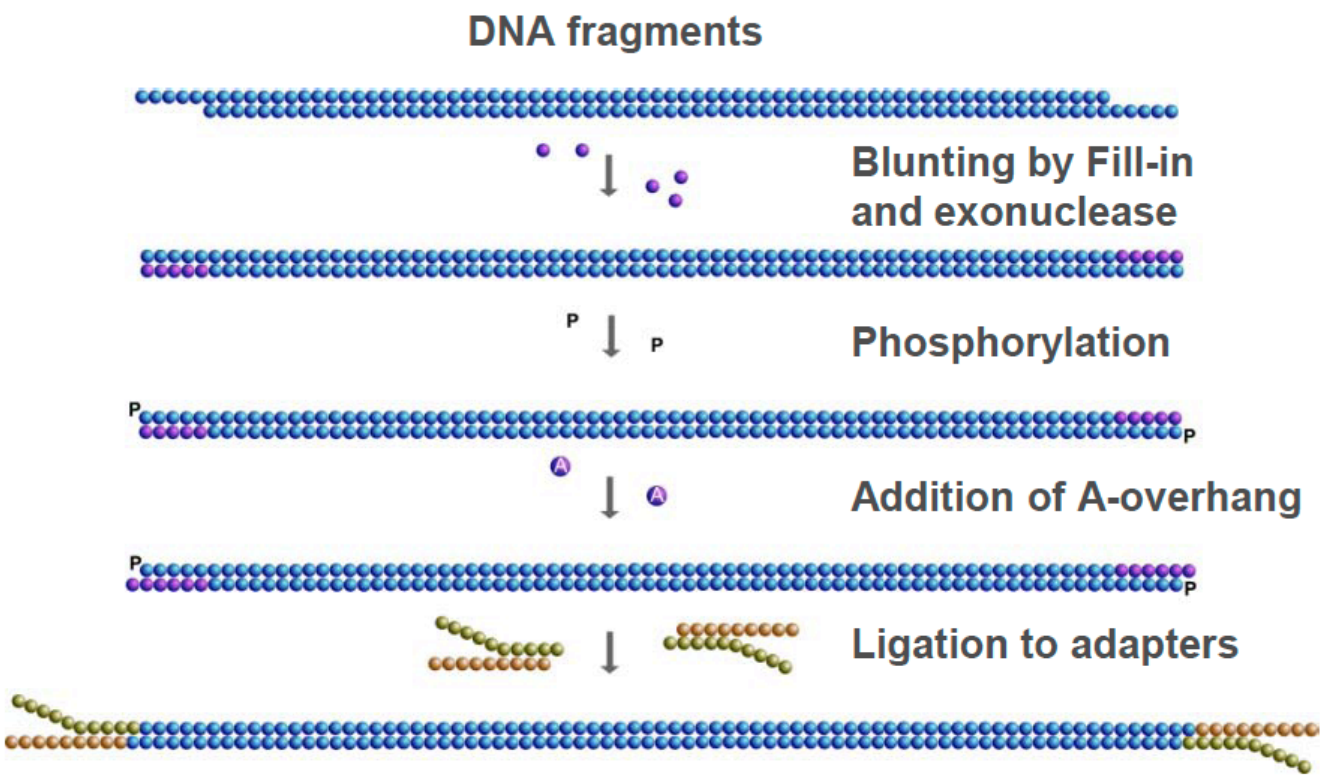


Human chromosomes (karyotype)



Data Generation

Library Construction for MPS



- Shear high molecular weight DNA with sonication
- Enzymatic treatments to blunt ends
- Ligate synthetic DNA adapters (each with a DNA barcode), PCR amplify
- Quantitate library
- Proceed to WGS, or perform exome or specific gene hybrid capture

Slide courtesy of Elaine Mardis (Nationwide Children's Hospital)

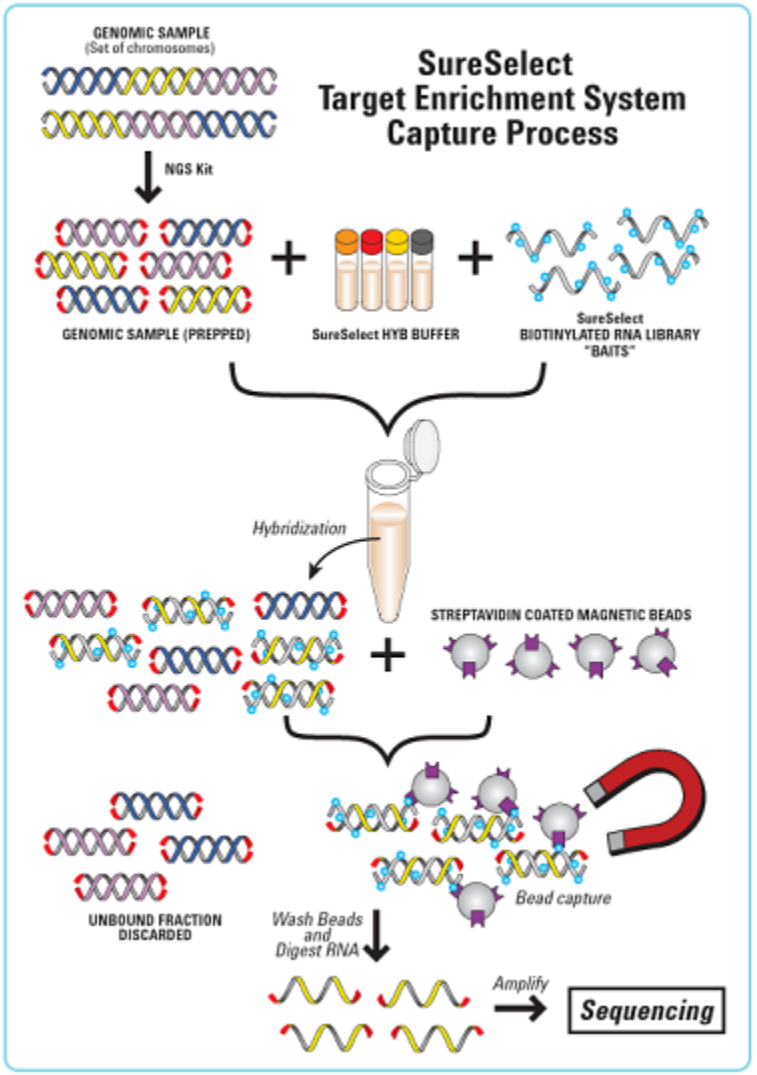
PCR-related Problems in MPS

- PCR is an effective vehicle for amplifying DNA, however…
- In MPS library construction, PCR can introduce preferential amplification ("jackpotting") of certain fragments
  - Duplicate reads with exact start/stop alignments
  - Need to "de-duplicate" after alignment and keep only one pair
  - Low input DNA amounts favor jackpotting due to lack of complexity in the fragment population
- PCR also introduces false positive artifacts due to substitution errors by the polymerase
  - If substitution occurs in early PCR cycles, error appears as a true variant
  - If substitution occurs in later cycles, error typically is drowned out by correctly copied fragments in the cluster
- Cluster formation is a type of PCR ("bridge amplification")
  - Introduces bias in amplifying high and low G+C fragments
  - Reduced coverage at these loci is a result

Slide courtesy of Elaine Mardis (Nationwide Children's Hospital)

Hybrid Capture

- **Hybrid capture** - fragments from a whole genome library are selected by combining with probes that correspond to most (not all) human exons or gene targets.
- The probe DNAs are biotinylated, making selection from solution with streptavidin magnetic beads an effective means of purification.
- An "**exome**" by definition, is the exons of all genes annotated in the reference genome.
- **Custom capture reagents** can be synthesized to target specific loci that may be of clinical interest.



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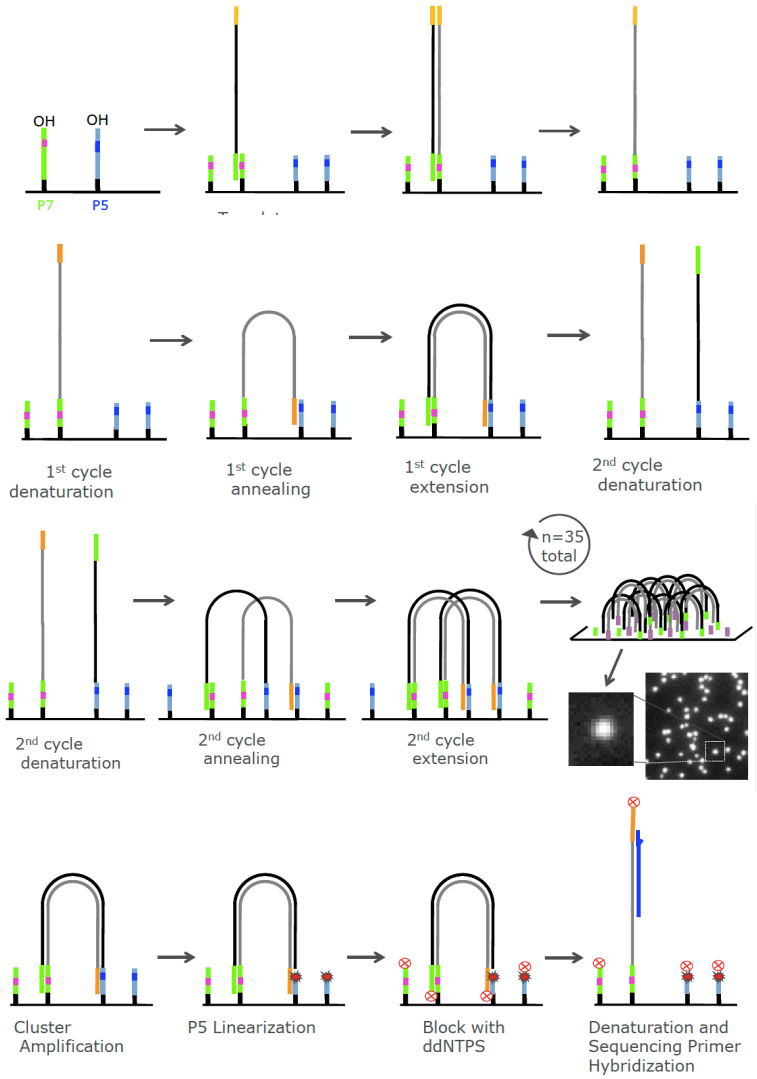
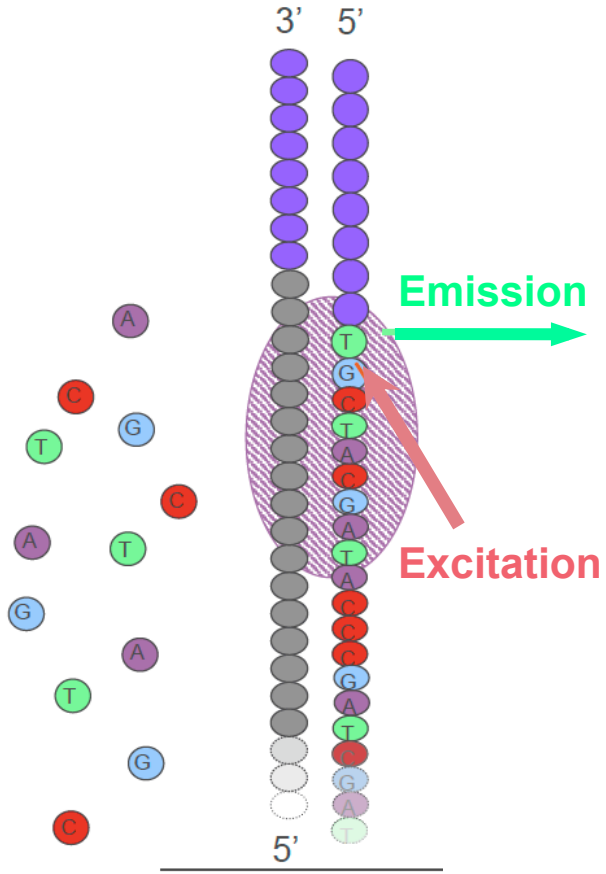
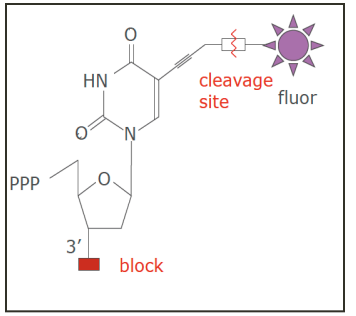
Massively Parallel Sequencing by Synthesis







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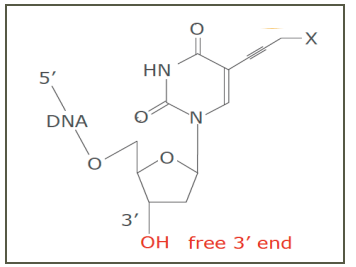

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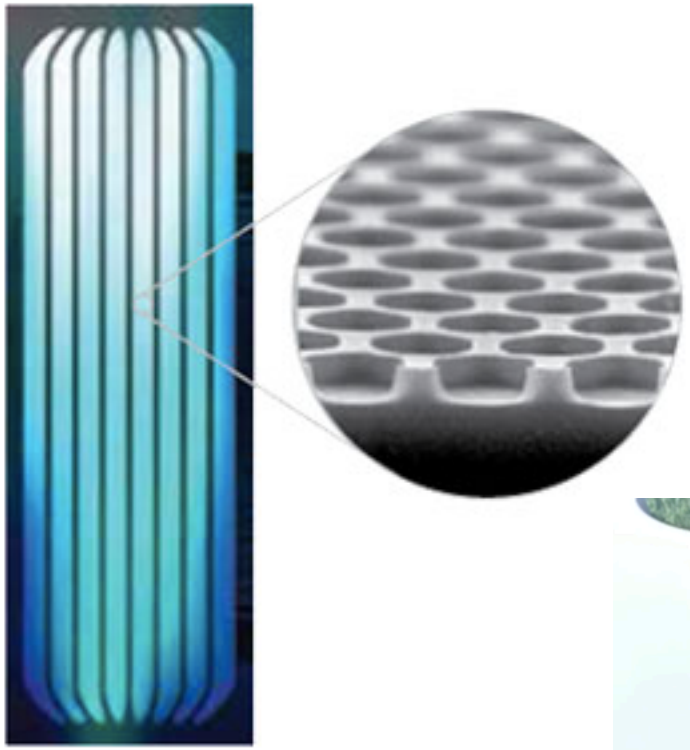
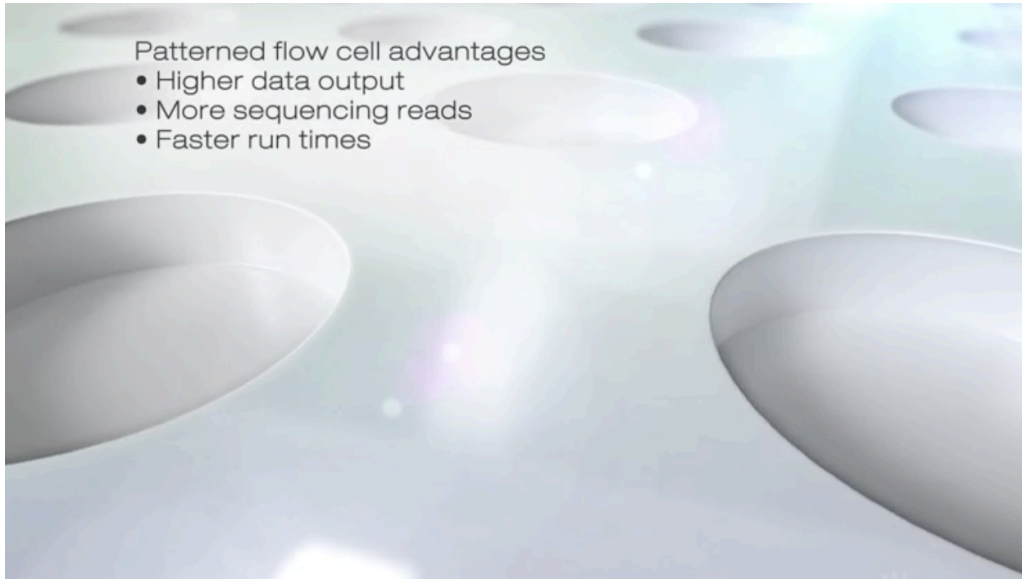
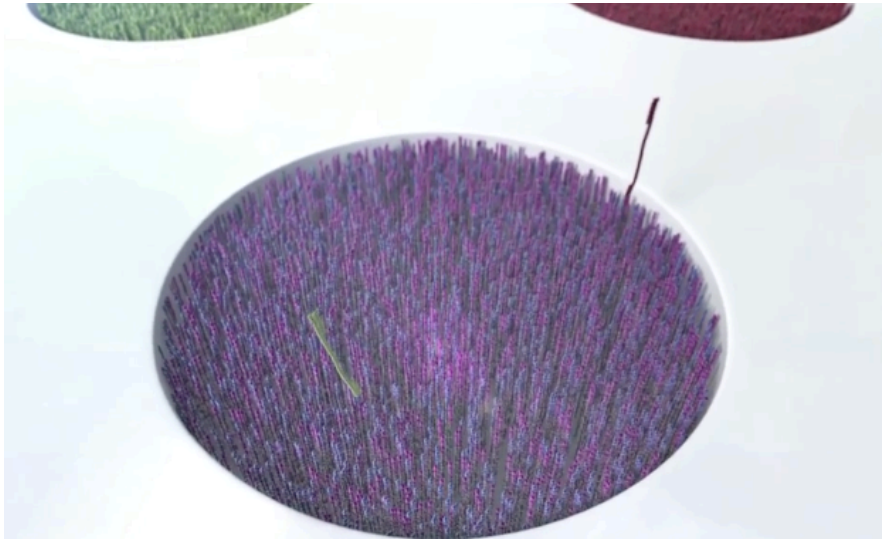
Illumina Patterned Flow Cell







Slide courtesy of Elaine Mardis (Nationwide Children's Hospital)

Platforms: Illumina

	NextSeq*	HiSeq 4000*	NovaSeq 6000††*	HiSeq X Ten†
Output Range	20-120 Gb	125-1500 Gb	167-6000 Gb	900-1800 Gb
Run Time	11-29 hr	<1-3.5 days	19-40 hr	< 3 days
Reads per Run	130-400 million	2.5-5 billion	1.4-20 billion	3-6 billion
Maximum Read Length	2 x 150 bp	2 x 150 bp	2 x 150 bp	2 x 150 bp
Samples per Run†	1	6-12	4-48	8-16
Relative Price per Sample†	Lower Cost	Lower Cost	Lower Cost	Lower Cost
Relative Instrument Price†	Higher Cost	Higher Cost	Higher Cost	Higher Cost

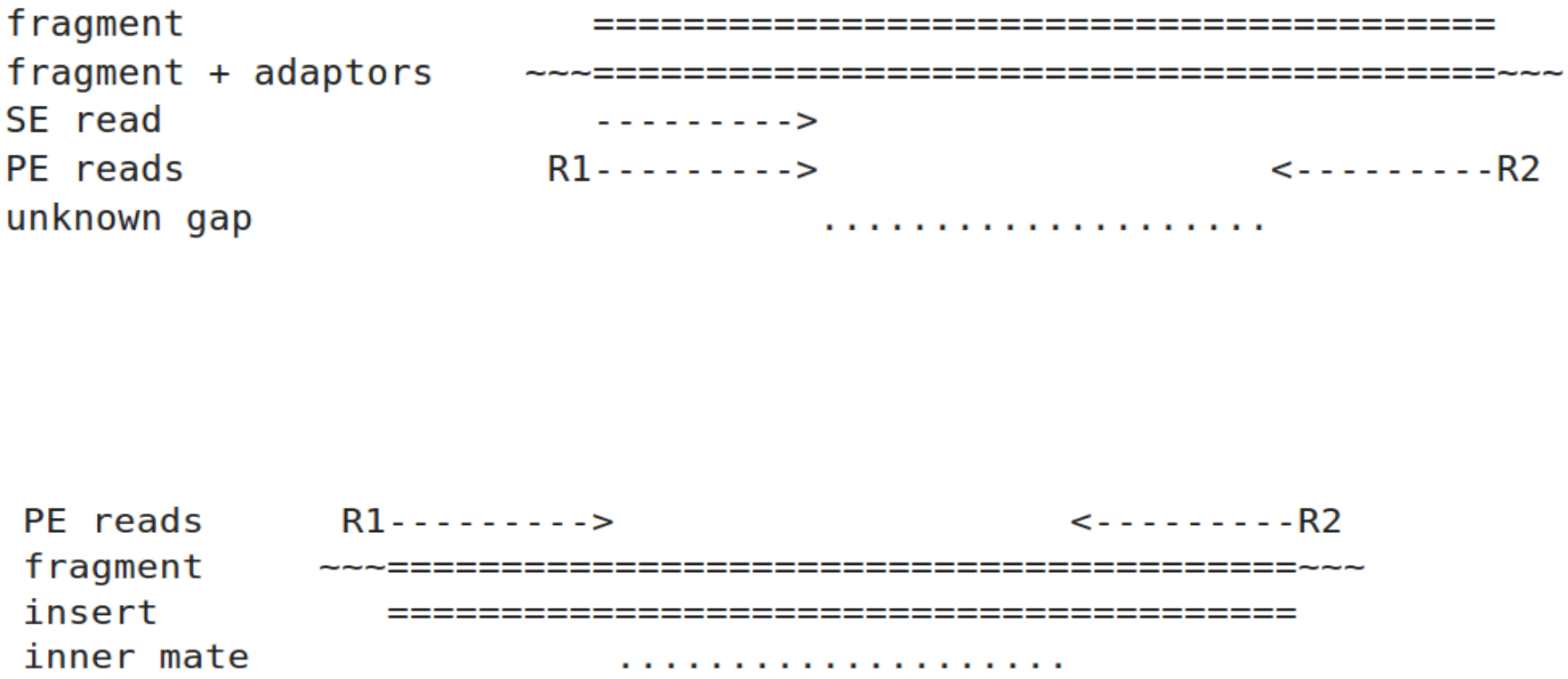
- High accuracy, range of capacity and throughput
- Longer read lengths on some platforms (MiSeq)
- Improved kits, improved software pipeline and capabilities, cloud computing in BaseSpace

Slide courtesy of Elaine Mardis (Nationwide Children's Hospital)

Our reference genome

- All reference files were obtained from the 1000 genomes project
  - The GRCh38 build of the human genome is used
  - This is the latest version of the human reference
- For the tutorial, two chromosomes are used (chr. 6 and chr. 17)
  - The reason for this is to reduce run time for the tutorial
  - Performing this analysis on the complete genome reference would require only minor modification of the commands
    - Would also require more storage, compute resources, and time

Insert size terminology explained



http://thegenomefactory.blogspot.ca/2013/08/paired-end-read-confusion-library.html