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MYELOMA INSTITUTE FOR RESEARCH AND THERAPY

UNIVERSITY OF ARKANSAS FOR MEDICAL SCIENCES

# Revealing the Inherent Heterogeneity of Human Malignancies by Variant Consensus Strategies Coupled with Cancer Clonal Analysis

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# Outline

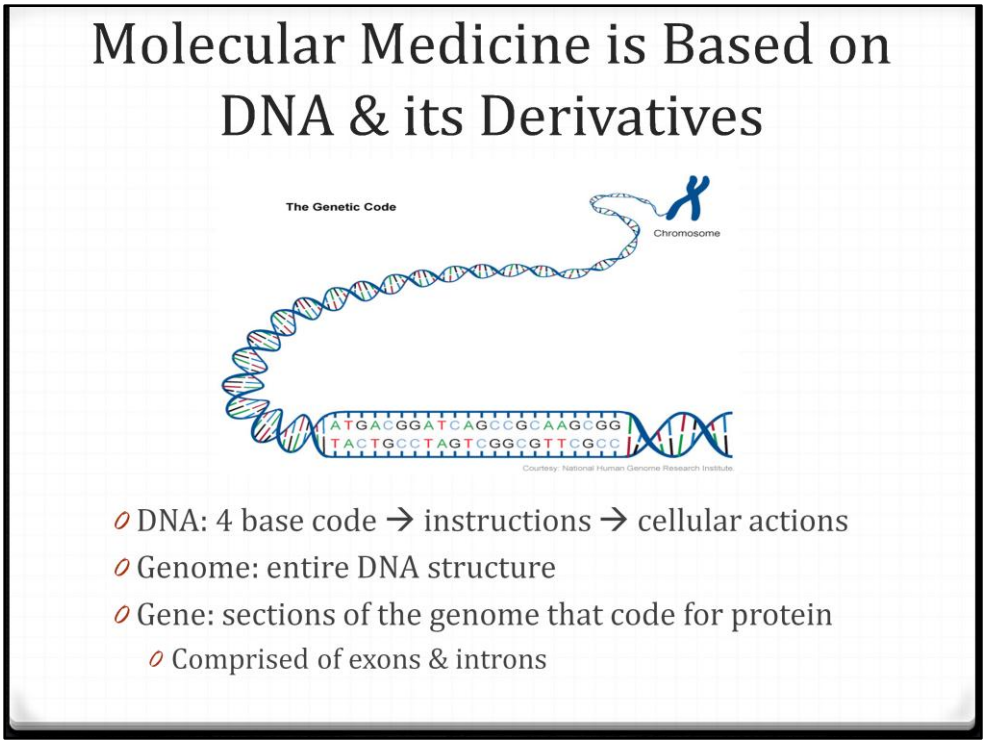
- Background
- Whole Exome Sequencing (WES) & Variant Consensus Analysis
- Tumor Clonality Analysis

# Biomedicine: Unprecedented Views of Our Molecular Make-up & Disease

*There are three things extremely hard, Steel, a Diamond, and to "know one's self"*

- Benjamin Franklin, Poor Richard's Almanac

- While Mr. Franklin was speaking about knowing one's self in the philosophical sense
  - Here we are talking about truly understanding our genetic make-up at the molecular level (the lowest meaningful level)
- Understanding disease in a way that was not previously possible.
- We have learned in the last 10-15 yrs. that cancer is a disease of the genome at the cellular level.
- We've now the technology to see genomic aberrations & derangements at the base level.




- Please excuse me if this is too elementary, but I just want everyone on the same page
- Contained within the chromosomes (proper) is the entirety of the genome (or our genetic code)
    - DNA is coded using a 4 base code (ATCG)
        - It is the DNA bases which code instructions
        - Instructions confer cellular action
    - 1% of the genome codes for proteins (we call these sections exons)

# Fruits of the Human Genome Project

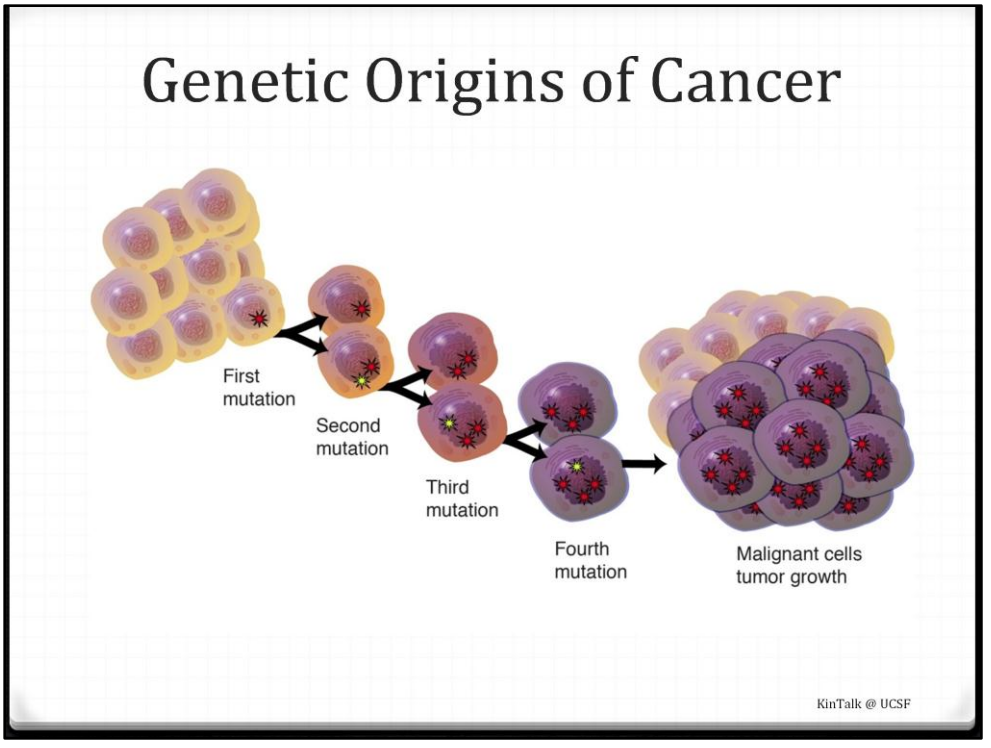
- Human Genome Project :
  - Near complete map of human genome

| Time | Cost |
|---|---|
| 1988 – 2003 (~15 yrs.) | $2.7 billion |

- Today:

| Time | Cost |
|---|---|
| 1 day | $1,000 |

- HGP yielded a nearly complete map of the human DNA sequence
- Illumina just announced a few days ago, WGS can be done in 1 day at a cost of $1K
- Because of the cheapness, it will soon enter clinical practice
- Oncology is seeing some of the biggest impacts:
  - In the form of new classes of therapeutics / drugs
  - Current treatments are far too toxic and we are finding tumors too late

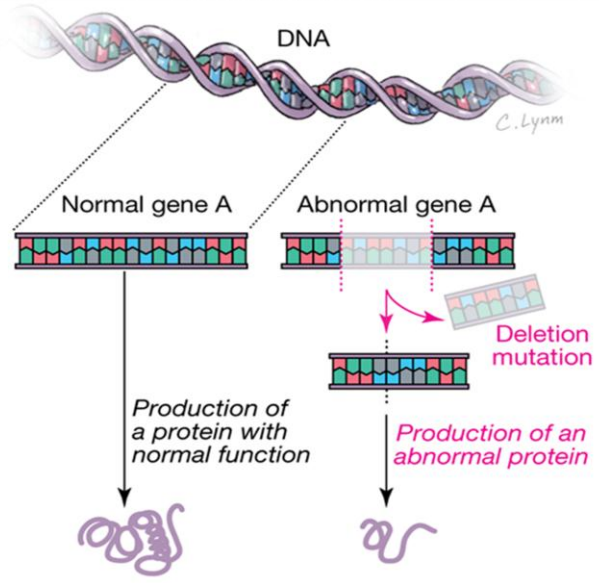



- This is just a graphic
- Cancer is a disease of the genome at the cellular level
- Each cancer has an entire genome in it, only it is mutated
    - That is, within a someone with cancer there exist two genomes; one from the tumor (somatic), the other he/she was born with (germline)
- Next-generation sequencing and its associated analyses can differentiate between somatic and germline mutations

# Variability in the Human Genome

- For everyone here has a DNA sequence that is 99% identical
    - If we were all dogs, we would all be of the same breed
- However, it is the variability that defines the human population
- Underlies different prognoses & outcomes in disease states
- A primary reason why categorical therapeutic approaches do not always work (lack specificity)

# Example of a DNA Deletion Mutation

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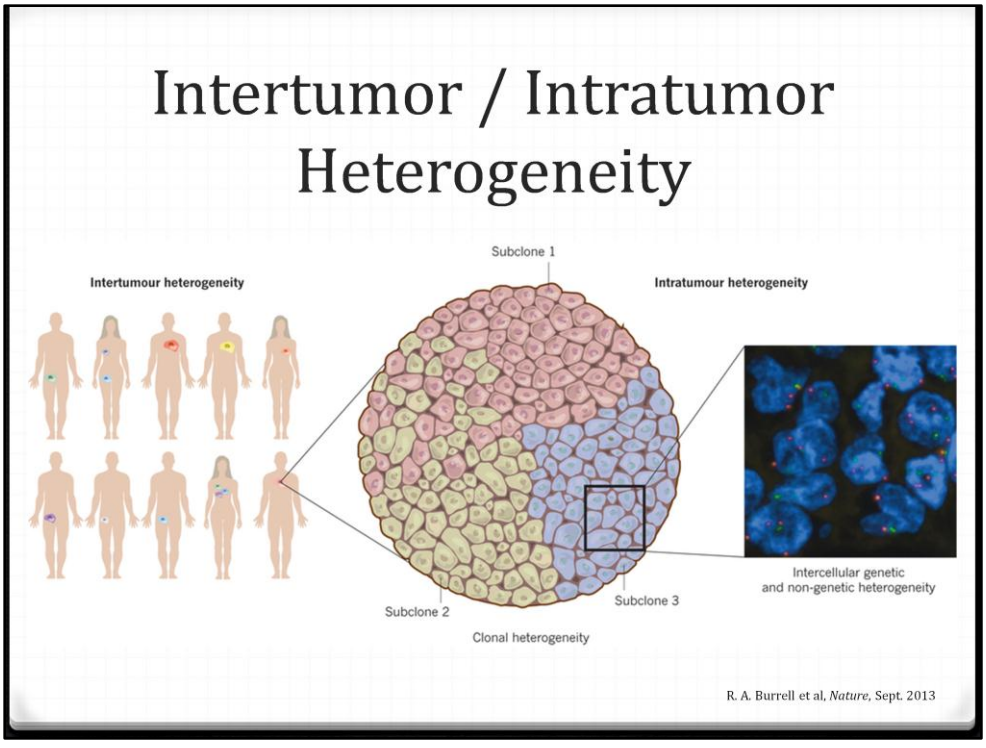
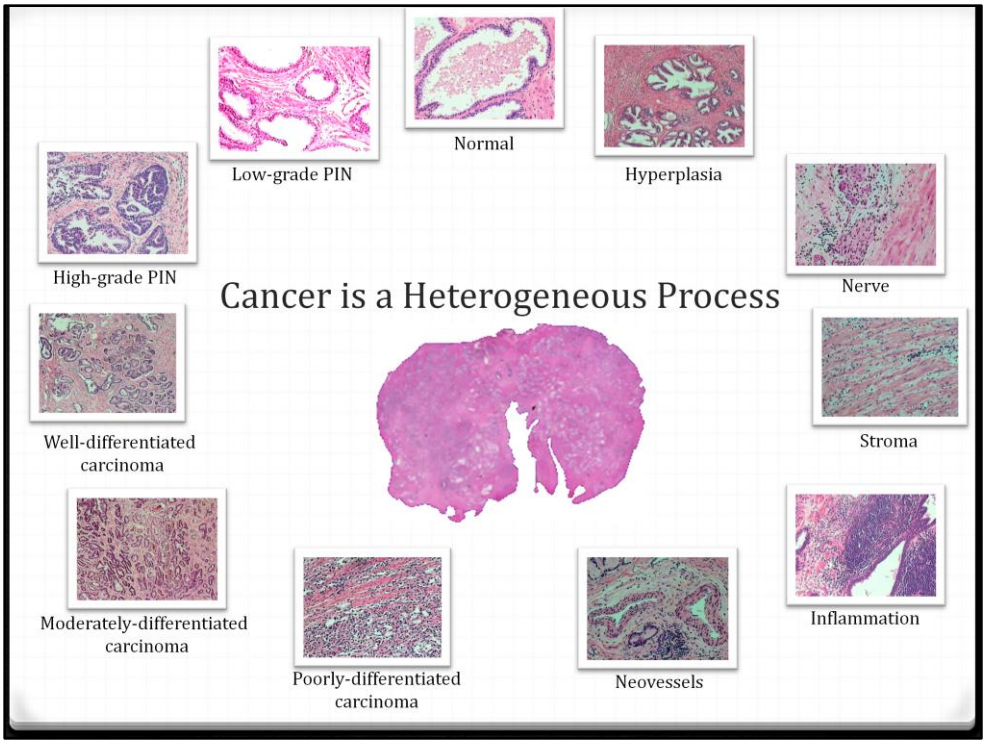
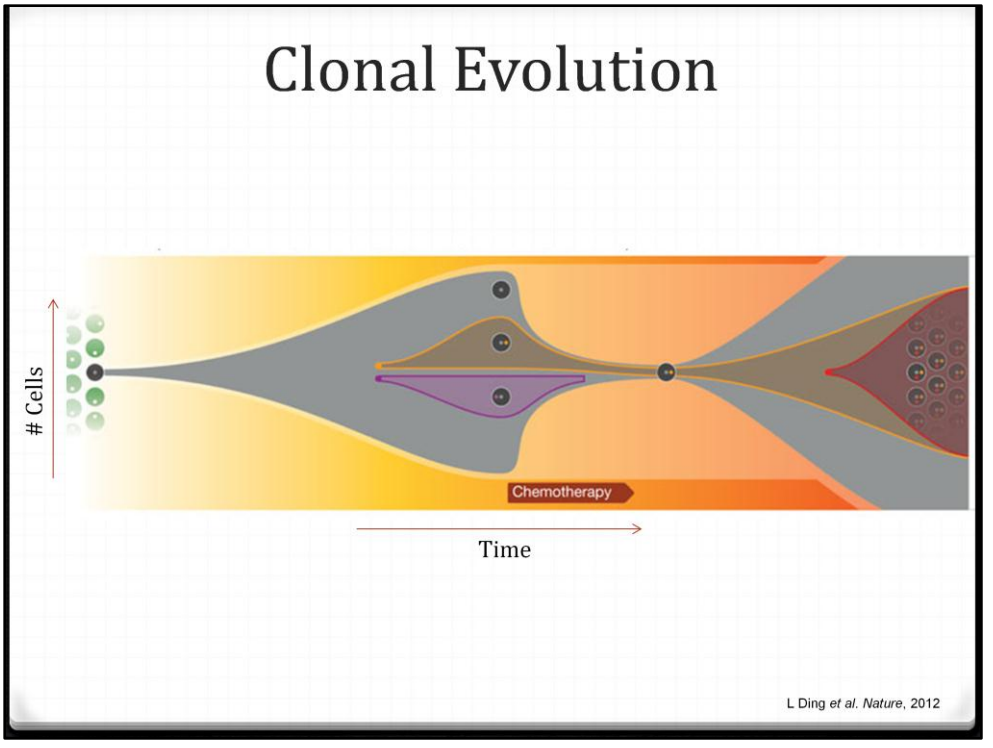



- This graphic illustrates the heterogeneity that exists not only between tumor, but within a particular person's tumor
- We know that not every tumor cell within a particular tumor are identical
- Tumors are a mosaic.




- Here we are looking at the cancer at a tissue and morphological level
- In the center, we see a tissue cross-section of a human prostate gland that is cancerous
- Around the periphery are the tissue subtypes within the cancerous prostate gland
    - “PIN” is not normal but not cancerous
    - “Differentiated carcinoma” show different levels of aggressiveness of the cancer

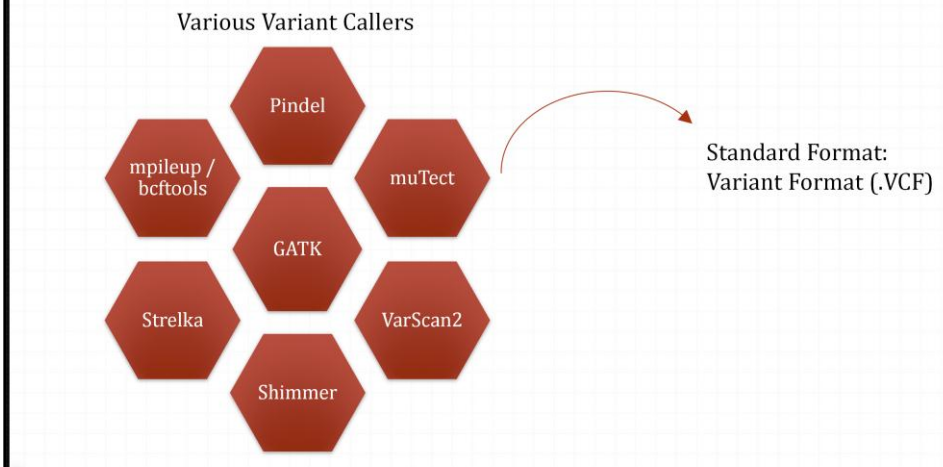



- Here we are looking at the clonal / molecular level
    - At the clonal evolution of cancerous cells
- X-axis shows time, Y-axis shows the expansion of mutated cells
- Over time we see that a so-called “founding clone”, that initiated the cancer, gains mutations over time
- At presentation, a sample of the patient’s tumor will appear as a cross-section
    - Exhibiting different abundances of various subclones
- During treatment a “selection event” occurs, some mutations are killed, others survive
- Some mutations might be a result of treatment (chemo) and ultimately proliferate and kill the patient

# Whole Exome Sequencing (WES) Methods

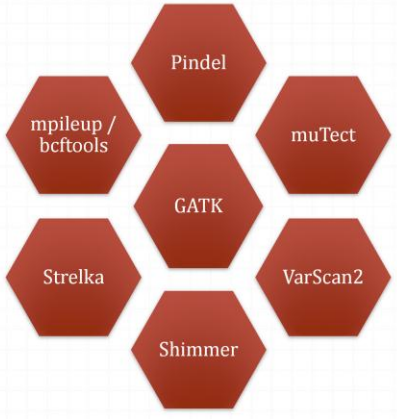
- Germline & Tumor DNA extracted from a Multiple Myeloma patient
- Whole Exome Sequencing (WES) was performed:
  - Illumina HiSeq 2500
- Paired End Alignment of Reads:
  - BWA / STAMPY
- Base recalibration with GATK
- Variant calling performed:
  - GATK, VarScan2, and Strelka
- VCF files used as input to Variant Consensus Reporter

# The Good


Various Variant Callers
Pindel
mpileup / bcftools
muTect
GATK
Strelka
VarScan2
Shimmer
Standard Format:
Variant Format (.VCF)

# The Bad

Various Variant Callers




Different Computation Algorithms

Vis-à-vis

Different Results

# One Solution: Consensus Analysis

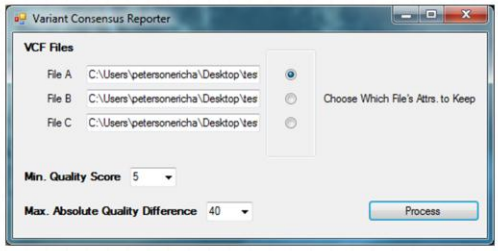
- Why: Common Approach used in Machine Learning
- Variant Consensus Reporter (VCR)
  - Input: Various .vcf files from variant callers
  - Output: Union and/or intersection of records in .vcfs
    - Output could be fed to functional annotators (i.e., ANNOVAR, snpEff)

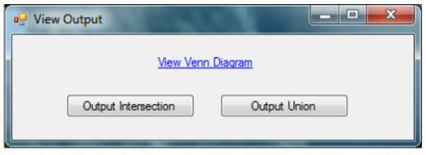
# Essential Contents of a VCF File

- Chromosome
- Position within chromosome
- Reference base(s)
- Alternate base(s)
- Quality score

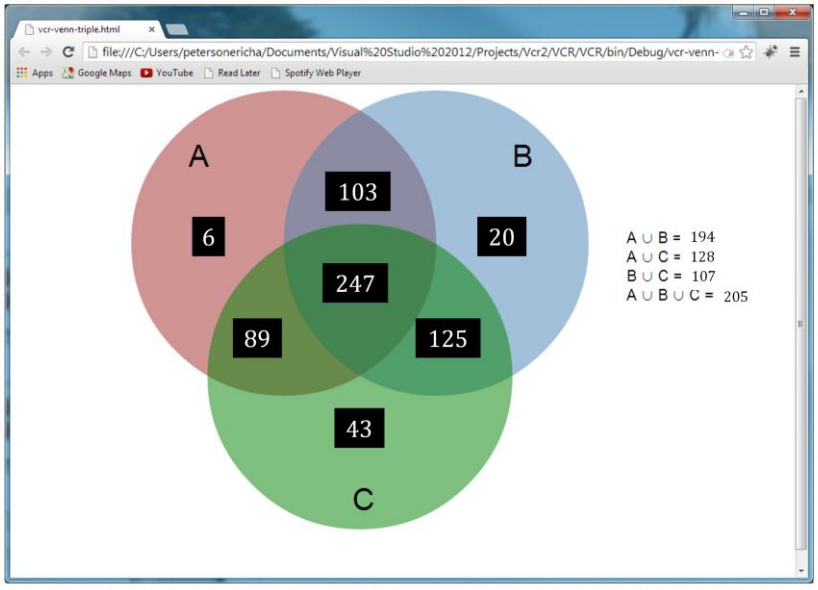
- Chr 1
- 101550
- C
- AT
- 40

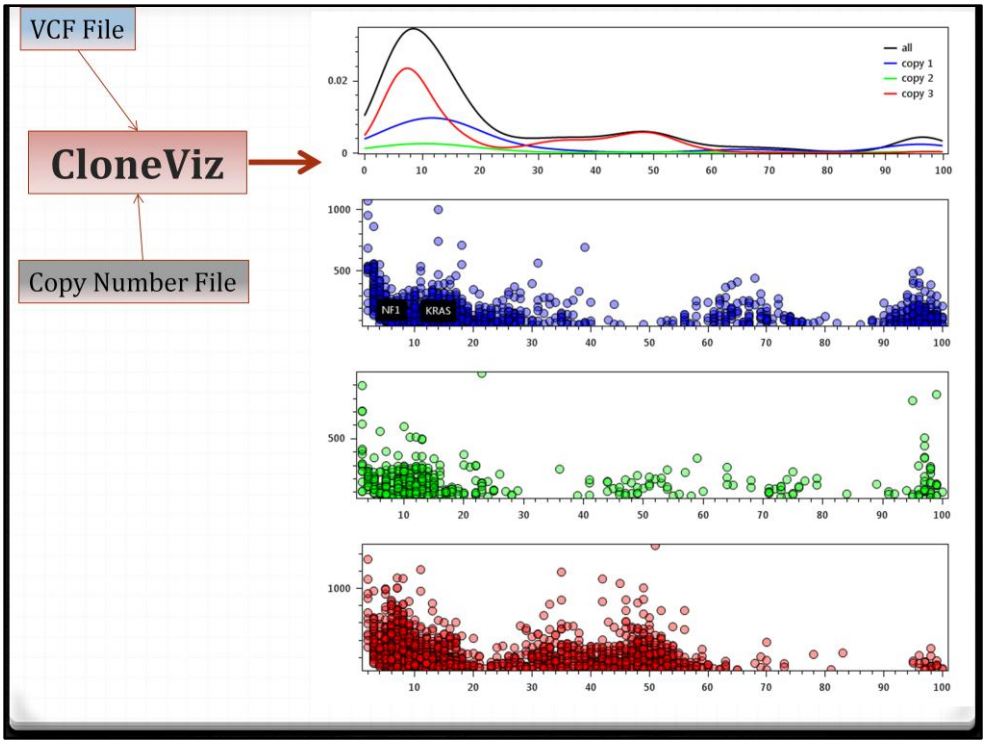
# Interactive User Interface



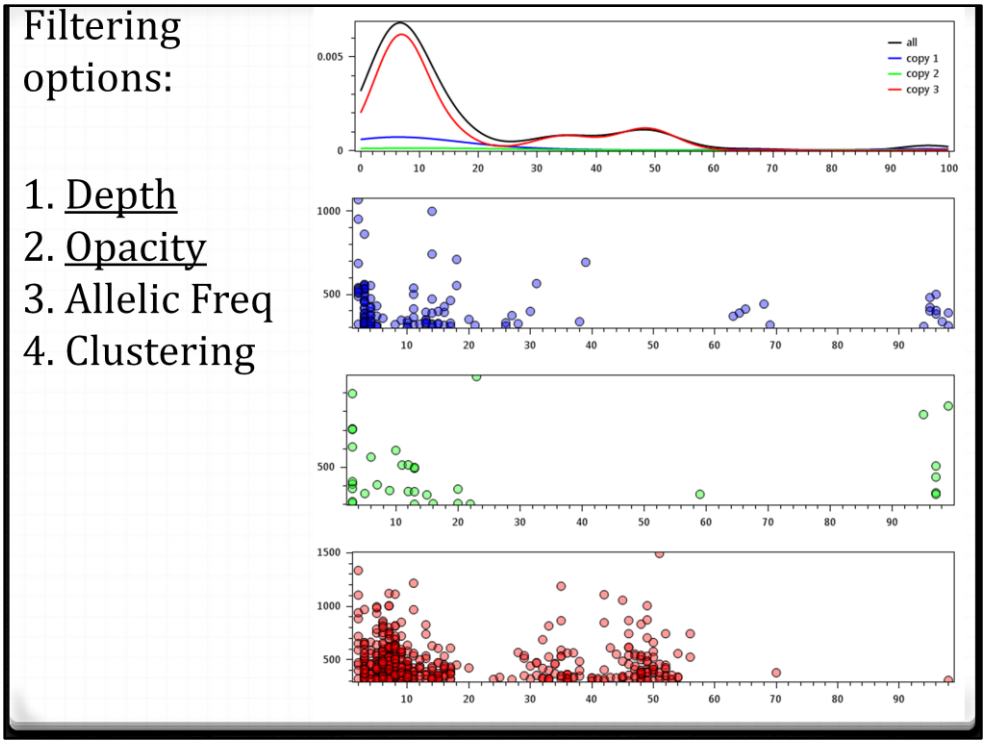



# Automatic Graphical Results

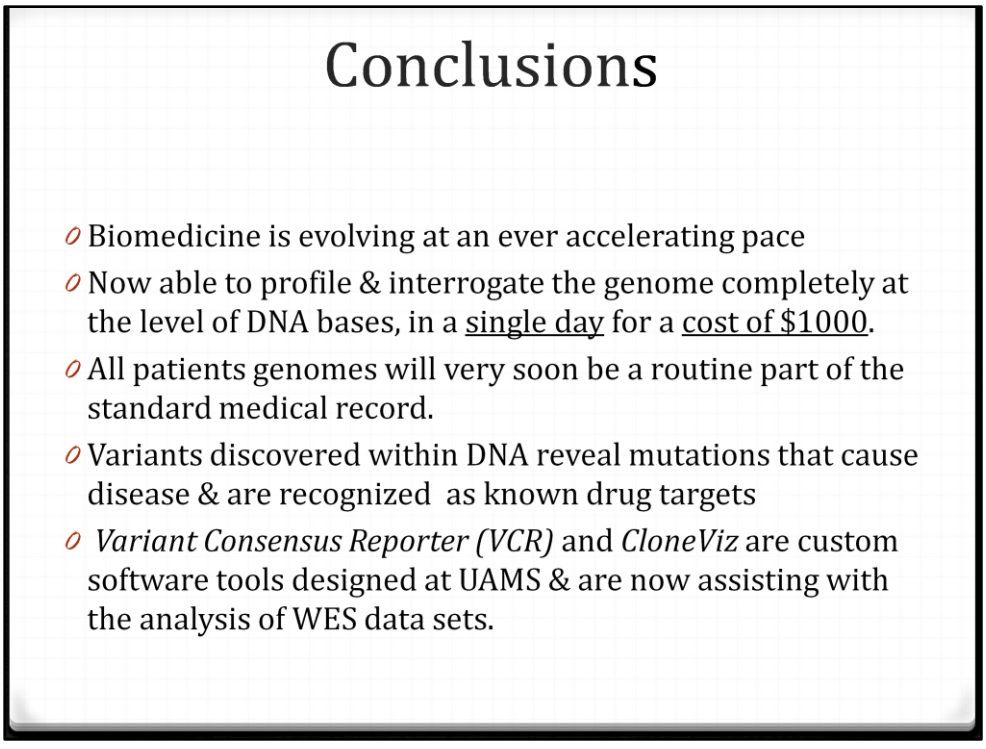





- X-axis in all plots is the variant allele frequency
    - It is basically the relative abundance of the variant within the sample
- Y-axis is depth in the copy number plots and density in the top plot
- Each dot is a variant / mutation found within the sample
- We can label variants in found in certain genes, by providing a custom gene list we are interested in annotating




- Filtered on minimum and maximum depth
- Everything is re-calculated and displayed interactively
- Future work:
    - Filtering on allelic freq.
    - Clustering of subclones




- Biomedicine is evolving at an ever accelerating pace
    - Human Genome Project established the foundation of modern molecular medicine.
    - Subsequent fruits of this public-private partnership continue to advance technology that is now changing the practice of medicine, especially cancer.
- Now able to profile & interrogate the genome completely at the level of DNA bases, in a single day for a cost of $1000.
- All patients genomes will very soon be a routine part of the standard medical record.
- Variants discovered within DNA reveal mutations that cause disease & are recognized as known drug targets
- *Variant Consensus Reporter (VCR)* and *CloneViz* are custom software tools designed at UAMS & are now assisting with the analysis of WES data sets.

# Acknowledgments

Collaborators

Shweta S. Chavan

Michael A. Bauer

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