GBM Mutation Analysis and New Sequencing Technologies

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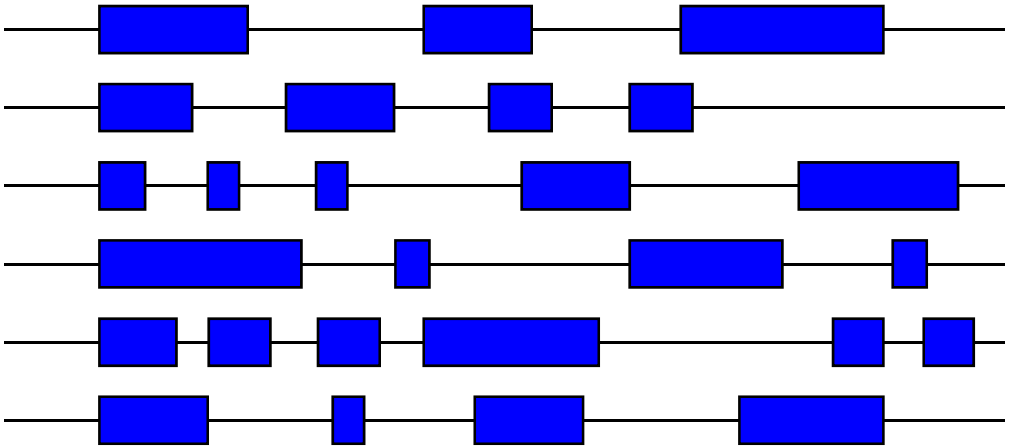
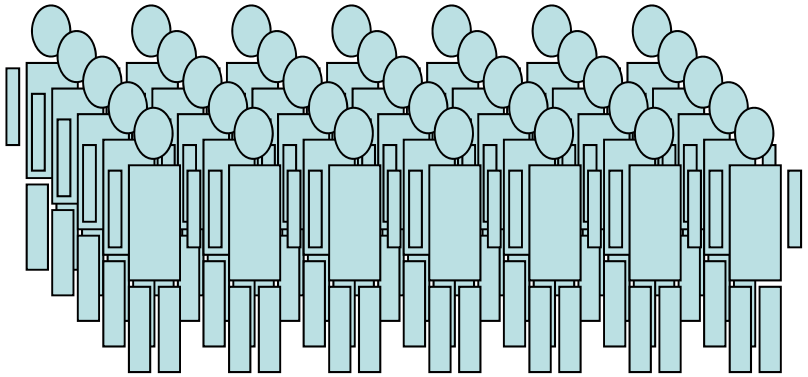
Targeted re-sequencing in TCGA

THE CANCER GENOME ATLAS

~1500 genes of interest





~200 GBM tumors

(& matching normals)

- **Sequencing Centers: BCM-HGSC, BI, WUGSC**
- **Funded by NCI & NHGRI**

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Summary of Mutations in GBM

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- **A total of 90 Mbp sequenced**
- **A total of 454 somatic non-synonymous mutations identified**
- **94 GBM tumors sequenced: mutations found in 85 tumors**
- **601 candidate genes sequenced: mutations found in 233 genes**
- **A total of 13 recurrent sites identified: 4 are novel**

Types	Missense	Nonsense	Splice_site	Insertions	Deletions
# of mutations	356	37	23	1	36

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Significantly Mutated Genes in GBM

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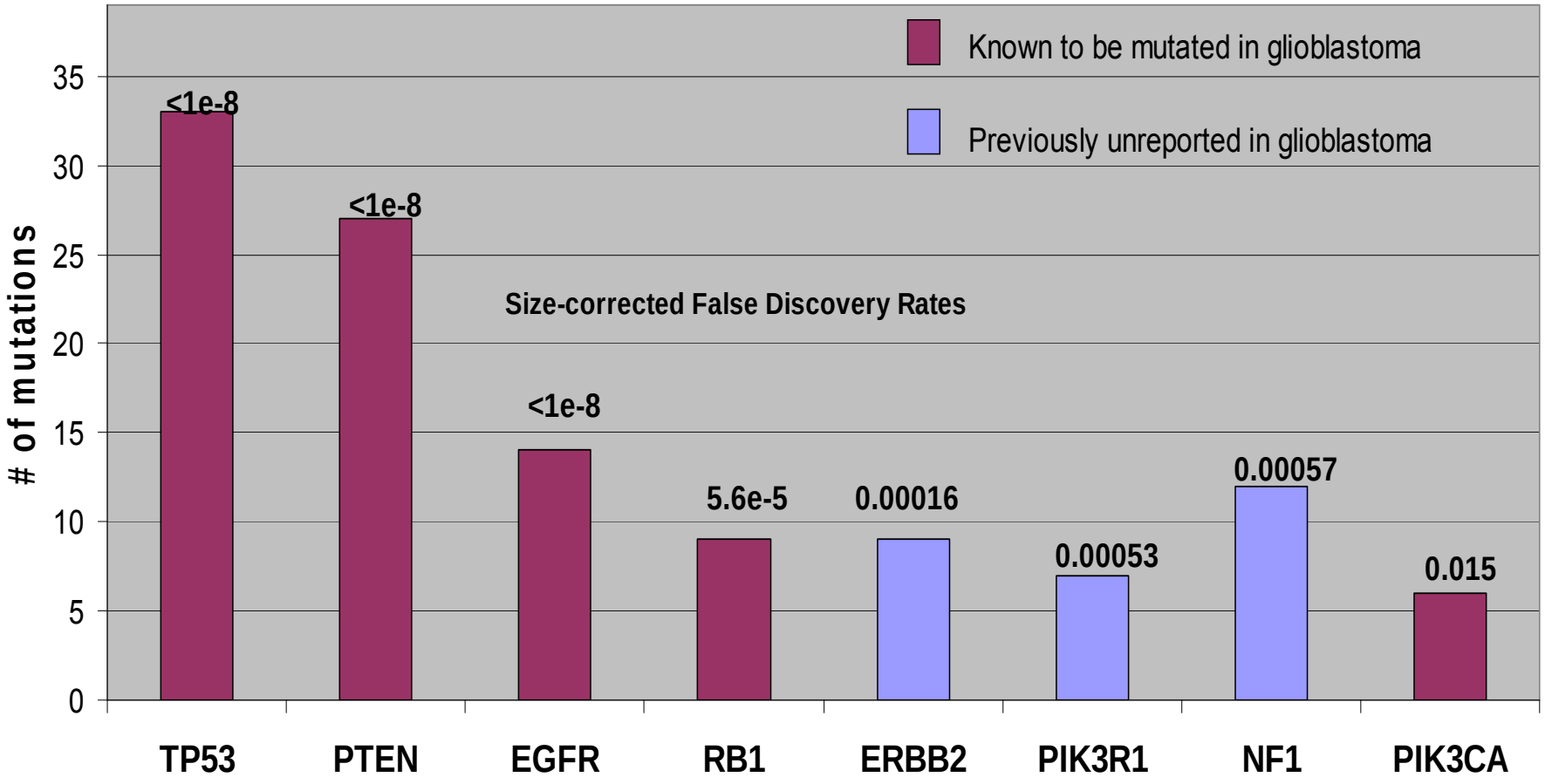

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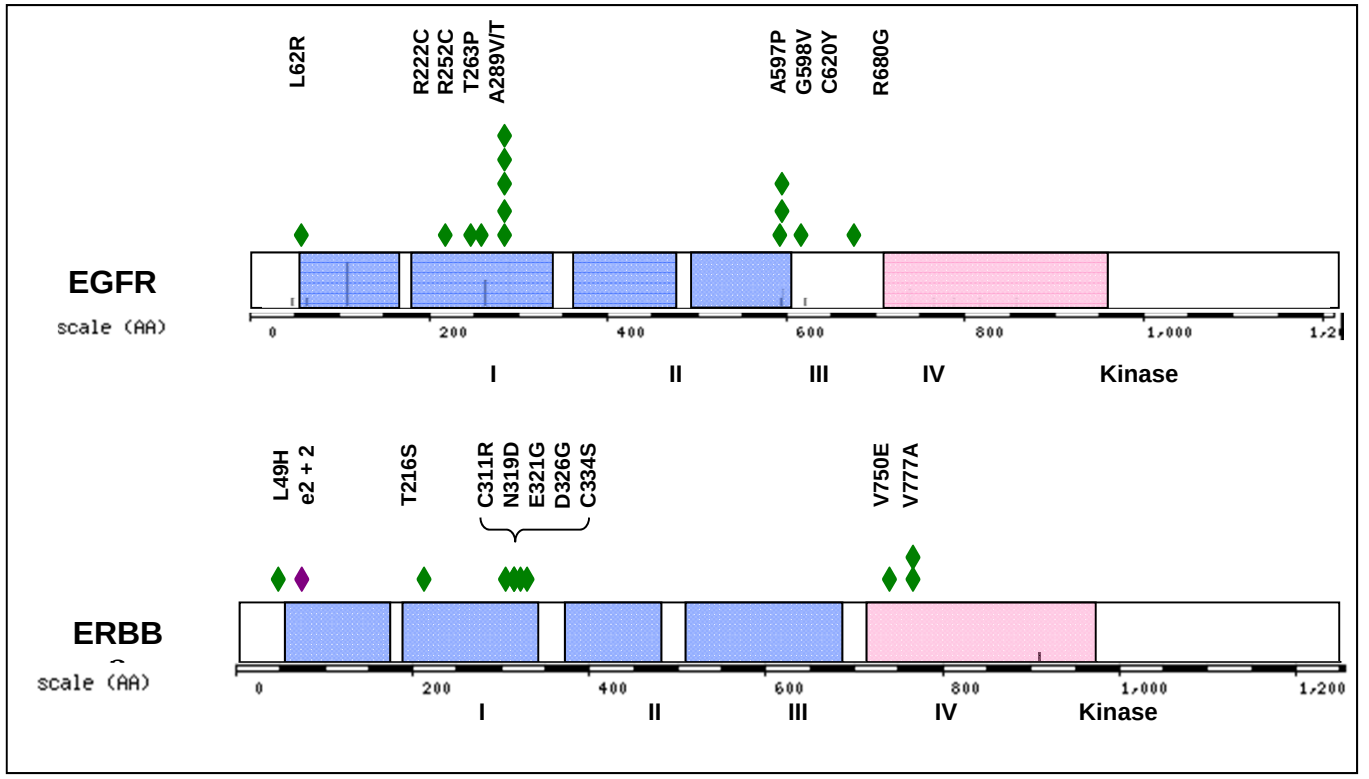
Mutations in GBM



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Mutations in GBM

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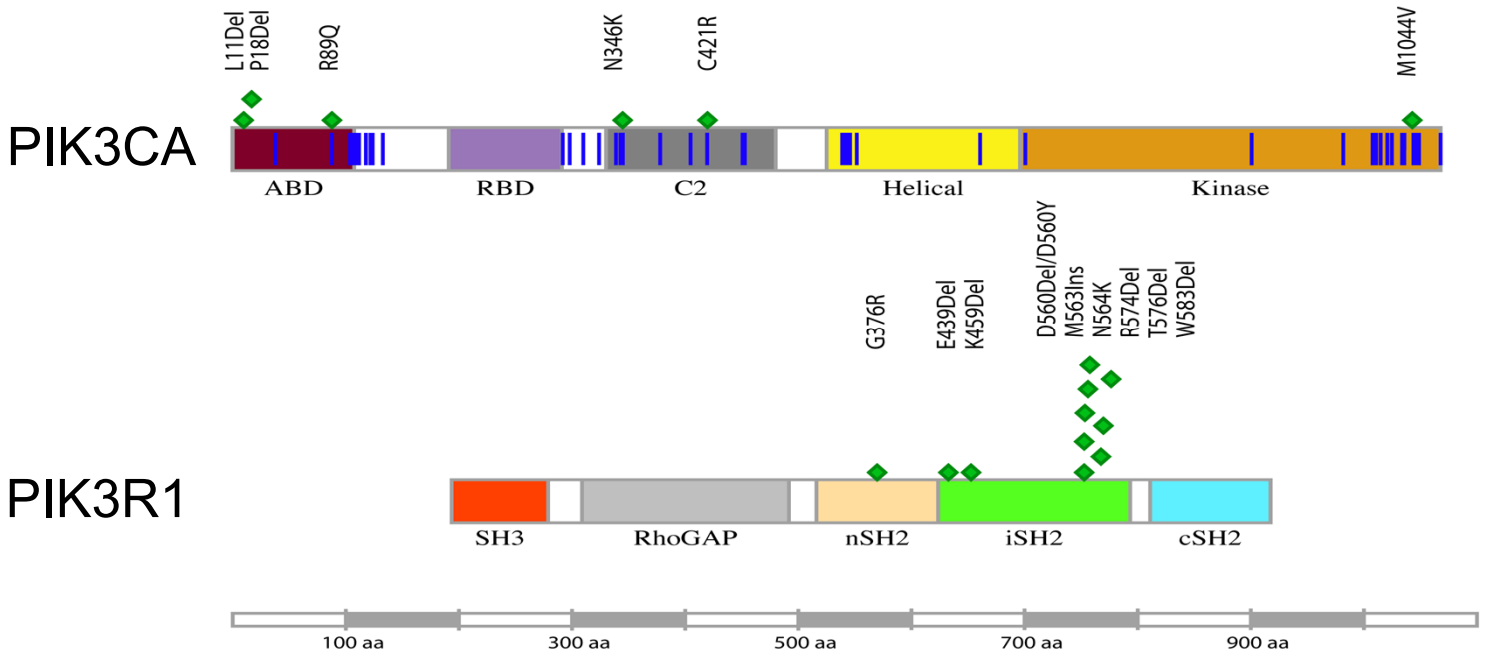

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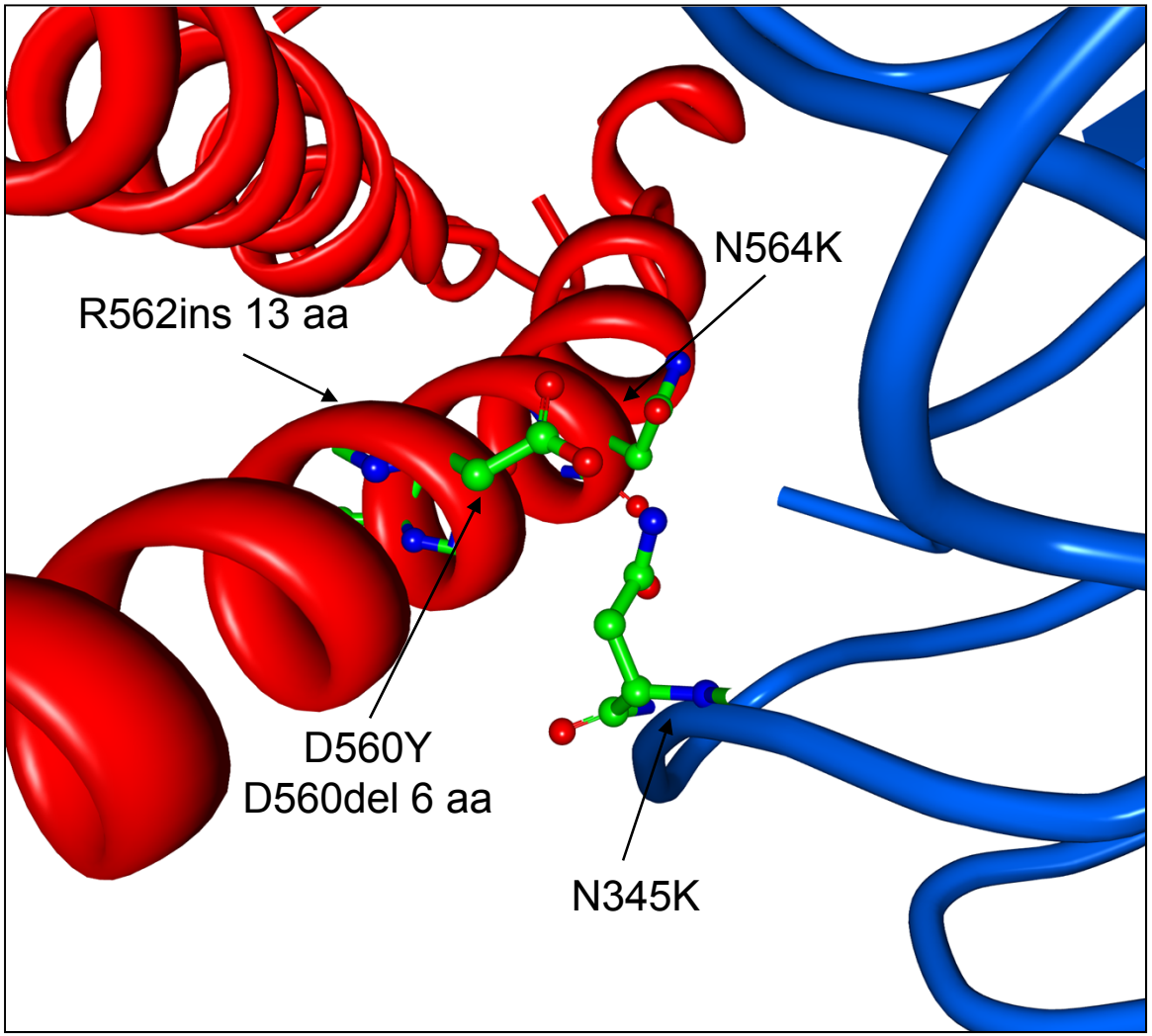
PIK3CA/PIK3R1 Mutations



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GBM and Lung Adeno Mutations

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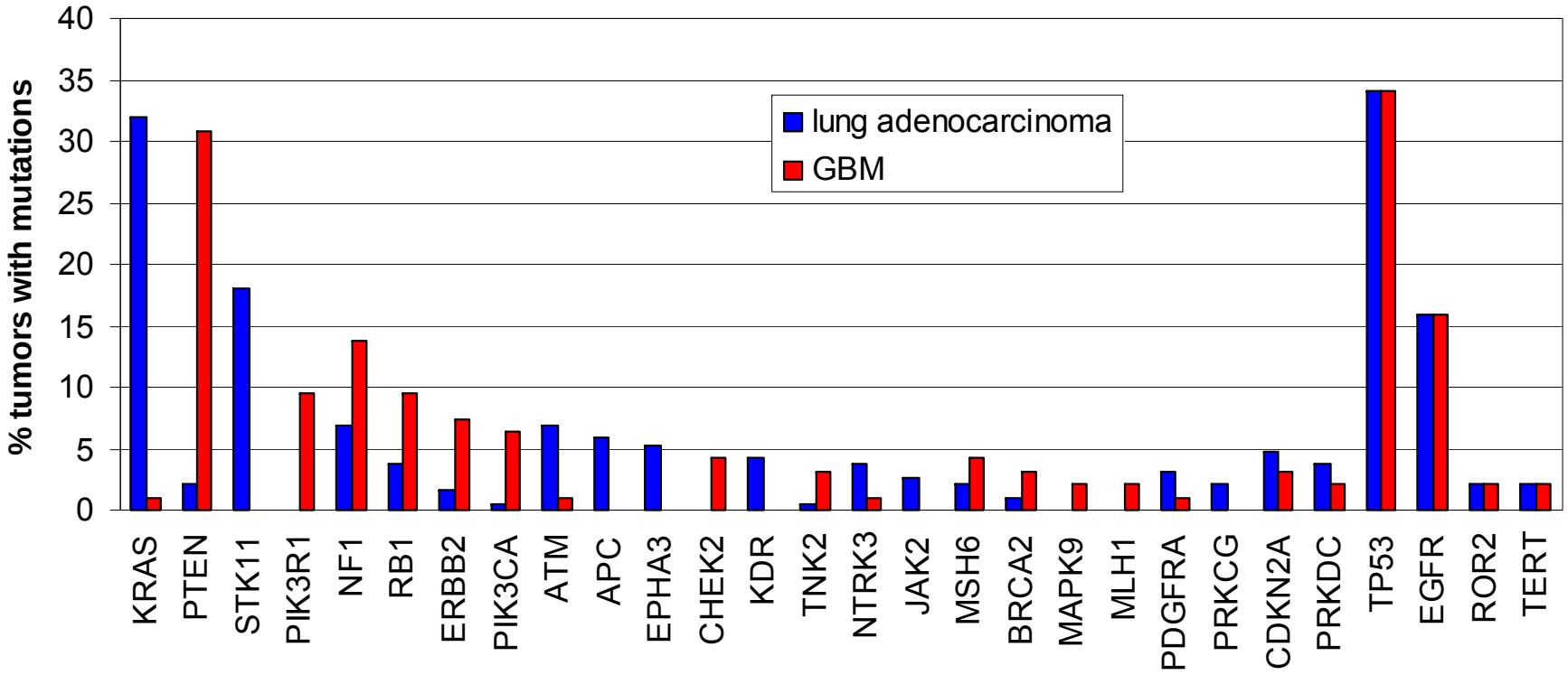

Lung Adeno mutations: KRAS, STK11, APC, EPHA3, ATM, and KDR

GBM mutations: PTEN, ERBB2, PIK3R1, CHEK2, and PIK3CA

Overlap: TP53, RB1, EGFR, CDKN2A, and NF1

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Limitations of our current approach?

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- **Hypothesis-driven (biased):**
  - Gene sets with related functions: “kinome”, “phosphatome”
  - Genes mutated in other cancers
  - Closely related genes
  - Investigator-driven ideas
- **Data-driven (unbiased):**
  - Use genomic platforms to identify loci with recurrent somatic alterations
    - Array-based RNA profiling, exon arrays
    - Array CGH (LOH and CNV analysis)

But… What are we missing outside of exons?
How does the cost and complexity of PCR limit us?
How can we effectively “shake the whole tree”?

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Next Gen Sequencing Technology

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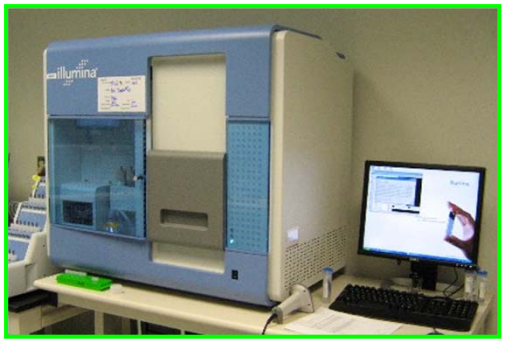
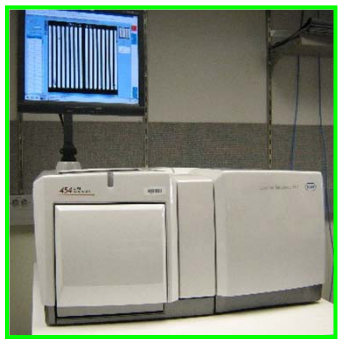
Genome Size:		3000 Mb		
Coverage:	6	12	20	25
	3730	**454 XLR**	**Illumina GA II**	**SOLiD**
bp/read	600	400	50	35
reads/run	96	1,000,000	52,000,000	80,000,000
bp/run	57,600	400,000	2,000,000,000	4,000,000,000
Total Runs	312,500	90	30	19
Cost/Run	$48	$6,800	$9,300	$15,000
Total Cost	**$15,000,000**	**$612,000**	**$280,000**	**$281,000**

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Next Gen-based mutation detection

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DNA from X tumor samples

↓ Pooled with equal concentration

PCR amplification with Y primer pairs

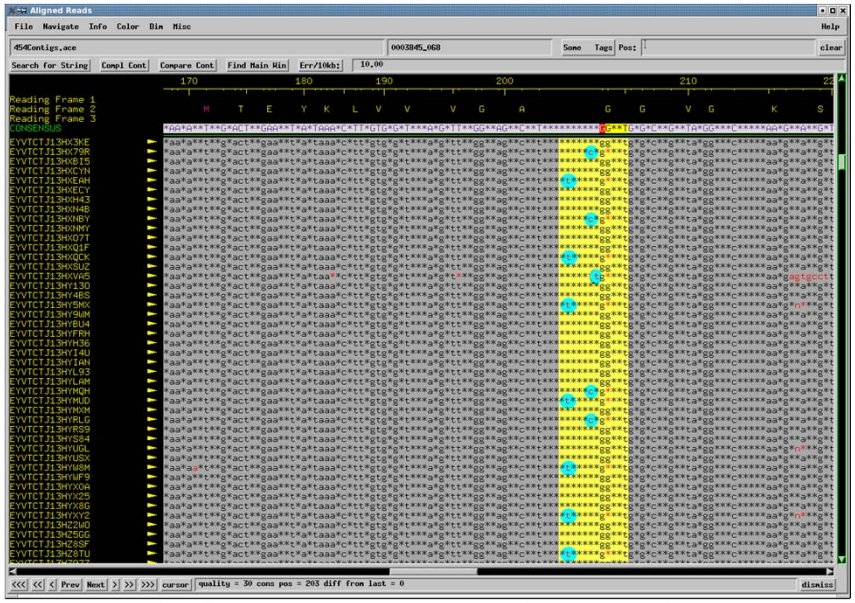
↓ Pool PCR products

454 sequencing

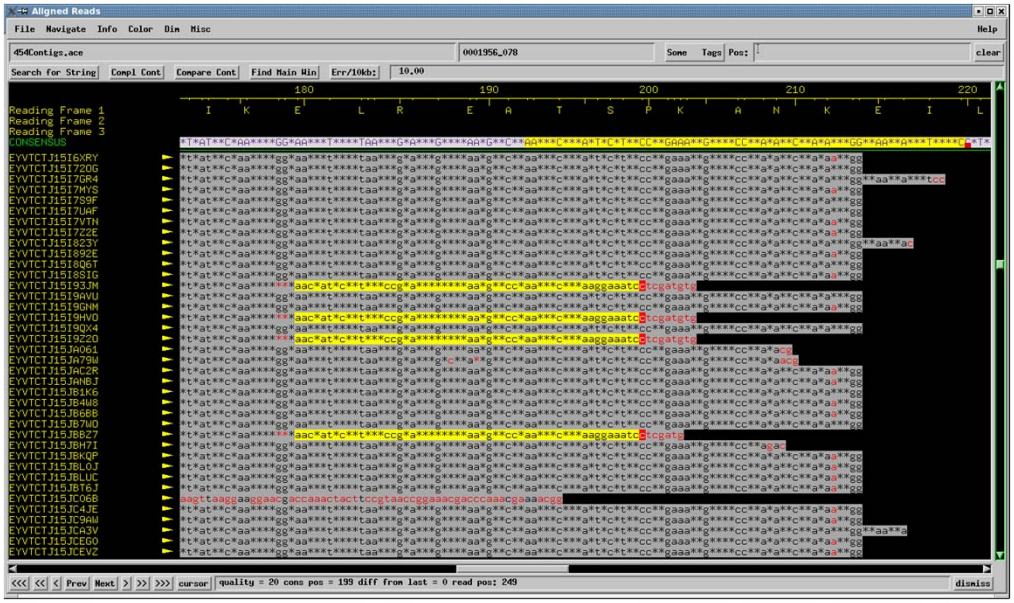
↓

SNP/Indel Detection Using ssahaSNP and BreakPointRead

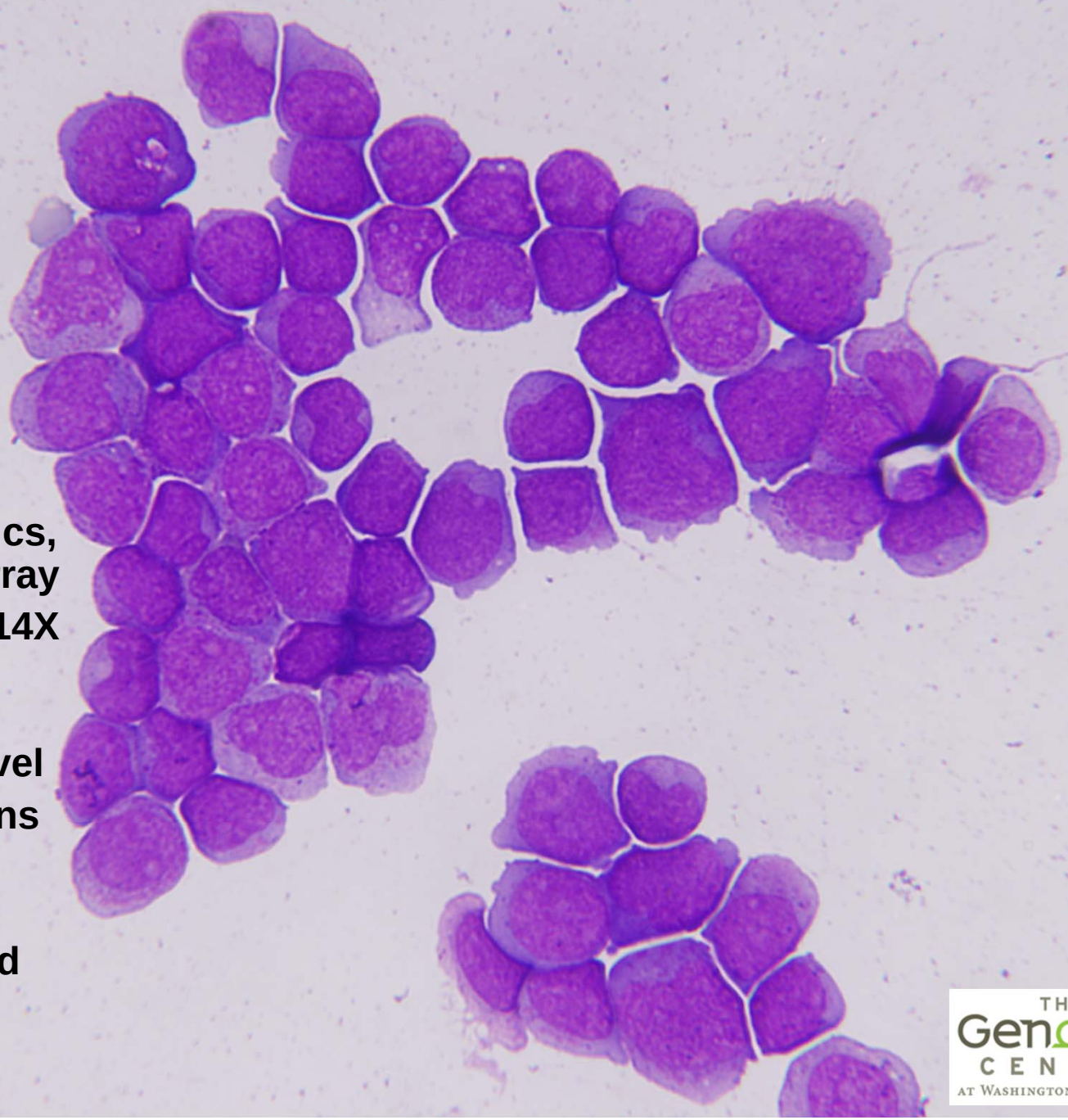
Reads with G12 mutation in KRAS



Reads with 15 bp deletion in EGFR



"AML1" genome sequence



- 57 y/o Caucasian female
- *De novo* M1 AML
- Normal cytogenetics, no CNV/LOH by array
- WGS: 32X tumor, 14X normal (Illumina)
- 3.7M variants detected, 1.3M novel
- 8 somatic mutations validated:
  - FLT3, NPMc
  - 3 cancer-related genes
  - 3 novel genes

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Next Gen Sequencing in TCGA

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- Three glioblastoma samples (tumor + matching normal) have been selected for whole genome sequencing.
- Broad and Wash U will utilize the Illumina technology, Baylor will utilize 454 + SOLiD.
- Twelve GBM samples have been selected for transcriptome analysis by cDNA sequencing.
- All three centers are currently working on applications to perform directed (targeted) sequencing using the various next gen platforms. This will likely be done first for the second gene set for the ovarian samples.

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Acknowledgments

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  Genome sequencing centers:

  - Baylor Human Genome Sequencing Center
  - Broad Institute of Harvard & MIT
  - The Genome Center at Washington University

- **The Genome Center**

  - Li Ding, Elaine Mardis, Tim Ley, David Dooling

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