

# Genomic Analysis of Ovarian Cancer

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# UCSF Comprehensive Cancer Center Genome Analysis Core Facility

Recharge facility to support Cancer Center members and UCSF investigators for Genomic services including :

DNA Sequencing (w/ Automated BLAST and PHRED) 1-day turn-around time (ABI3700)

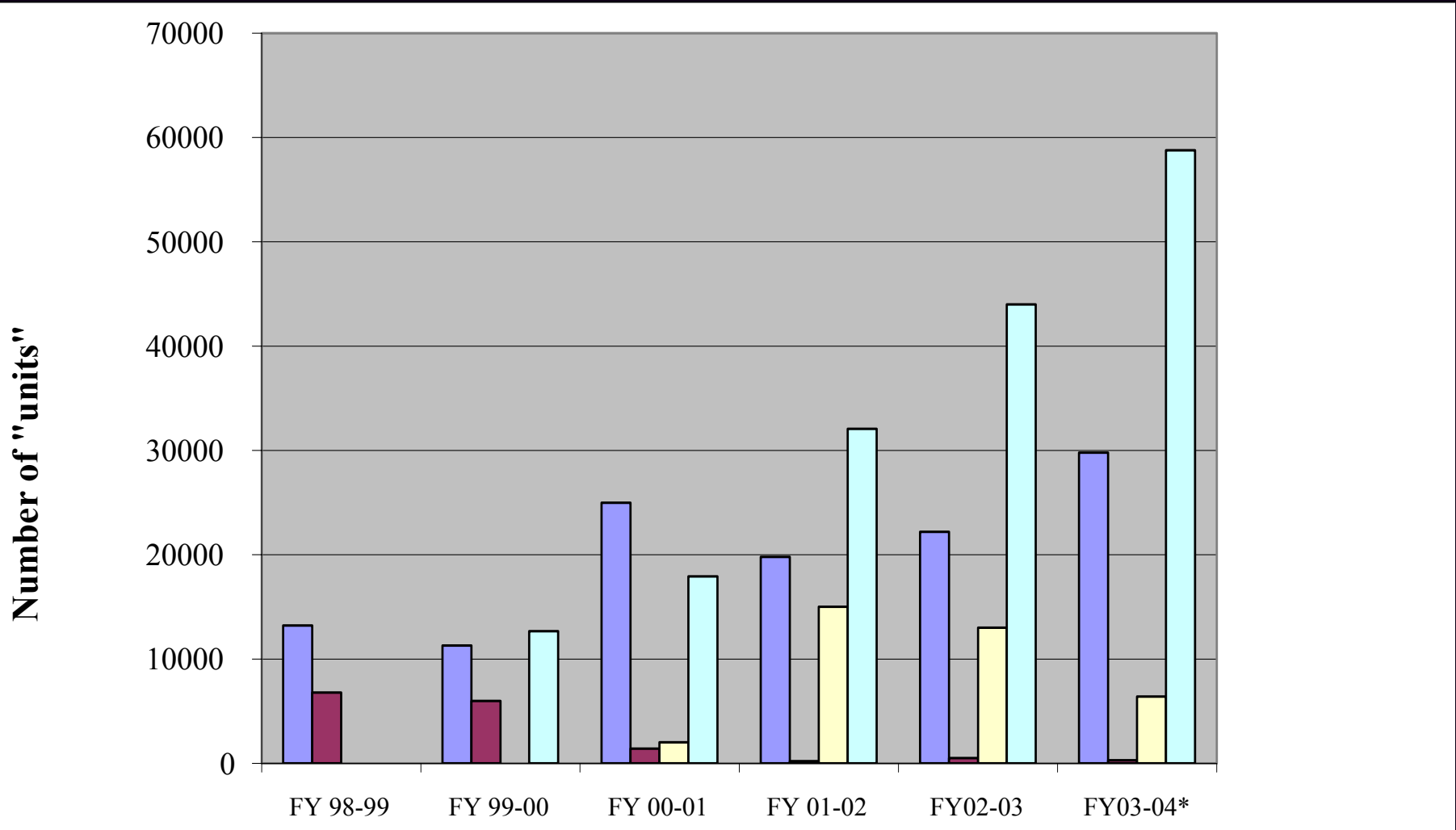
DNA Fragment Analysis, (LOH, Linkage etc.)

SNP Analysis, SNaPshot, AD assays (others?)

Real-time Q-PCR Analysis and Training  
(2-ABI7700s, ABI7900-384well, Cepheid, tested many others)

Bioanalyzer, Nanodrop micro-spectrophotometer

# UCSF-CCC Core Usage Summary



1.5 FTE  
~\$250K budget

4 FTE  
>\$650K budget

# Real-time PCR in the Core

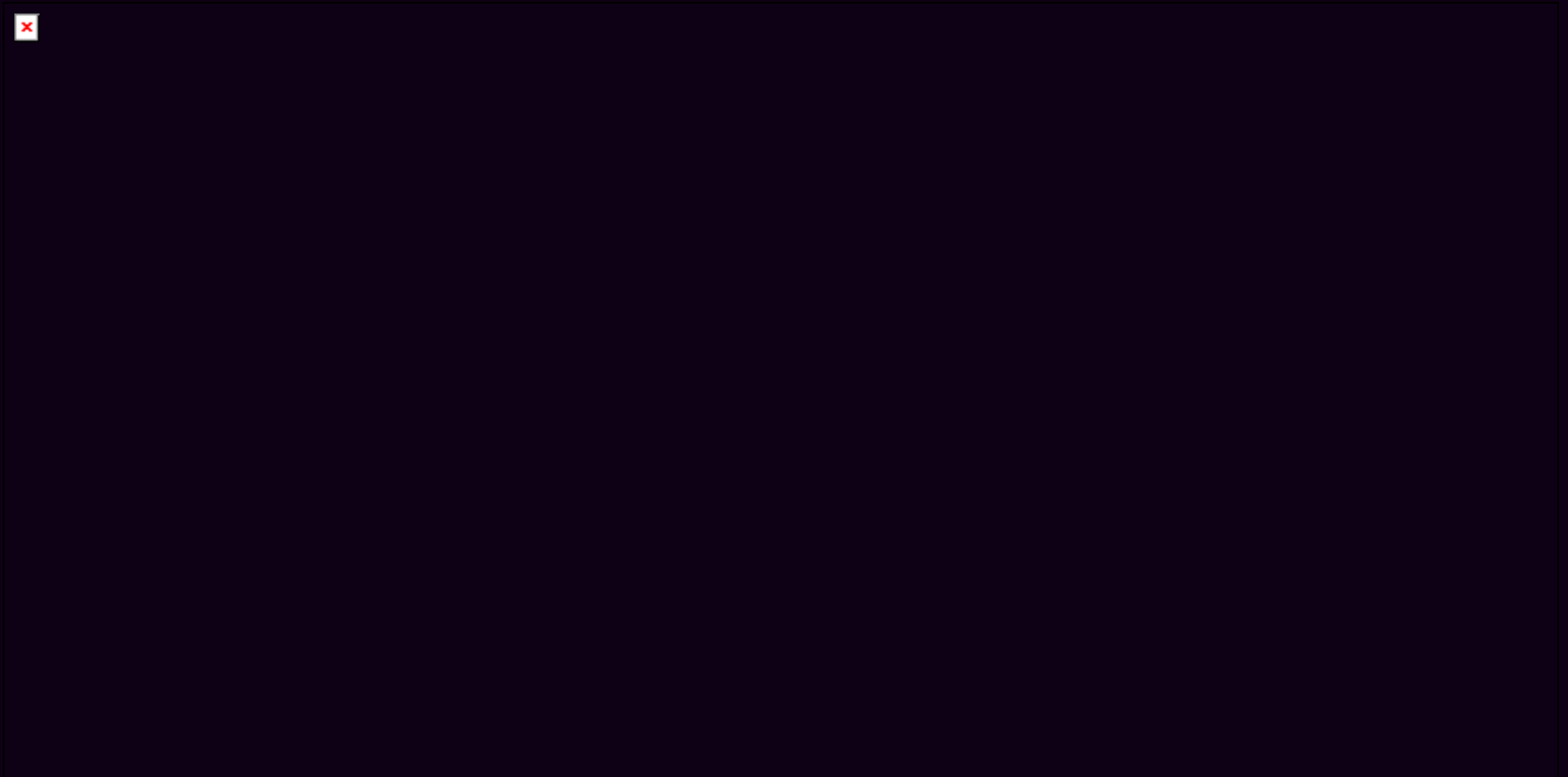
## -expression analysis

### Our methods include:

- Careful analysis/Qtn of RNA -Bioanalyzer, NanoDrop.
- Use validated high quality cDNA synthesis system Bio-Rad iScript! Must be linear over range of input RNA
- Carefully choose “control gene” - test a panel for least variant genes and use more than one (3)
- Use high quality TaqMan based assays - about 1000 assays designed to date. Or AoD's from ABI (collaborative agreement)

# Linearity of Reverse Transcription

-700-22ng



# RT- linearity w/ Bio-Rad iScript



# Choosing the Best “Control Gene” for Ovarian Cancer

Sample	18s	Gus	Histone	ORF2	GapDH	H.Cyclophilin
1	13.15	24.38	19.92	18.86	18.44	25.23
2	13.43	24.95	21.24	19.22	18.36	25.64
3	13.48	24.42	20.65	17.94	18.75	25.54
4	13.46	24.14	20.16	18.26	18.55	26.02
6	13.34	25.09	21.21	19.02	18.90	25.72
7	13.17	23.85	21.00	17.70	18.48	25.55
8	13.40	24.08	20.69	18.27	18.34	26.31
9	12.90	24.37	21.09	18.52	18.68	26.06
Avg Ct	<b>13.29</b>	24.41	20.75	18.47	<b>18.56</b>	<b>25.76</b>
Std Dev	<b>0.20</b>	0.42	0.49	0.53	<b>0.20</b>	<b>0.35</b>
	<b>0.21</b>	0.4	0.57	0.55	<b>0.24</b>	<b>0.37</b>

# Ovarian Cancer

## -introduction

- The leading cause of gynecologic cancer death in women in US
- The fourth leading cause of cancer death for women
- **Insidious onset with vague symptoms**
- The least understood disease among all major human malignancies
- The American Cancer Society (ACS) estimates that  
25,400 new cases of ovarian cancer in 2003  
Accounts for ~4% of all cancers among women  
14,300 deaths projected from ovarian cancer in 2003  
Lifetime risk of ovarian cancer is projected at 1.7%

# Ovarian Cancer

## - lifetime risk

The lifetime risk of ovarian cancer in the general population is 1.4% (1 in 70 women).

BRCA1 mutations confer a lifetime risk of ovarian cancer of 40%-50%

BRCA2 mutations confer a lower lifetime risk of 20-30%.

# Staging of Ovarian Cancer

		<u>5-year Survival</u>
<b>Stage I</b>	<b>Cancer involves the ovary but has not Spread.</b>	<b>90%</b>
<b>Stage II</b>	<b>Cancer has spread to nearby areas but is Still inside the pelvis.</b>	<b>70%</b>
<b>Stage III</b>	<b>Cancer has spread outside of the pelvis To other parts of the abdomen. This includes ascites in the abdomen.</b>	<b>15 –20%</b>
<b>Stage IV</b>	<b>Cancer has spread to other parts of the Body.</b>	<b>1% to 5%</b>

Estimated that more than half (60%) of patients with ovarian cancer are stage III or stage IV at time of diagnosis.

Harrison's Principles of Internal Medicine, 1998

NCI; [http://www.nci.nih.gov/cancer\\_information](http://www.nci.nih.gov/cancer_information)

# Ovarian Cancer Project- SPORE

Collaboration - UCSF/LBNL, Duke, MD Anderson and Northwestern (OvCA-SPORE) + others

- Array CGH: long vs short survival time
- Affy Expression Analysis correlation w/ CGHa  
Confirmation with Real-time PCR
- Early Detection of High Risk Women by FISH  
Northwestern U: Ovarian “pap slides”

# Genomic Analysis of Ovarian Cancer

## - "the game plan"

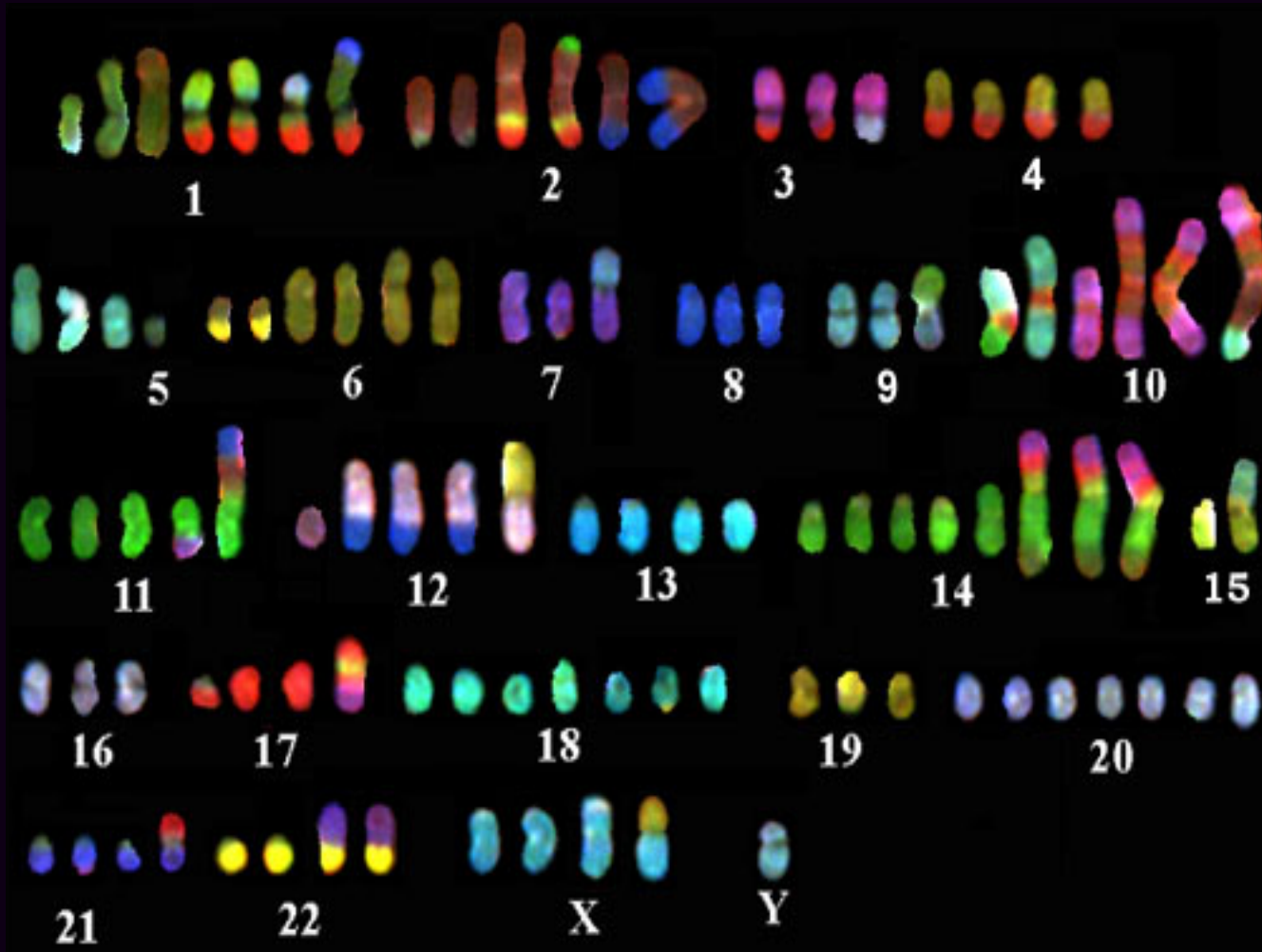
- Array CGH: Define Recurrent CNA regions
  - cell-lines (20) and tumors (30+)
- Affy Expression Arrays
- Confirmation with Real-time PCR >50 assays
  - correlation between Affy and Q-PCR
- Correlations with survival

# Genomic Imbalance Drives Tumor Evolution

Tumorigenesis and cancer progression are a result of the accumulation of DNA copy number aberrations which in turn drive the expression of genes controlling cell growth and death.

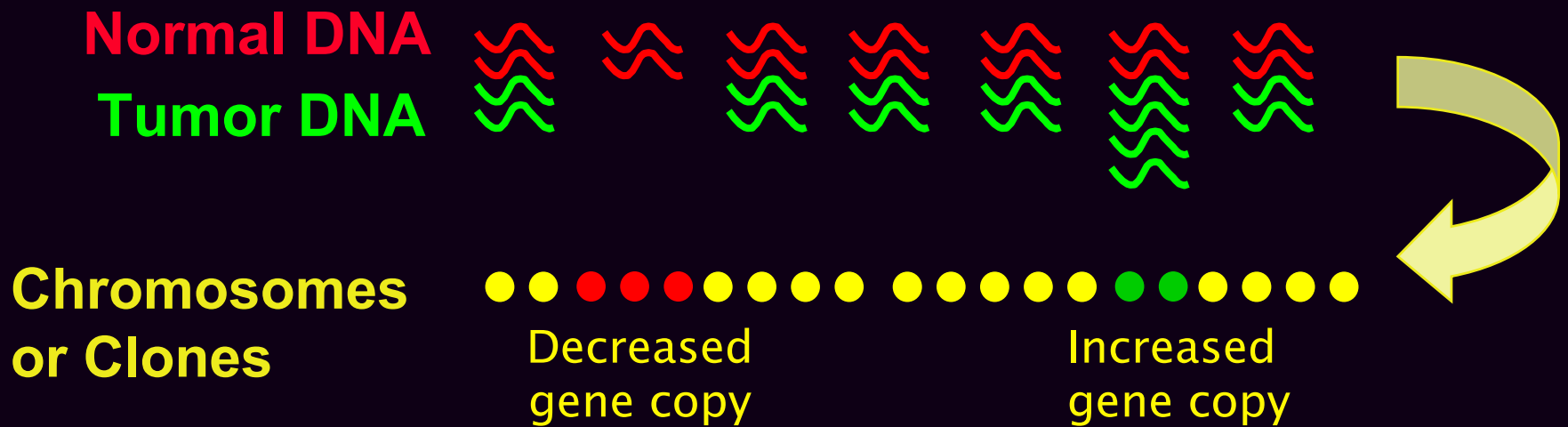
Disruption of normal cellular controls provides the tumor a growth advantage - Darwinian “oncoevolution”

# Cancer Is A Disease Of The Genome



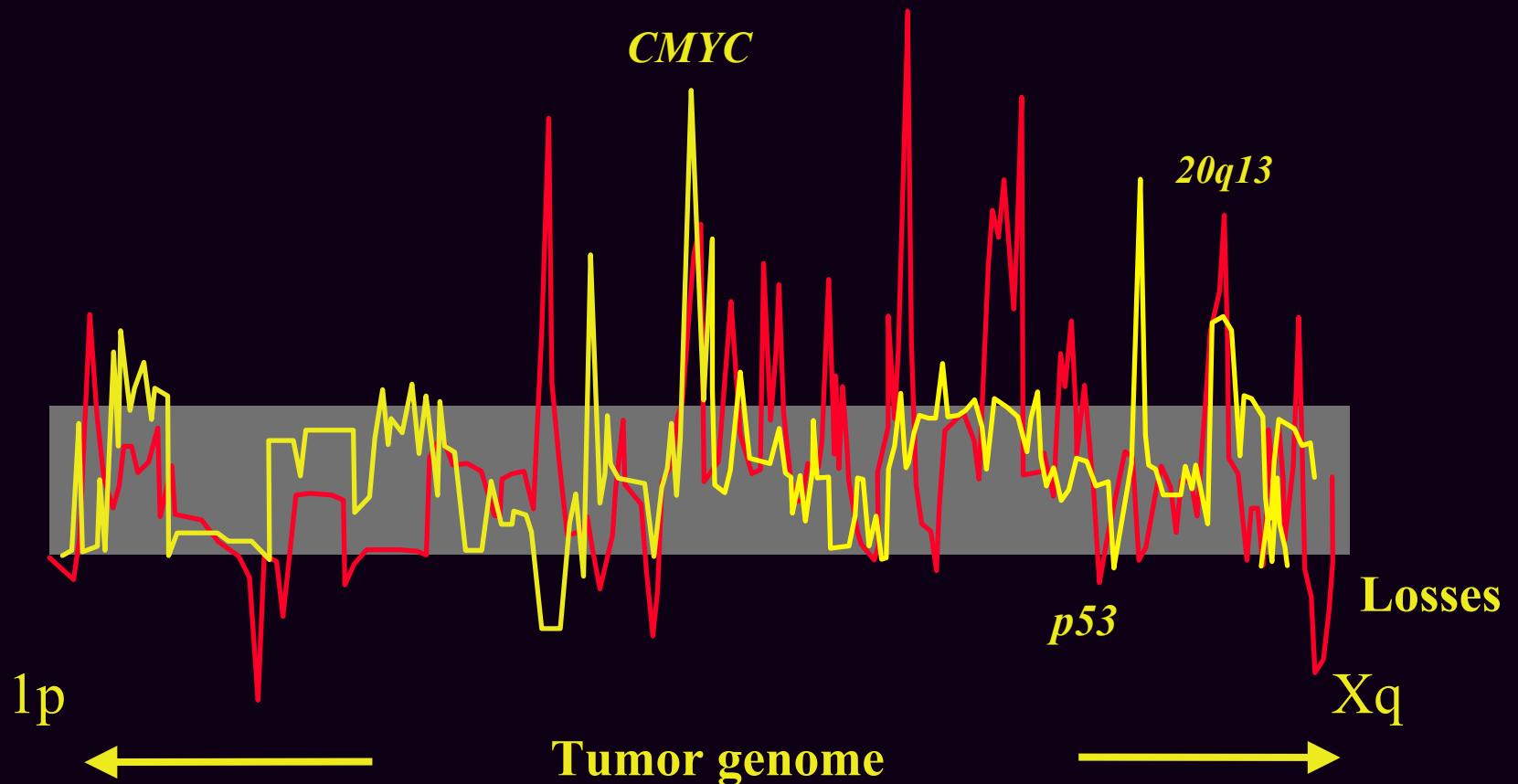
# Array based CGH

## - Comparative Genomic Hybridization

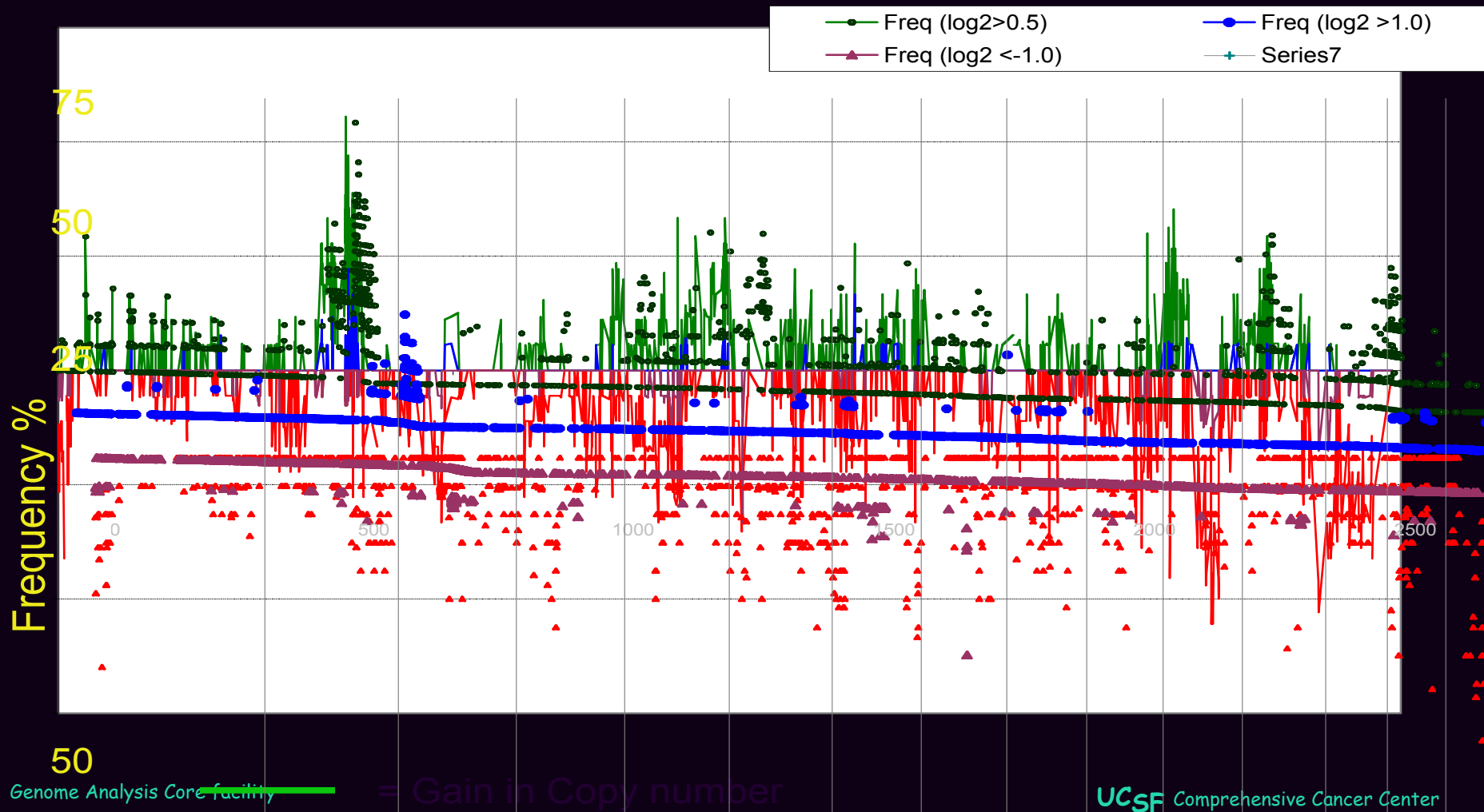


For more information, please see CGH array workshop Tuesday lead by Donna Albertson

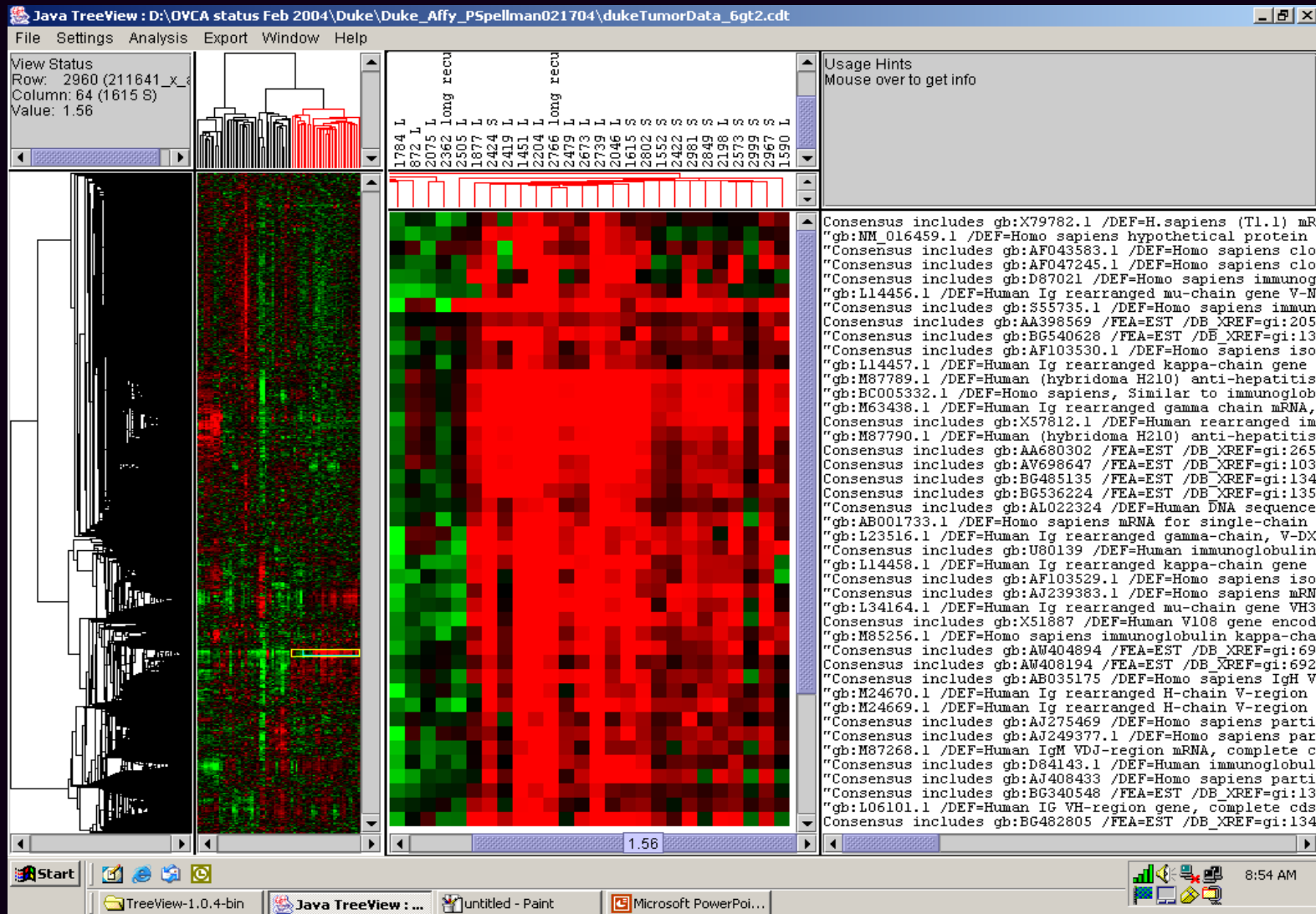
# Recurrent DNA Copy Number Aberrations as Measured by CGHa



# Recurrent CNA in 20 Ovarian Cancer cell lines



# Expression Profiling using Affymetrix Chips - overlap with CNA regions



# Correlation of Affymetrix Expression Analysis with CGHa

Analysis still underway, but preliminary analysis indicates that about 1/2 the regions of CNA show altered expression.

Coverage of Affy targets?

Do we have the right genes covered?

Other changes, methylation etc.

Which gene is driving the evolution of the tumor and which is along for the ride?

# Correlation of Affymetrix Gene Expression with Real-Time PCR

Affy data normalized to control genes

Of 50 genes tested 48 are on Affy Chip

Only 29 of 48 genes have all Affy targets  
to the same transcript.

Person's correlation coefficient for median of all  
Genes = 0.74

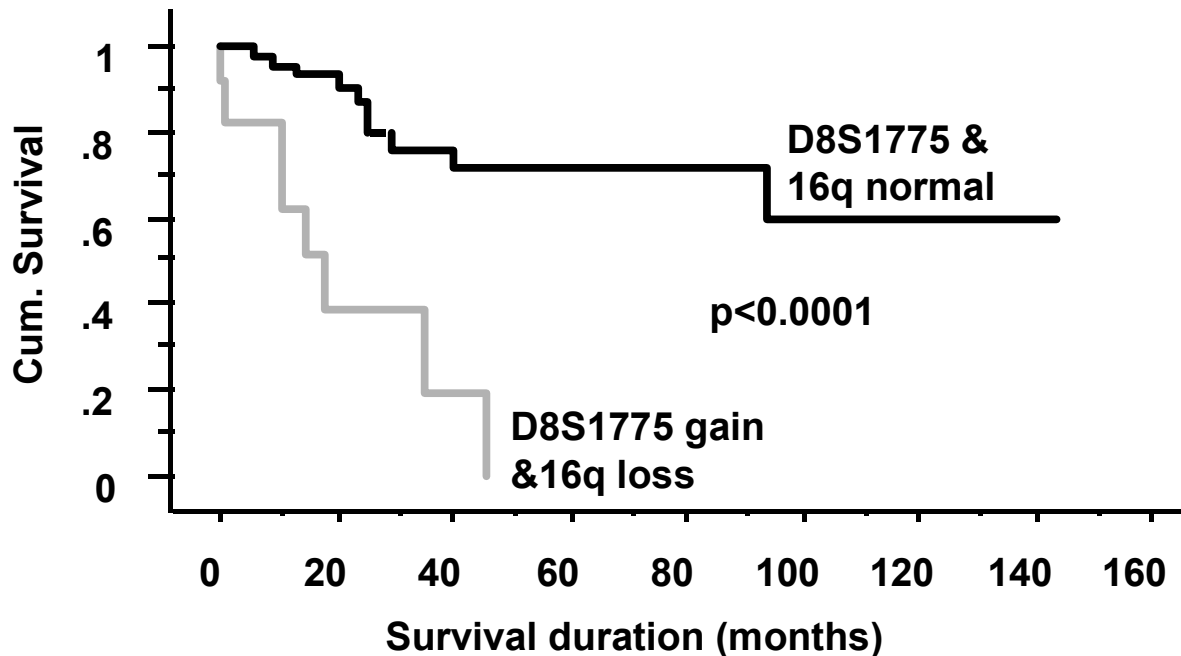
Median of only those target w/ all target

Matched = 0.77

# Correlations with survival

- not done yet with this data set

**Concomitant Chromosome 16q loss and 8q gain are associated with poor survival in women with Ovarian Cancer**



From Suzuki et al., (2000). *Cancer Res.* 60(19): 5382-5.

# Summary

Considerable effort is under way to gain deeper understanding of the genomic changes underlying ovarian cancer

Recurrent copy number aberrations are clues to the genes driving the evolution of the tumor

Current genomic technologies are relatively consistent

# Acknowledgements

UCSF/LBNL

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Bio-Rad

-Ayoub Rashtchian

-David Schuster

-Keith Hamby

# “Safety in Numbers?”

An Idea to consider:

ABRF members encompass many Core facilities  
(how many?)

Many of these facilities do real-time PCR  
(how many labs? Running how many assays?)

Applied Biosystems has 10’s of thousands of Assays

What about banding together to get a ABRF core  
discount for AoD’s???

How cheap? \$99/assay???????????