

Genomic and Precision Medicine

Week 3: Next-gen sequencing for solving
diagnostic dilemmas



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advancing health worldwide™

Lecture 3

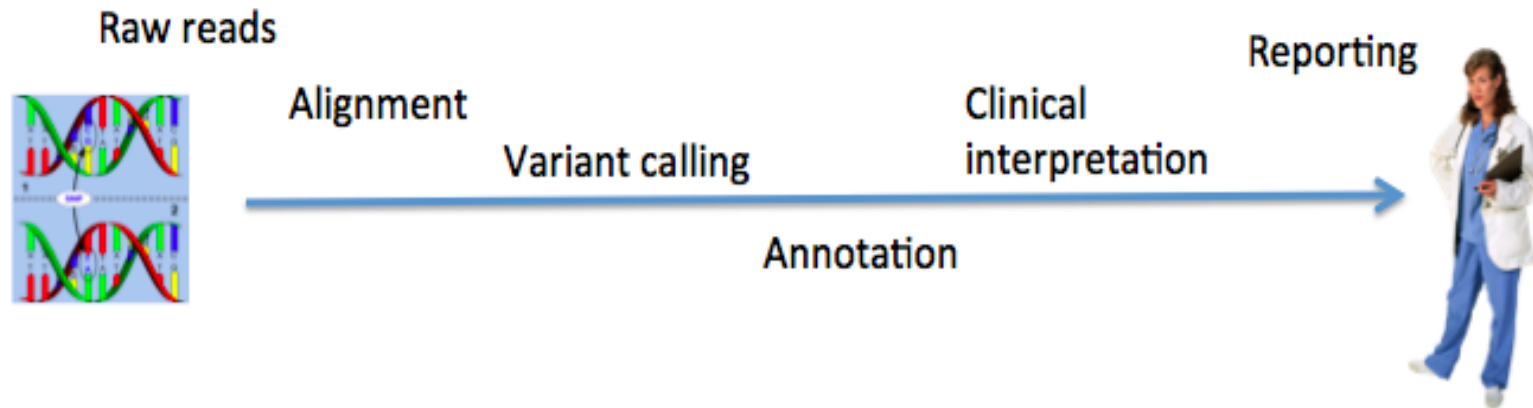
- Module 1: Whole Genome Analysis
- Module 2: Clinical interpretation of variants
- Module 3: Using NGS for diagnostic dilemmas
- Module 4: Practical issues

Module 1: Whole Genome Analysis

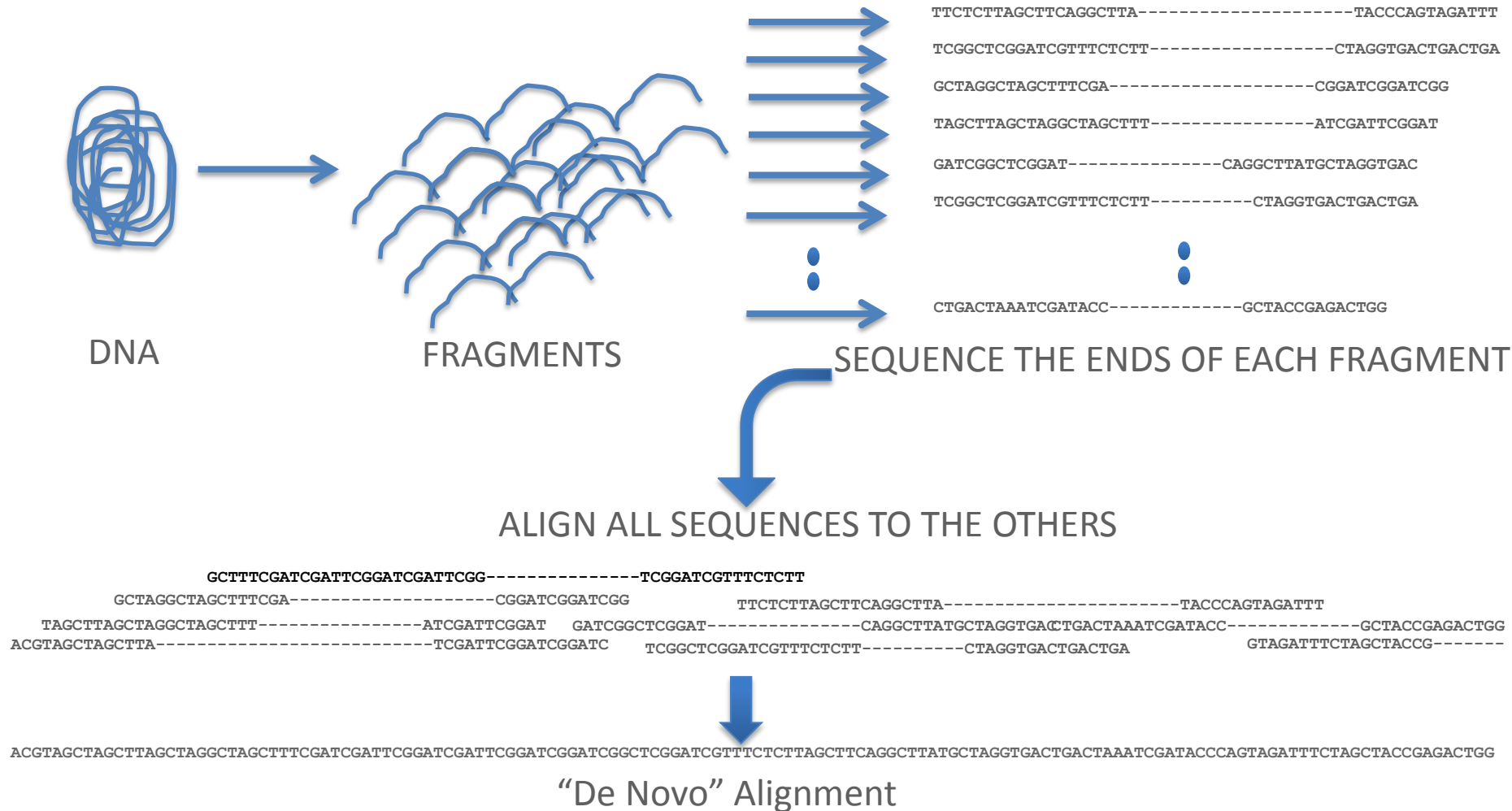
Whole Genome Analysis

- A genome-wide search for disease-causing variants
 - Karyotype – Chromosomes under the microscope
 - Cytogenomic Arrays for large deletions/duplications
 - Whole Exome Sequencing (WES)
 - Whole Genome Sequencing (WGS)

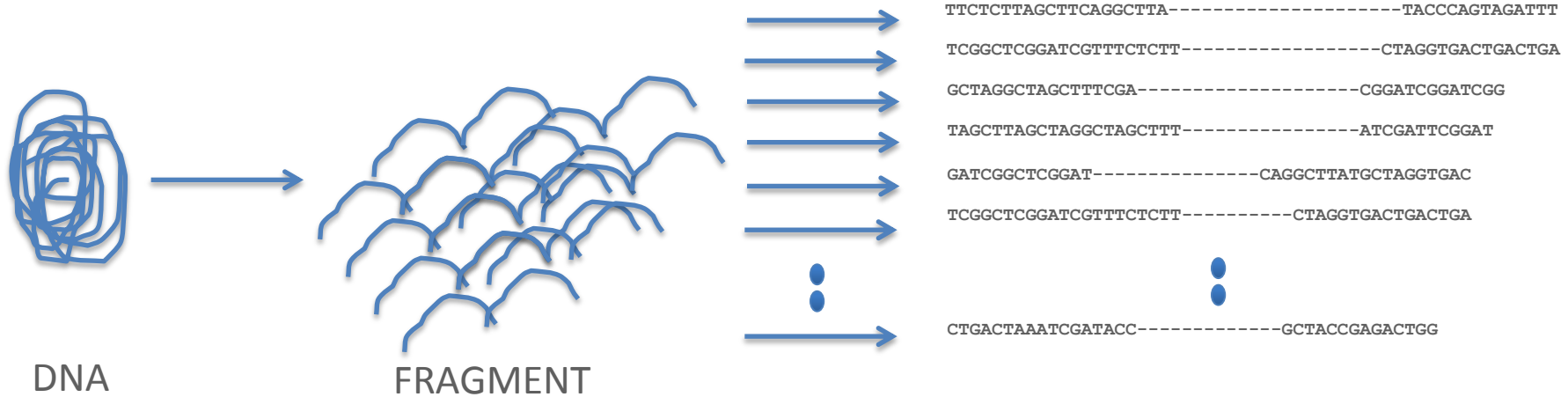
Going From Sequence to Clinical Use



Sequencing



Resequencing



ALIGN EACH SEQUENCE TO THE REFERENCE

Reference Sequence

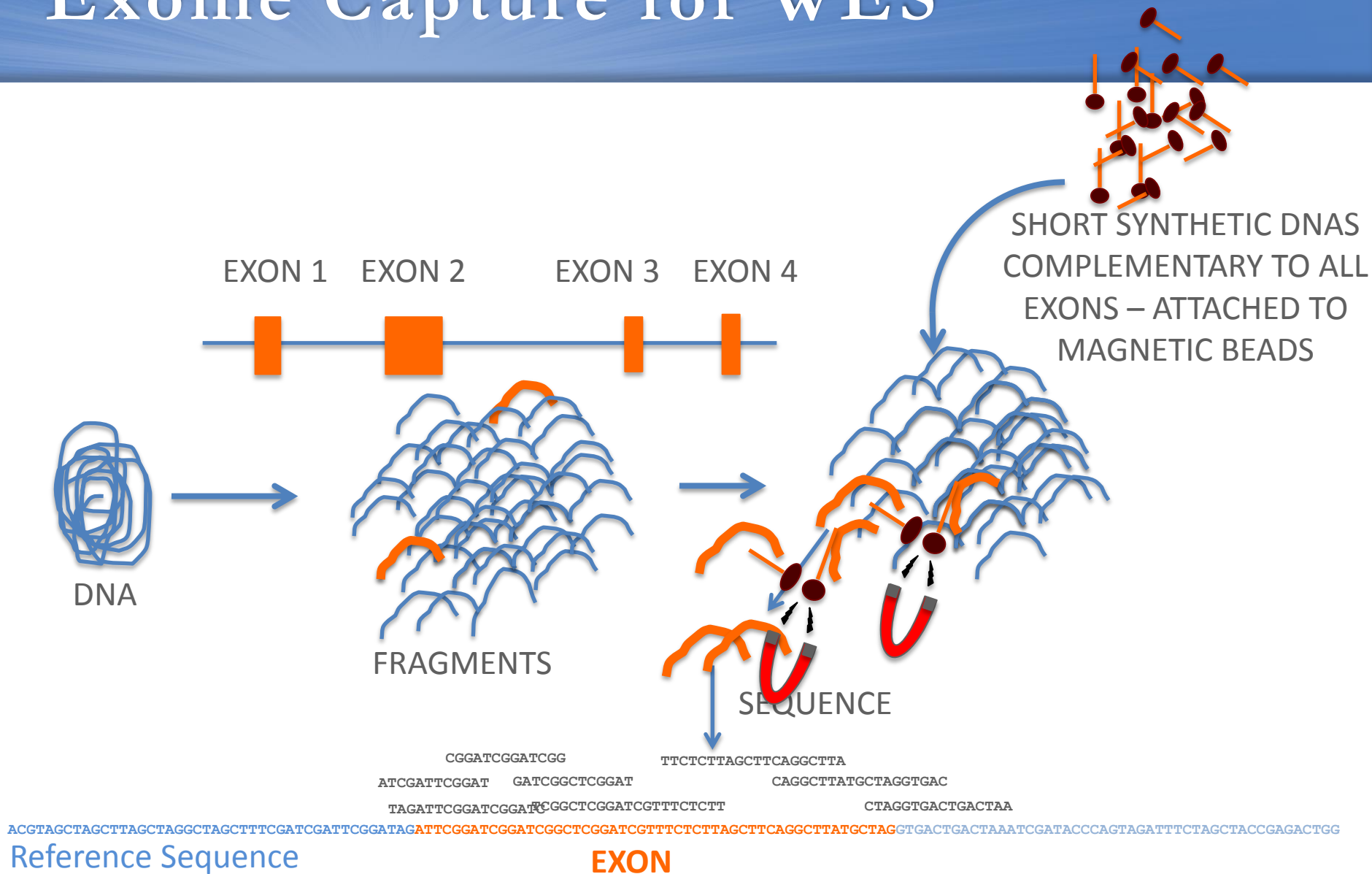
GCTTTCGATCGATTTCGGATCGATTTCGG
 GCTAGGCTAGCTTTCGA
 TAGCTTAGCTAGGCTAGCTTT
 ACGTAGCTAGCTTA
 ACGTAGCTAGCTTAGCTAGGCTAGCTTTCGATCGATTTCGGATCGATTTCGGATCGGATCGGCTCGGATCGTTTCTCTTAGCTTCAGGCTTATGCTAGGTGACTGACTAAATCGATACCCAGTAGATTTCTAGCTACCGAGACTGG

TCGGATCGTTTCTCTT
 CGGATCGGATCGG
 ATCGATTCGGAT
 TCGATTTCGGATCGGATTCGGCTCGGATCGTTTCTCTT
 GATCGGCTCGGAT
 TTCTCTTAGCTTCAGGCTTA
 CAGGCTTATGCTAGGTGAC
 CTAGGTGACTGACTAA
 TACCCAGTAGATTT
 GCTACCGAGACTGG

There is No Single “Human Genome”

- There is no “normal” or “control” human genome, there are billions of different genomes
- To provide some sort of standard, a “reference genome” was constructed as a consensus among multiple sequences
- Any person’s genome differs from the reference at millions of sites, ranging from single nucleotide differences up to hundreds of thousands, even millions of base pairs
- Reference still has gaps in regions where no sequence could be obtained

Exome Capture for WES



Whole genome vs whole exome sequencing

Why study just the exome?

- More predictable effect of mutations
- >85% of known mutations for rare Mendelian disorders occur in the exome
- Cheaper, faster and easier to analyze just 2% rather than the entire genome

What WES can reliably detect

- Small variants (SNVs or small indels) - Read Depth!
- Some CNVs
- Not larger indels or trinucleotide repeats
- Exon deletions are hit-or-miss using depth of coverage measures

WARNING: The technology is evolving rapidly and new advances will change this current snapshot

Read depth (coverage)

CCCACATCTTCTCCATCTCCGACAACGCCTATCAGTACATGCTGACAGGTGAAGGCCCTGGA Reference
GACAACGCCTATCAGTACATGCTGACAGGTGAA
CCATCTCCGACAACGCCTGTCAGTACATGCTGACAGGTGAAGGCCCTGGA
ATCTTCTCCATCTCCGACAACGCCTATCAGTAC

- Would you believe this person is heterozygous (A/G) for a variant with a read depth of 3?

How about Now?



CCCACATCTTCTCCATCTCCGACAACGCCTATCAGTACATGCTGACAGGTGAAGGCCCTGGATTTTGCA

Reference

GACAACGCCTATCAGTACATGCTGACAGGTGAA
CCATCTCCGACAACGCCTGTCAGTACATGCTGACAGGTGAAGGCCCTGGA
ATCTTCTCCATCTCCGACAACGCCTGTCAGTAC

CCGACAACGCCTATCAGTACATGCTGACAGGT
TCTCCGACAACGCCTGTCAGTACATGCTGACAGGTGAAGGCCCTGGATTT
ATCTTCTCCATCTCCGACAACGCCTATCAGTAC

CCGACAACGCCTGTCAGTACATGCTGACAGGT
TCTCCGACAACGCCTGTCAGTACATGCTGACAGGTGAAGGCCCTGGATTTTGC
TTCTCCATCTCCGACAACGCCTATCAGTACATGCTGACA

9/18 G

9/18 A

CCGACAACGCCTATCAGTACATGCTGACAGGT
TCTCCGACAACGCCTGTCAGTACATGCTGACAGGTGAAGGCCCTGGATTT
ATCTTCTCCATCTCCGACAACGCCTATCAGTAC

GACAACGCCTATCAGTACATGCTGACAGGTGAA
CCATCTCCGACAACGCCTGTCAGTACATGCTGACAGGTGAAGGCCCTGGA
ATCTTCTCCATCTCCGACAACGCCTGTCAGTAC

CCGACAACGCCTATCAGTACATGCTGACAGGT
TCTCCGACAACGCCTGTCAGTACATGCTGACAGGTGAAGGCCCTGGATTTTGC
TTCTCCATCTCCGACAACGCCTATCAGTACATGCTGACA