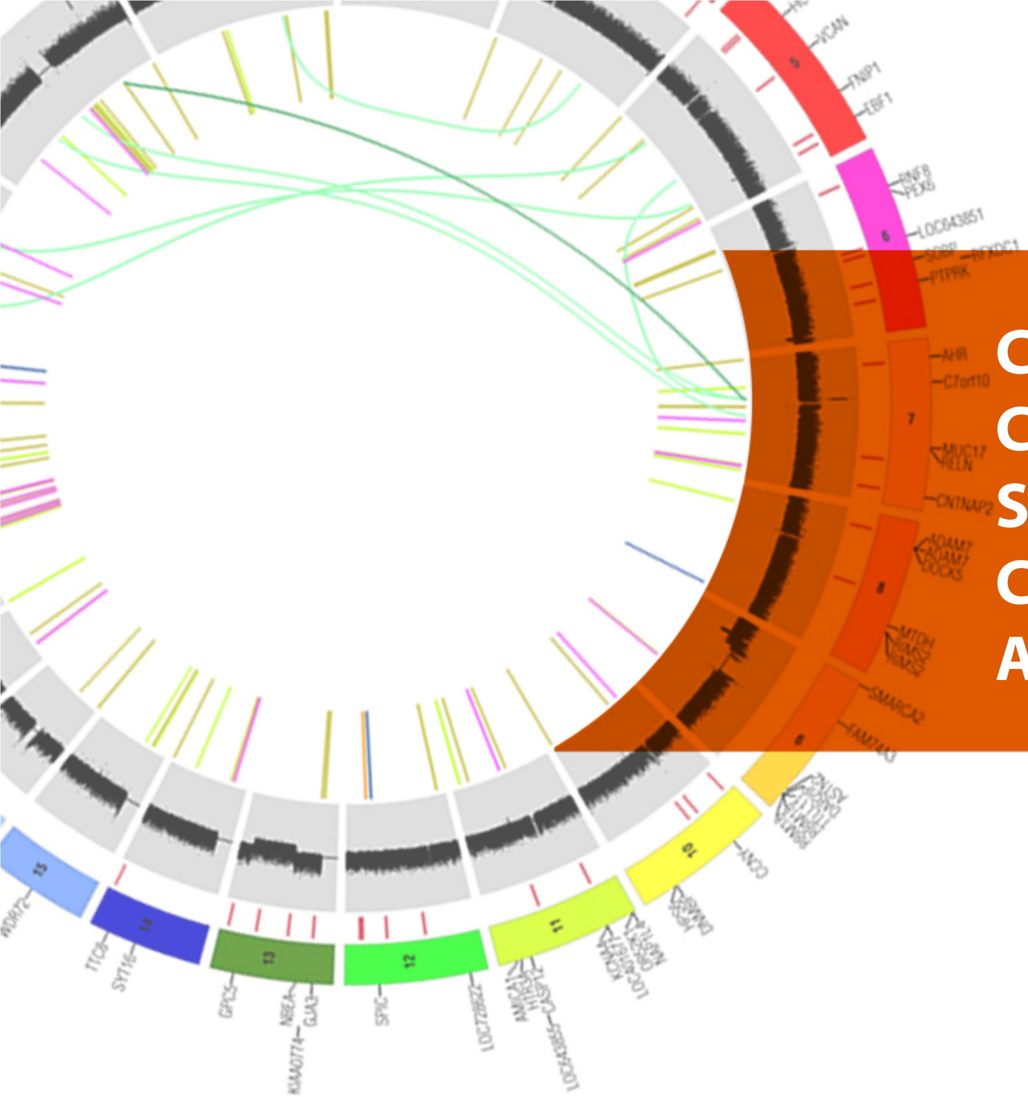
The Cancer Genome Atlas



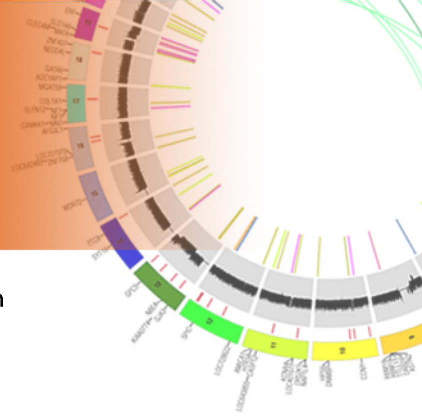
COMPREHENSIVE GENOMIC CHARACTERIZATION OF SQUAMOUS CELL CARCINOMA OF THE HEAD AND NECK

Neil Hayes, MD, MPH

The Cancer Genome Atlas 2nd Annual Scientific Symposium

11/26/2012

On Behalf of



Disease working group co-chairs

- Adel El-Naggar
- Jennifer Grandis

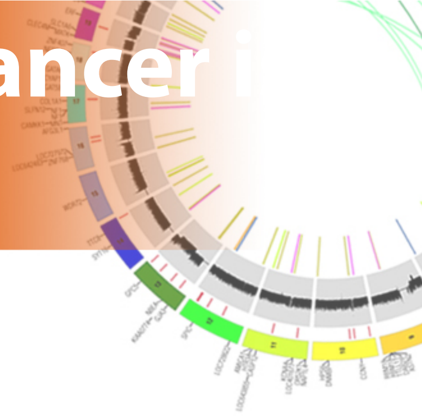
Representative Disease Working Group Members

- Jim Herman
- J. Jack Lee
- Jiexin Zhang
- Tom Carey
- Fei-Fei Liu
- Neil Hayes
- Johanna Gardner
- Candace Shelton
- Nishant Agrawal
- Patrick Ng
- Dean Bajorin
- Martin Ferguson
- Geoffrey Liu
- Brenda Diergaarde
- Tara Lichtenberg
- Tom Harris
- Robert Haddad
- Peter Hammerman
- Michael Parfenov
- Matt Wilkerson
- Andy Cherniack
- Carrie Sougnez
- Liming Yang
- Zhong Chen
- Anthony Saleh
- Han Si
- Tanguy Siewert
- Angela Hadjipanayis
- Ann Marie Egloff
- Curtis Pickering
- Paul Boutros
- Kenna Shaw
- Julie Gastier-Foster
- Raju Kucherlapati
- Leslie Cope
- Gordon Robertson
- Joseph Califano
- Lauren Byers
- Vonn Walter
- Ludmila Danilova
- Mitchell Frederick
- Maureen Sartor
- Carter Van Waes
- Angela Hui
- Yan Guo
- Alissa Weaver
- Margi Sheth
- Sergey Ivanov
- Michele Hayward
- Ashley Salazar

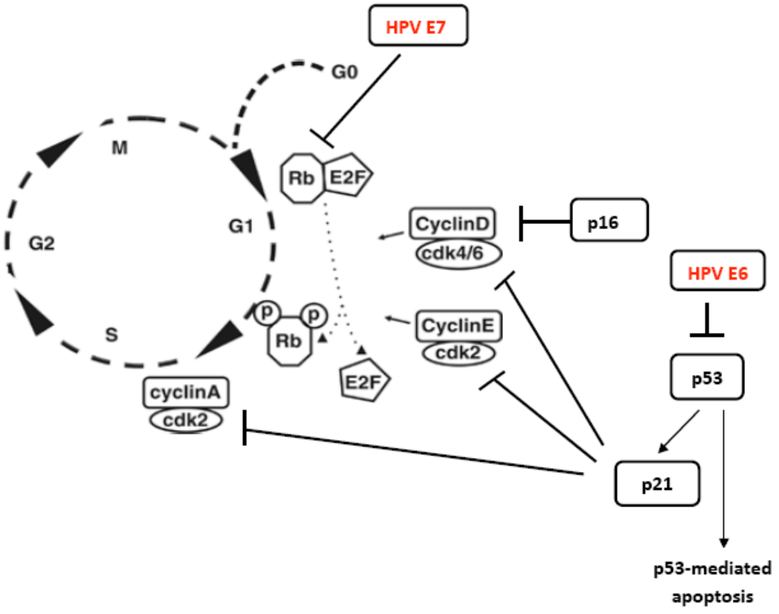
Institutions

- Albert Einstein
- BCGSC
- Broad
- Chicago
- Dana-Farber
- Harvard
- IGC
- Johns Hopkins
- MDACC
- Michigan
- NCH
- NIDCD
- Ontario
- Pittsburgh
- Princess Margaret
- UNC
- Vanderbilt
- Yale

Epidemiology: Head and Neck Cancer i a common disease

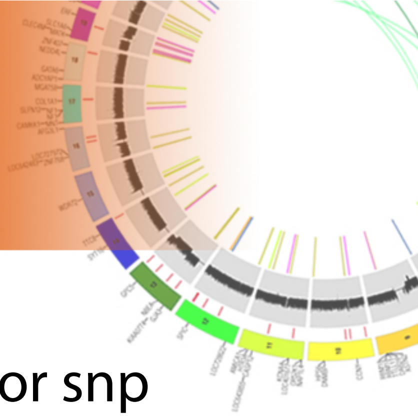


- 5th most common cancer worldwide
  - 500,000 cases / year
  - 200,000 deaths
- Most common cancer in central Asia
- 6th most common cancer in US
  - 45,000+ cases annually
- Risk factors
  - Smoking (80% attributable risk)
  - Human papilloma virus



Journal of Cancer Research and Therapeutics – April 2011

HNSCC - Data Freeze



- 279 samples = complete cases (exon sequencing, tumor snp chips, RNA sequencing, methylation, miRNA sequencing)

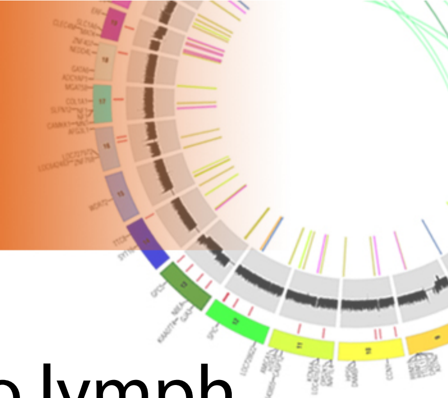
- 84/279 - have low pass tumor and normal
- 9/279 have a second matched normal
- 37/279 - have "matched normal RNA and miRNA"
- 253/279 - blood aliquot (+18 with tumor adjacent normal SNP)
- 9/279 - no matched snp chip
- 71/279 - tumor adjacent normal SNP
- 50/279 - "normal methylation“
- 212 – RPPA data

Demographics

- Median age 61
  - Versus 57 from SEER
- 10% minority
  - Mostly African American
- Smoking
  - Never = 20%
  - Light(<15 pack yr)28%
  - Heavy = 52%
- 73% male

- 11% HPV positive by sequencing analysis
- Tumor site
  - Oral cavity 62%
  - Larynx 26%
  - Oropharynx 11%
  - Hypopharynx 1%

Demographics

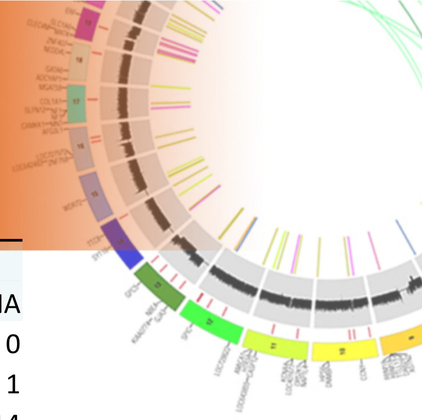


- Stage I – 5%
- Stage II – 20%
- Stage III – 16%
- Stage IVa – 57%
- Stage IVb – 2%
- Stage IVc <1%

- Alive – 44%
- Deceased – 66%

- Stage I-II = no lymph nodes, smaller tumors
- Stage III = larger tumors or single small lymph node
- Stage IV a & b = bone involvement, large tumors, and / or multiple nodes
- Stage IVc - distant metastases

HPV Status?



		Clinical p16		
		Negative	Positive	NA
Clinical ISH	Negative	31	0	0
	Positive	0	4	1
	NA	1	2	214
			DNA sequencing	
		Positive	NA	
Clinical ISH	Negative	0	31	
	Positive	5	0	
	NA	29	190	
		Positive	NA	
Clinical p16	Negative	0	32	
	Positive	6	0	
	NA	26	189	
		RNA sequencing		
		Definite(>=1000)	Some evidence (1-1000)	Negative (count = 0)
Clinical_ISH	Negative	0	8	23
	Positive	5	0	0
	NA	26	53	138
Clinical_p16	Negative	0	9	23
	Positive	6	0	0
	NA	25	52	138
LowPass	Positive	6	4	0
	NA	25	57	161
DNA sequence	Positive	26	6	0
	NA	5	55	161

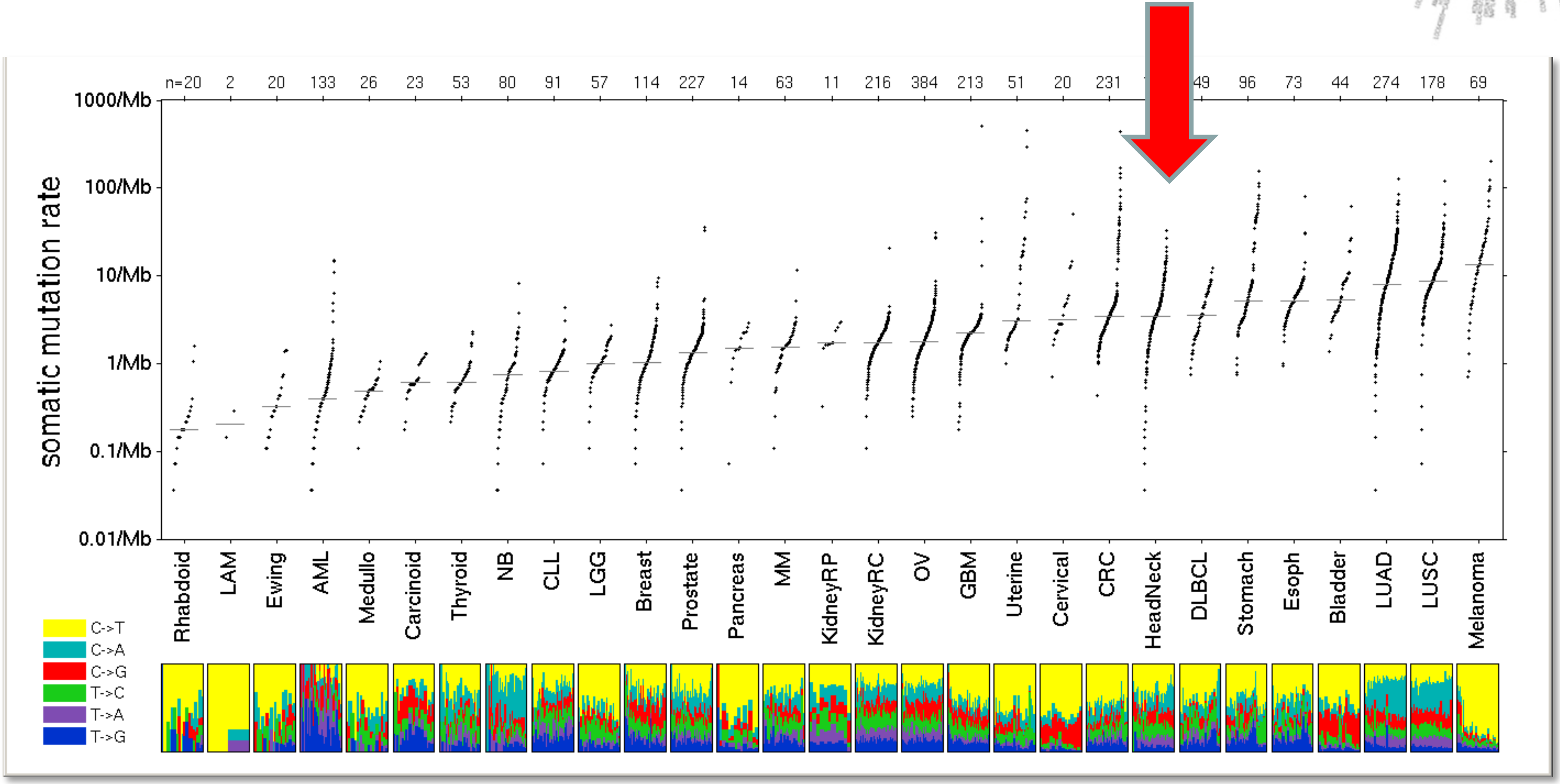
Tumor site

Smoking status

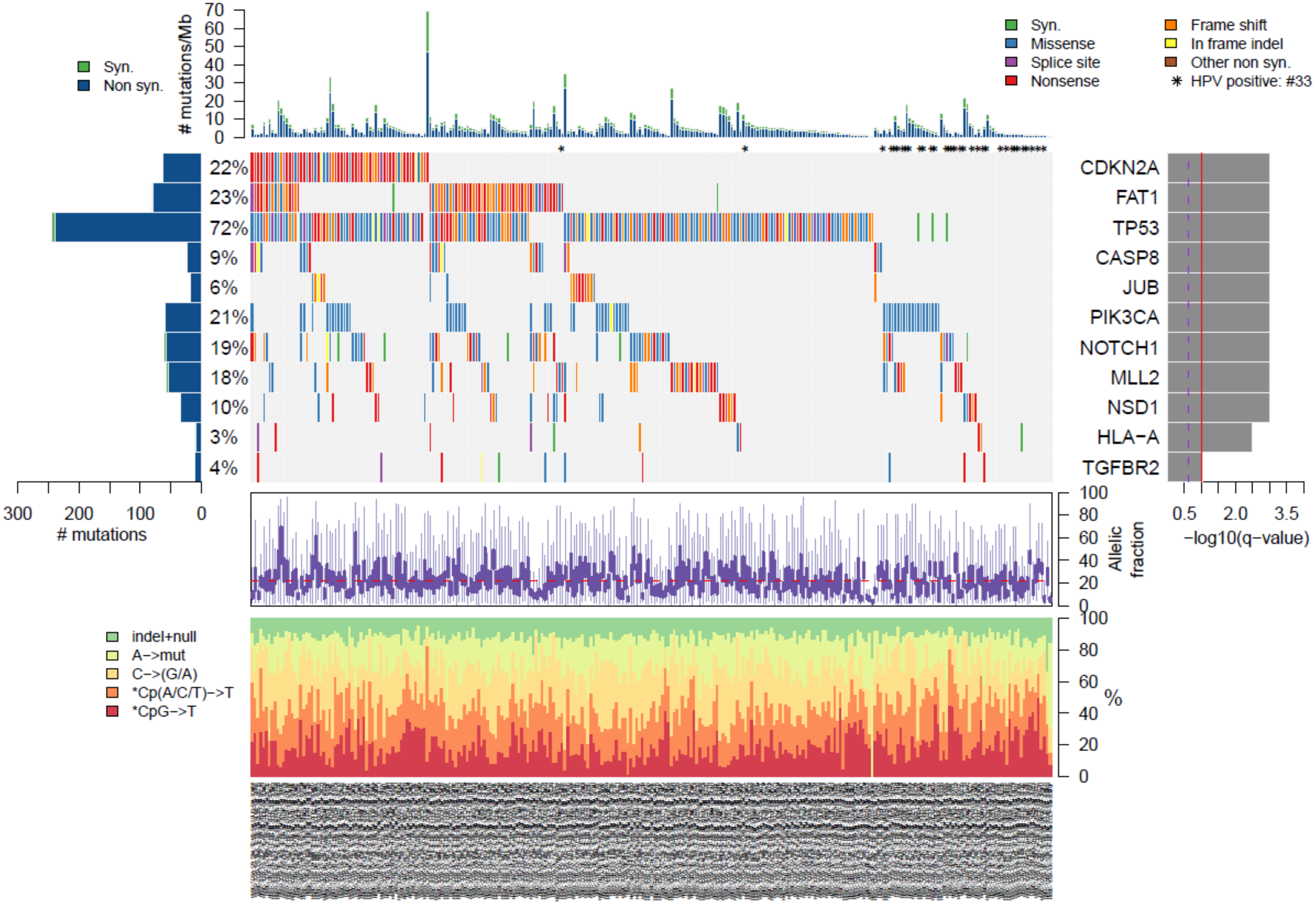
Conclusion for cohort

- Current data freeze is the largest genomic dataset ever assembled for each of the individual components by a factor of at least 2 (with >200 samples in the pipeline)
- Integrated
- Clinical data
- Limitations
  - Surgical cohort
    - Few oropharynx / HPV samples
    - Few small tumors
  - Relatively small “clinical” cohort given the heterogeneity of sites, stages, and risk factors
  - HPV assessment

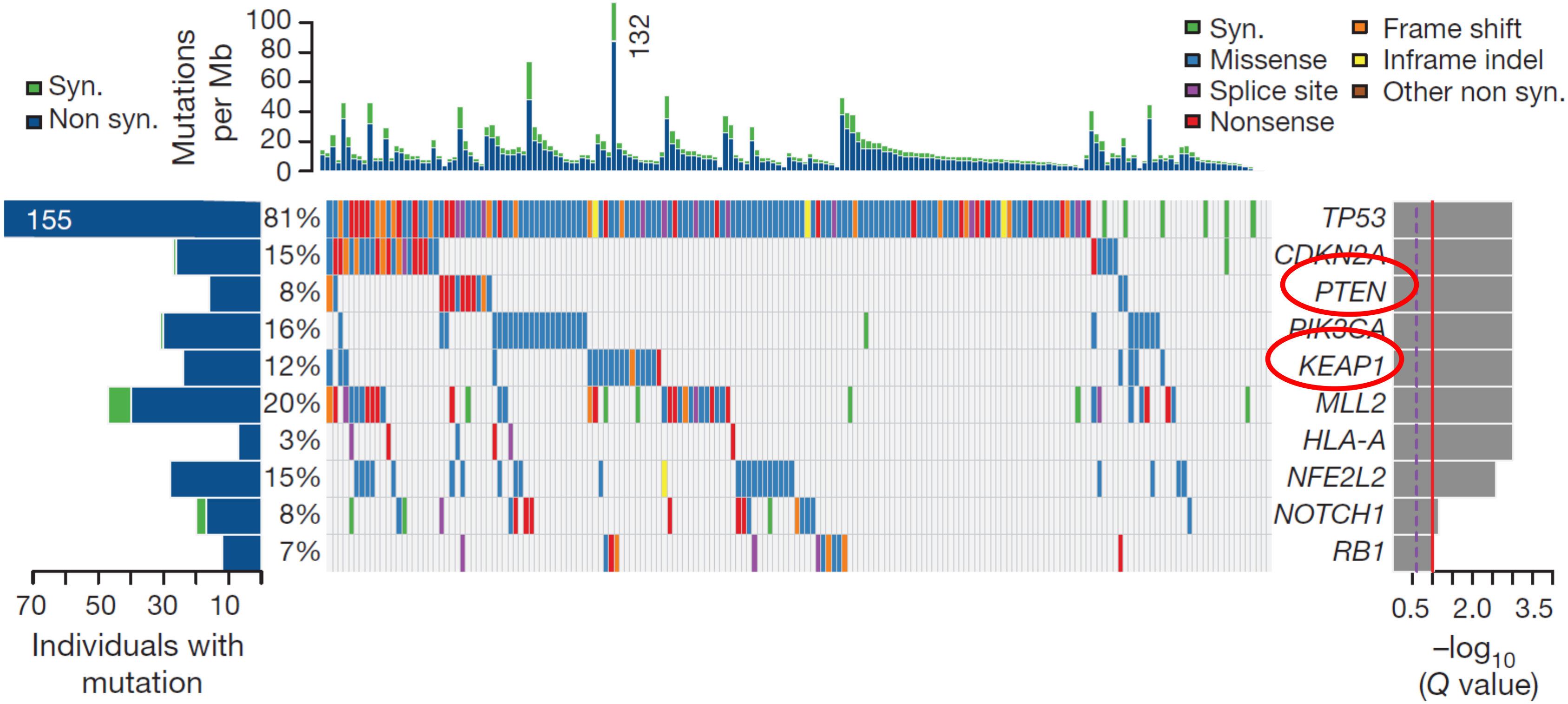
The big picture- NSCLCs are among the most genomically deranged of all cancers



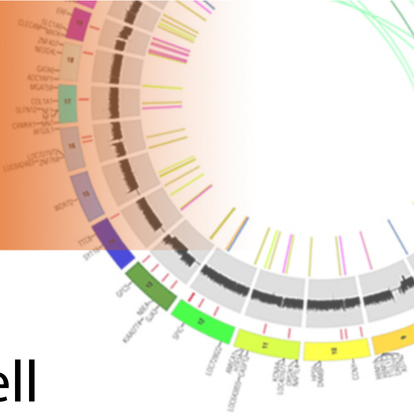
Significantly mutated genes



Lung Squamous Cell Carcinoma



Observation



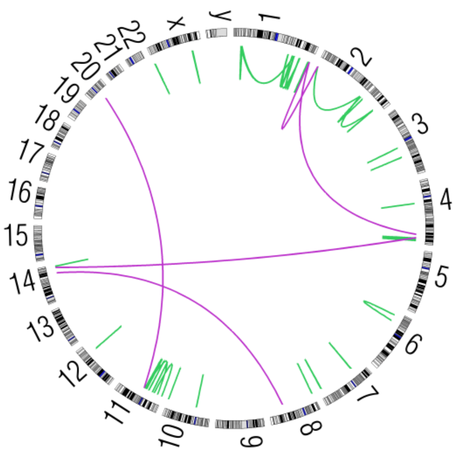
- HPV negative HNSCC looks a lot like lung squamous cell carcinoma
  - Mutations
  - Copy Number
  - Expression patterns
  - Pathways

- HPV positive HNSCC looks a lot like other HPV positive tumors (data not shown)

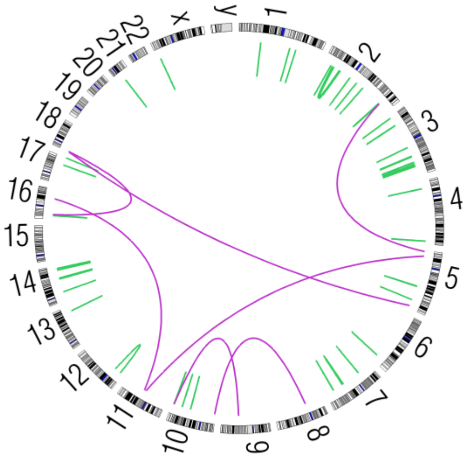
HPV+(n=34) vs. HPV- (n=254)

Significant difference in terms of mutation rate				
Common sig genes (4) HPV+ q < 0.25 (25) HPV- q < 0.1(48)	# Non Silent mutations		Mutation Rate	
	HPV+	HPV-	HPV+	HPV-
PIK3CA	12	49	0.353	0.193
MLL2	9	45	0.265	0.177
NSD1	6	28	0.176	0.11
MUC16	16	67	0.471	0.264
Wilcoxon Rank Sum Test	P value = 0.2 (Not significant due to small sample size)			
t. test	Not available due to small sample size			

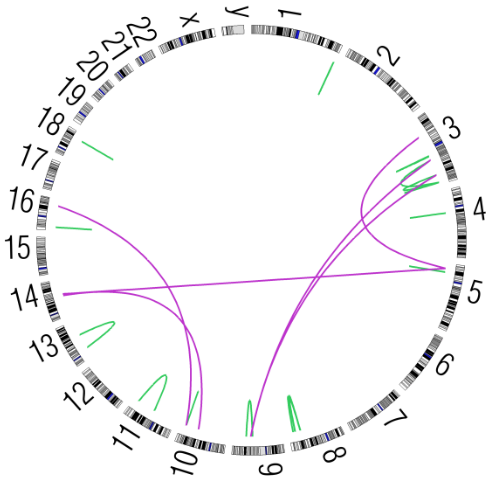
BB-4225 (50X)
73M BOT, HPV33,
Light tob



BA-4077 (26X)
47F HPV16 BOT
Light tob

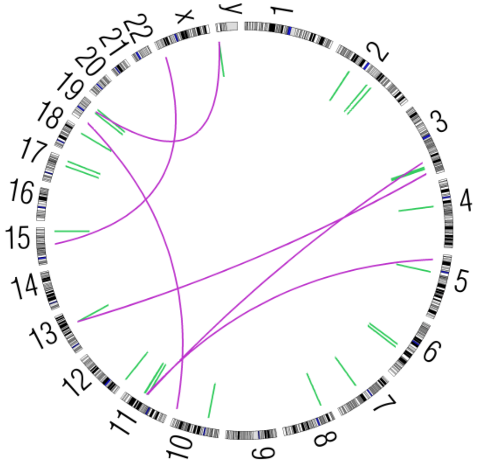


BA-5153 (31X)
51M tonsil, HPV16
No tob



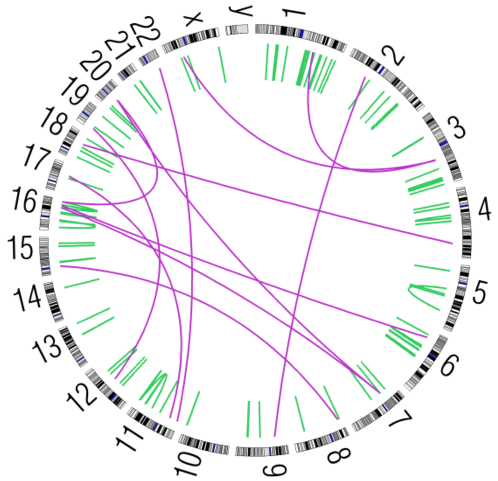
TRIO-PPP2R5E

CN-4741 (36X)
75M alveolar ridge, HPV16
Light tob



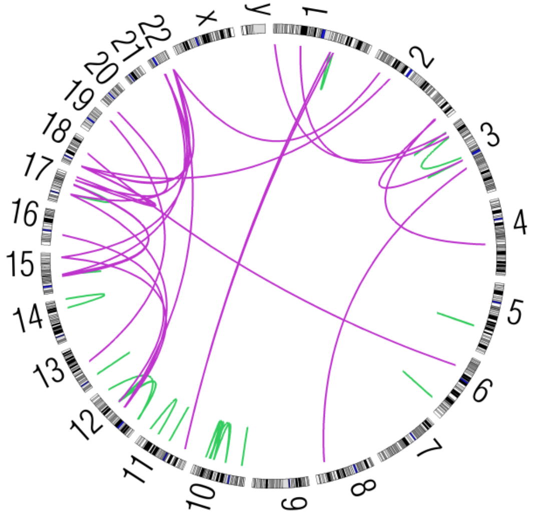
GPR149-RSF1, ERC1 del

CR-6472 (35X)
59M BOT HPV16,
No tob

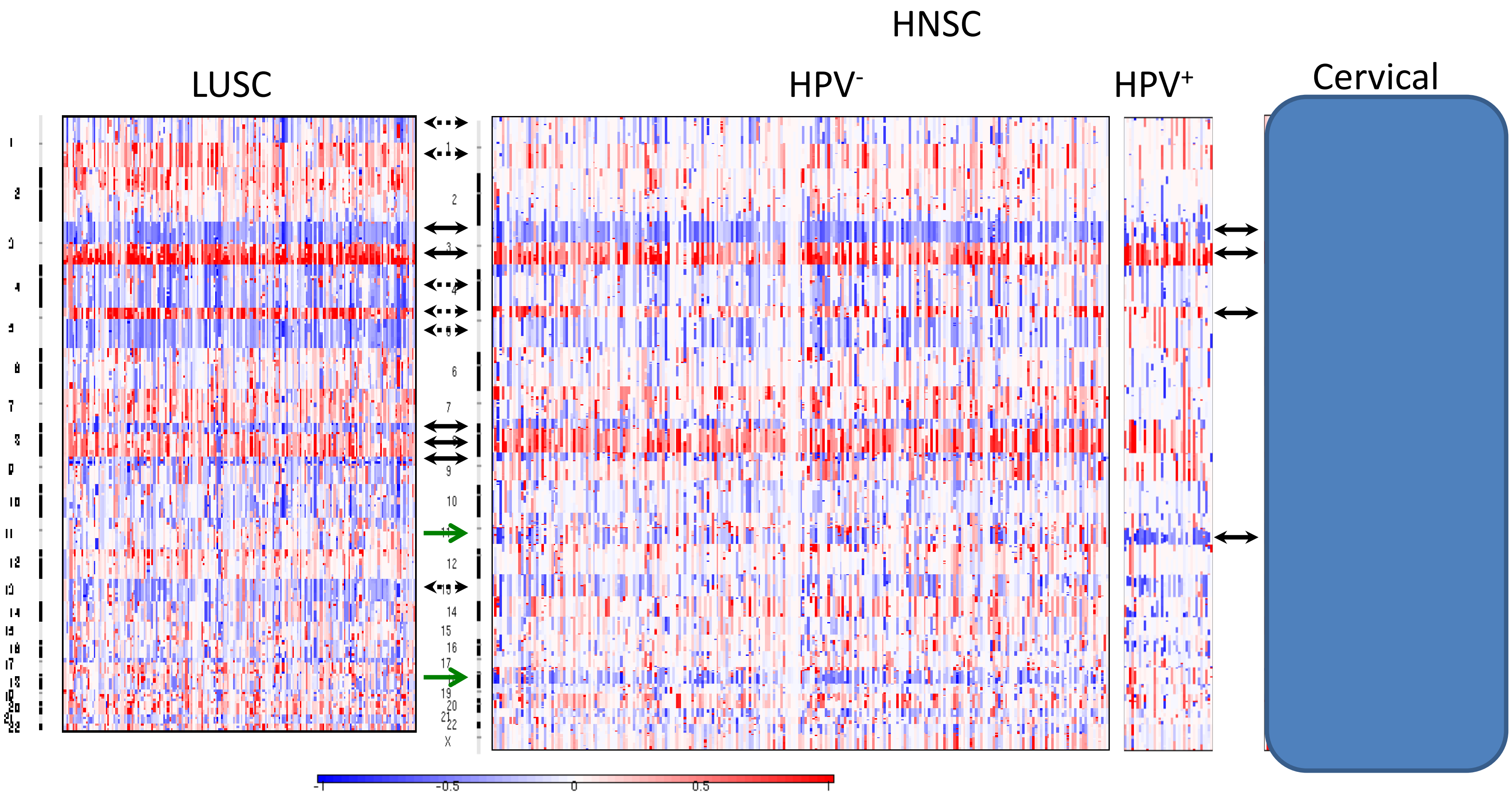


NFE2L3-CBX3, ETS1-ME3

CR-6480 (40X)
53M tonsil HVP 16
No tob



Pattern of SCNAs in HNSC are Similar to that in LUSC

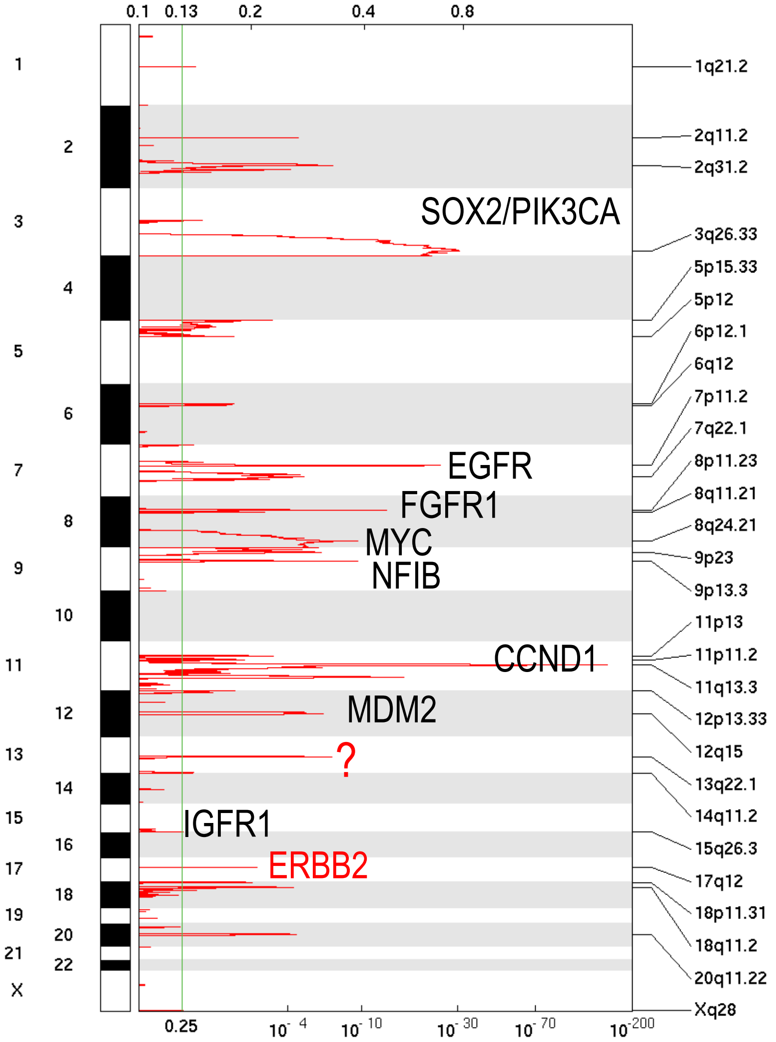


↔ Common to both

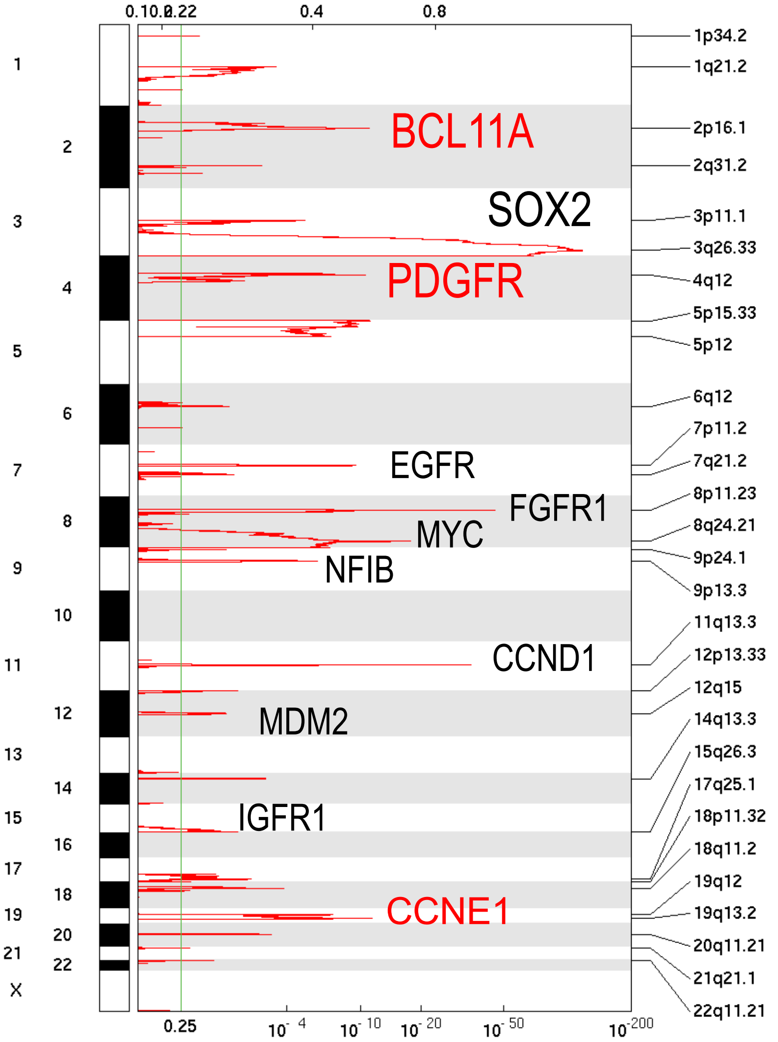
⇠⇢ Less frequent in HNSC

→ Distinctive to HNSC

Comparison of Reoccurring Focal Amplifications between HNSC and LUSC



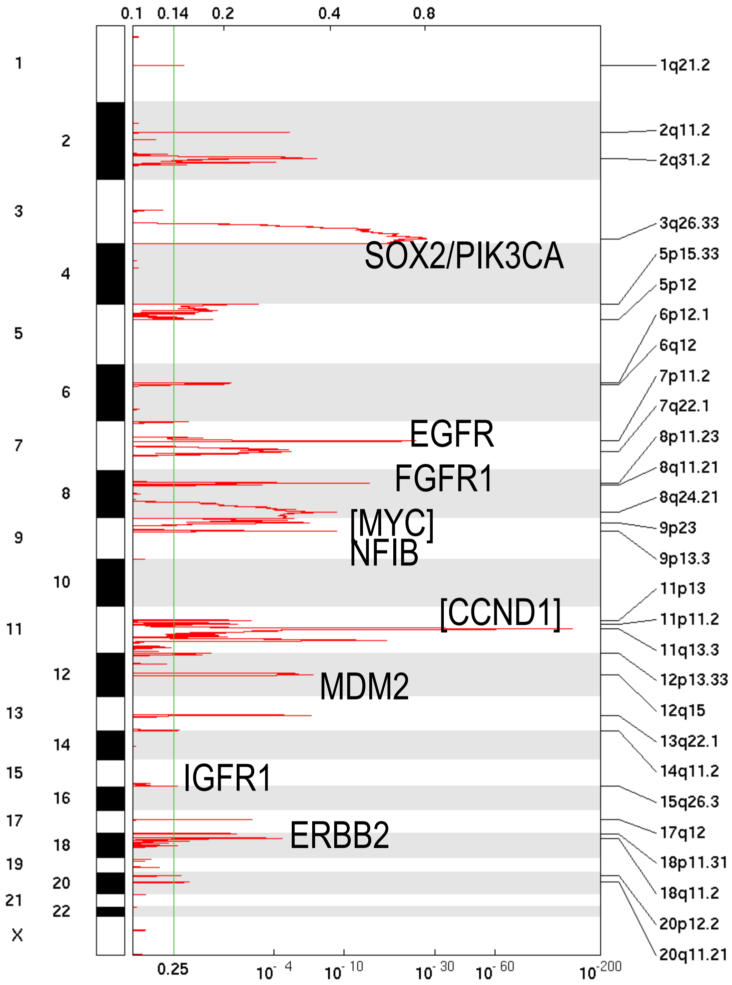
HNSC



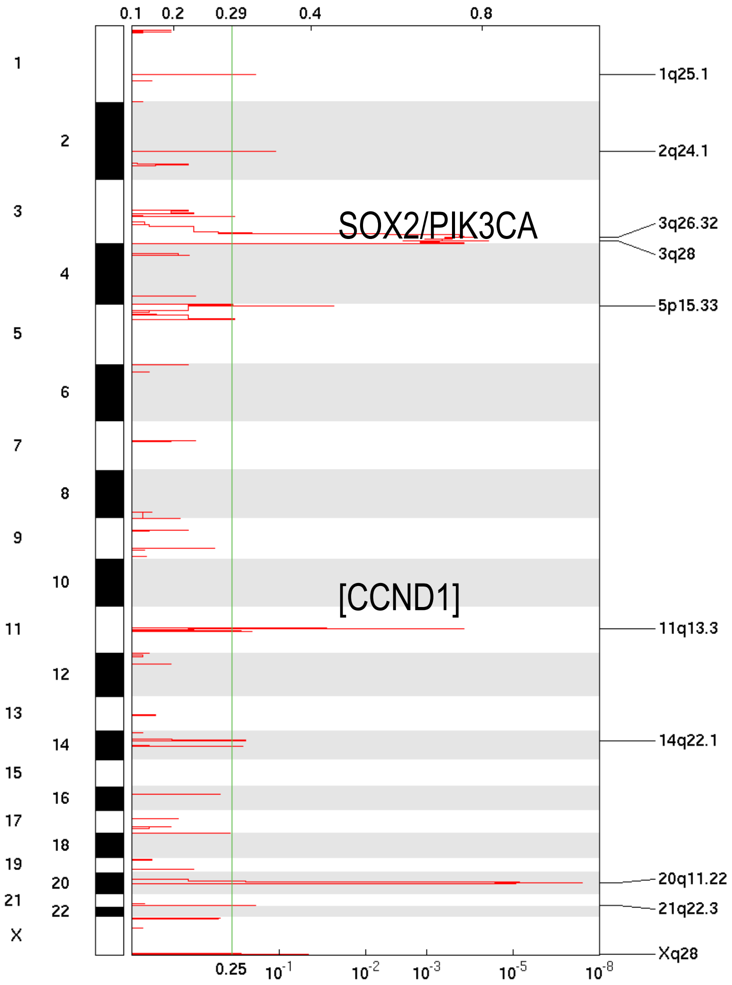
LUSC

HPV^{+} Tumors Lack Reoccurring Focal Amps with RTKs

HPV^{-}

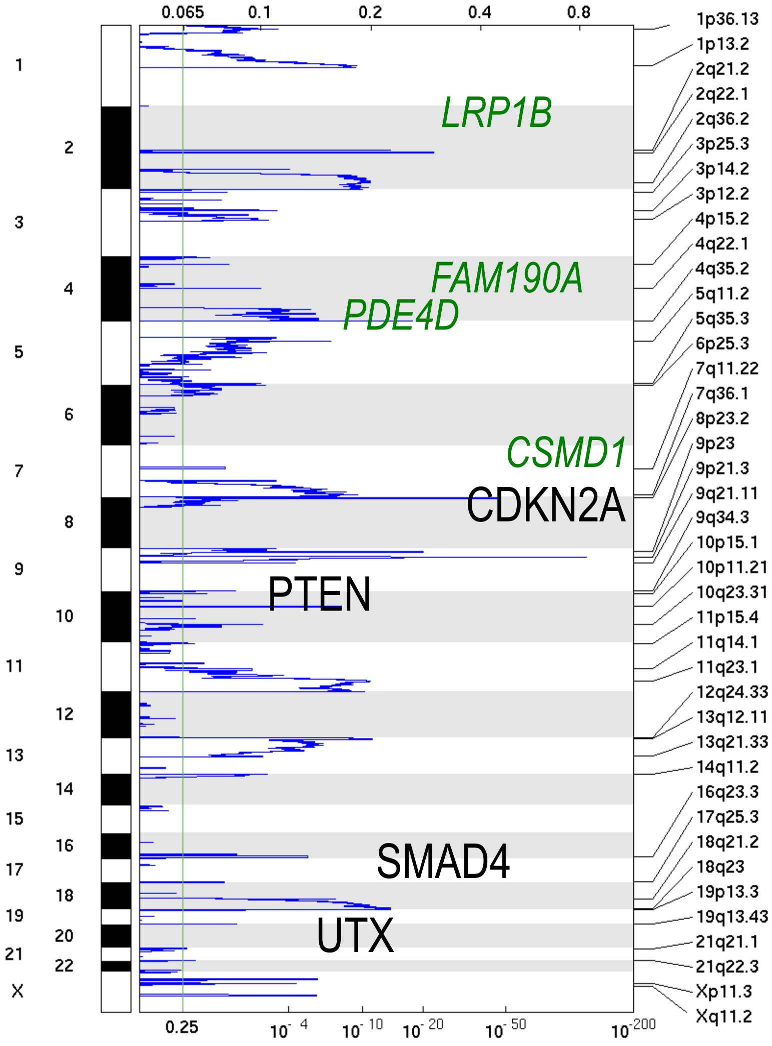


HPV^{+}

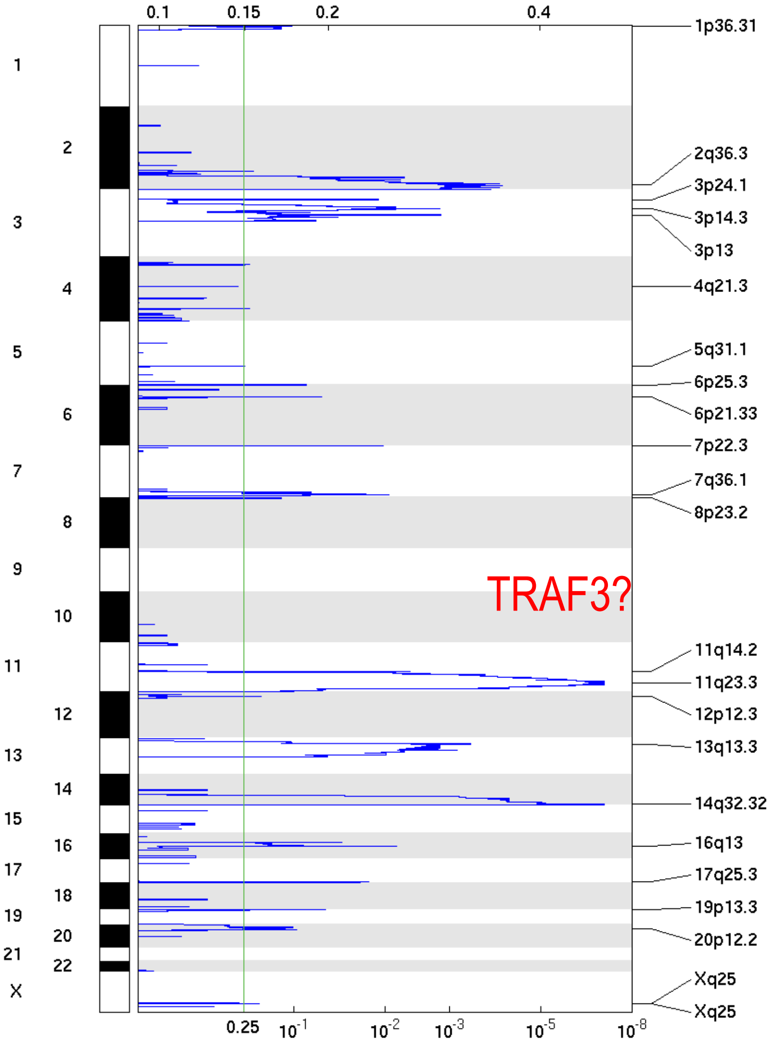


Comparison of Reoccurring Focal Deletions between HPV$^+$ and HPV$^-$ HNSC

HPV$^-$

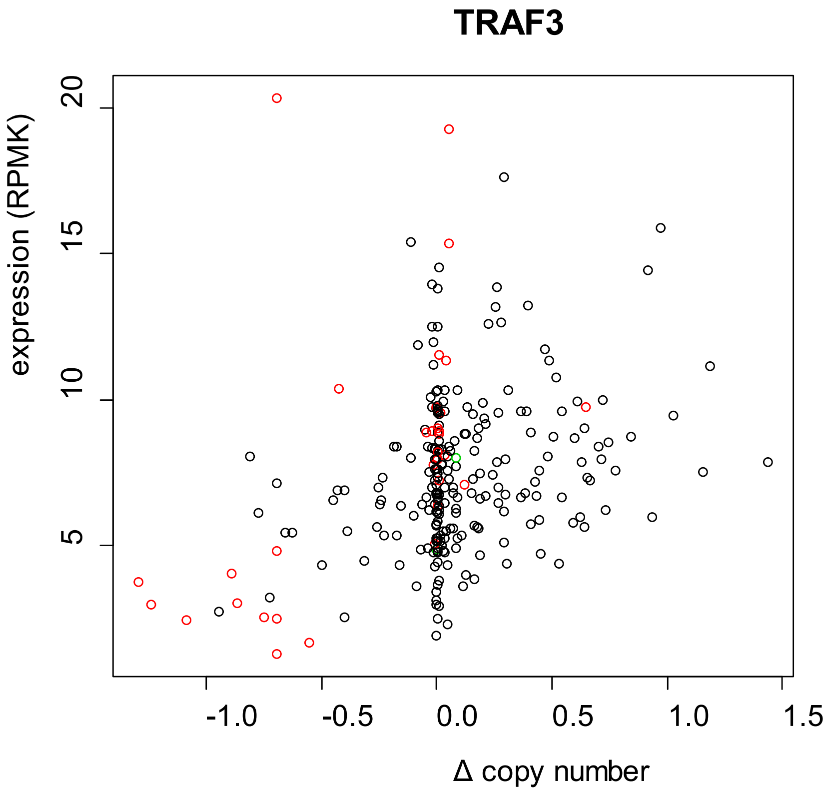


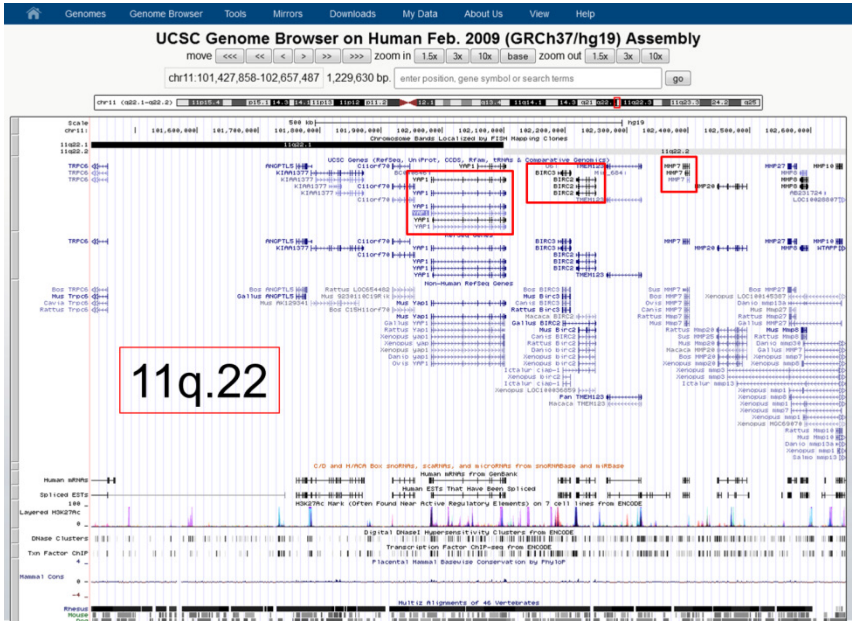
HPV$^+$



Black = Shared Tumor Suppressors

Green = Fragile Sites





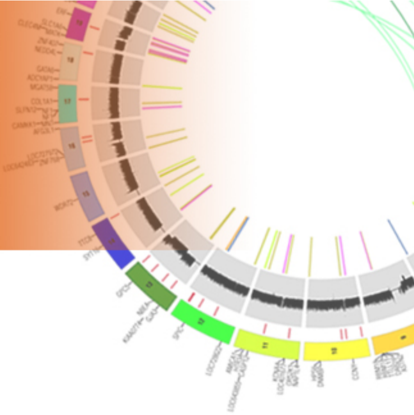
UCSC Genome Browser on Human Feb. 2009 (GRCh37/hg19) Assembly

chr11:101,427,858-102,657,487 1,229,630 bp.

enter position, gene symbol or search terms

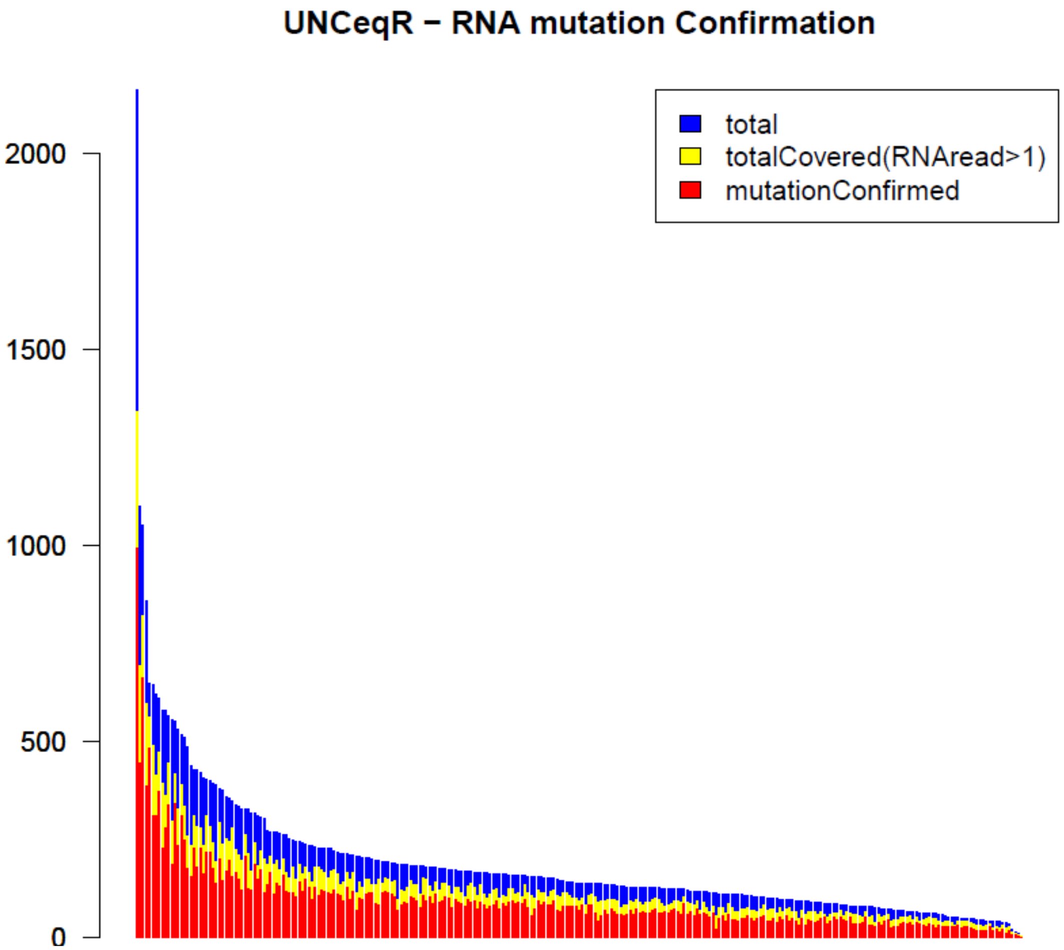
○ HPV+
○ Unknown

Observation

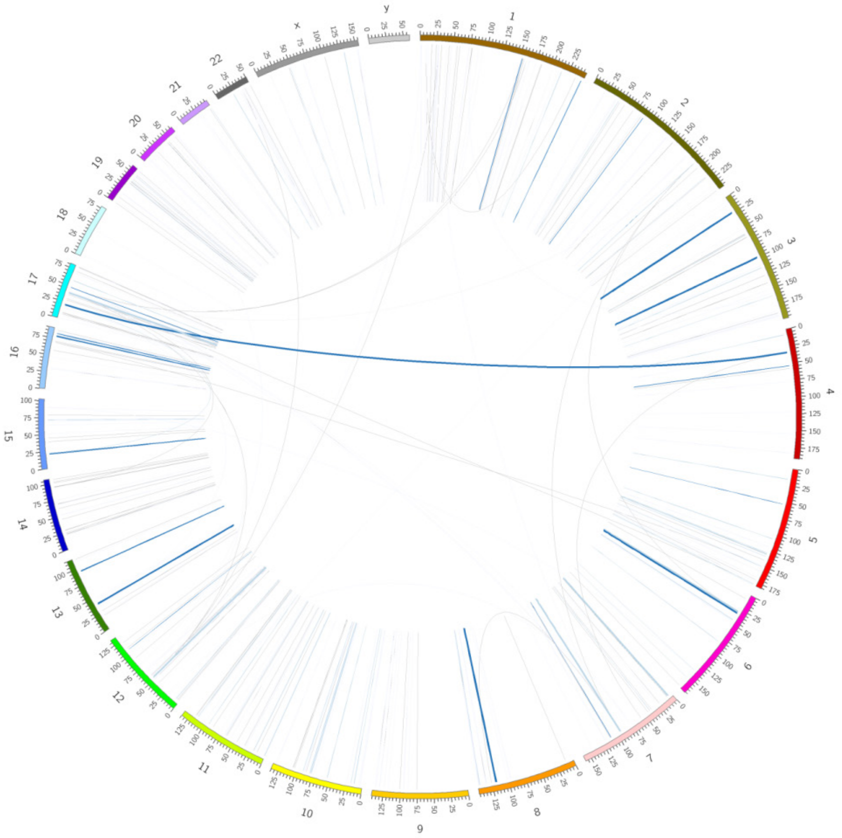


- Copy number landscape is rich for HNSC
- Confident attribution of the gene even in narrow peeks is difficult, akin to functional prediction for somatic variants

RNASeq: Mutation validation



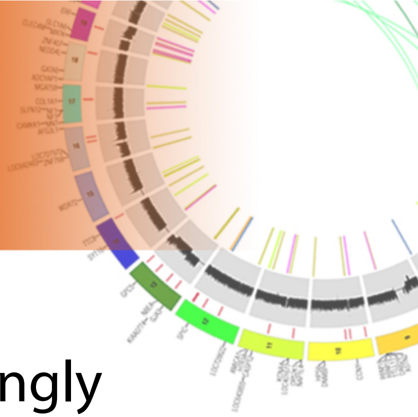
RNAseq: Structural variants and deeper coverage



Click anywhere on the chromosome to center view at that location.

KRT14 – ACO22596

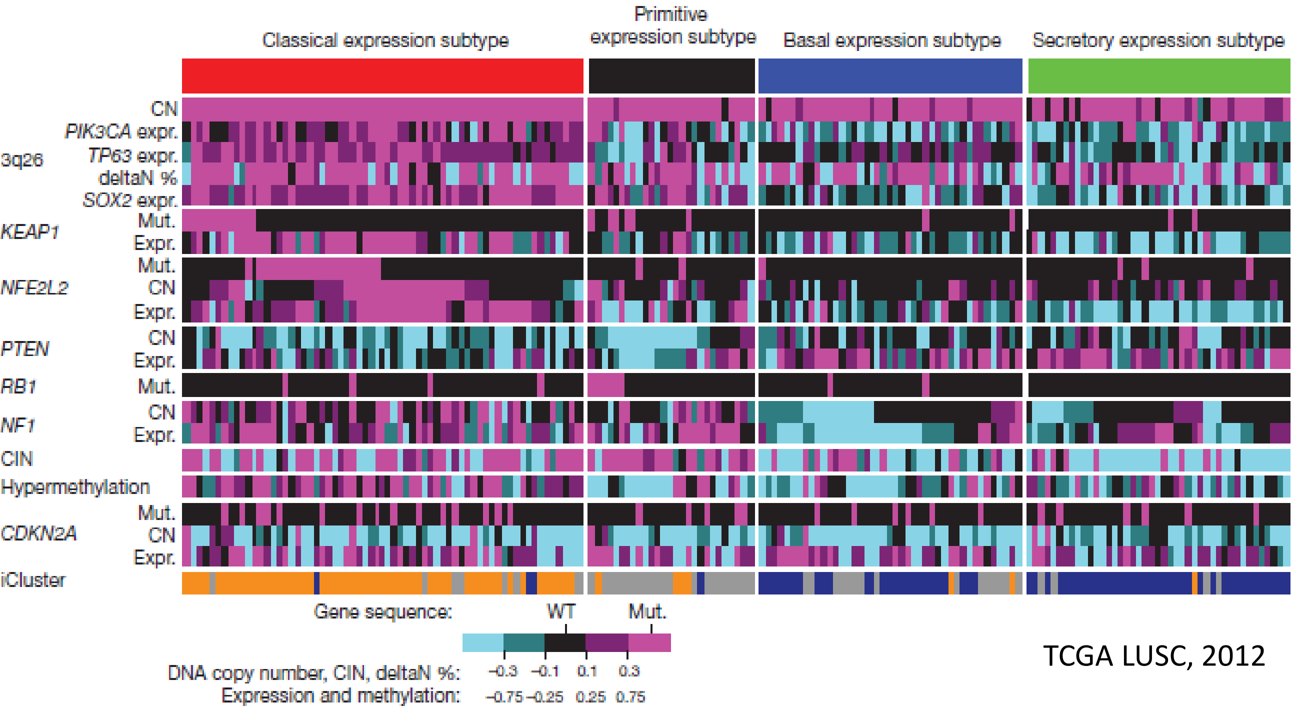
Observation



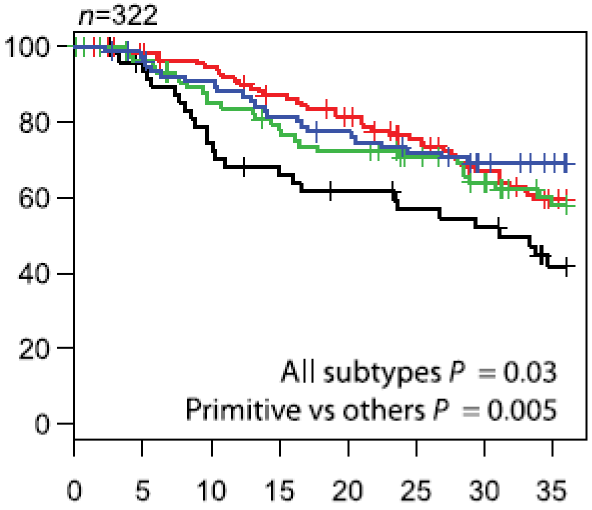
- Convincing evidence from early analysis does not strongly support recurrent in frame gene fusions
- Structural gene rearrangements are common
  - Functional events appear more likely to be inactivating events in tumor suppressor genes
  - Systematic annotation of these events are challenging

Expression Profiling: Background

- Patterns should be (i) statistically significant, (ii) reproducible/valid, (iii) have genomic/clinical relevance

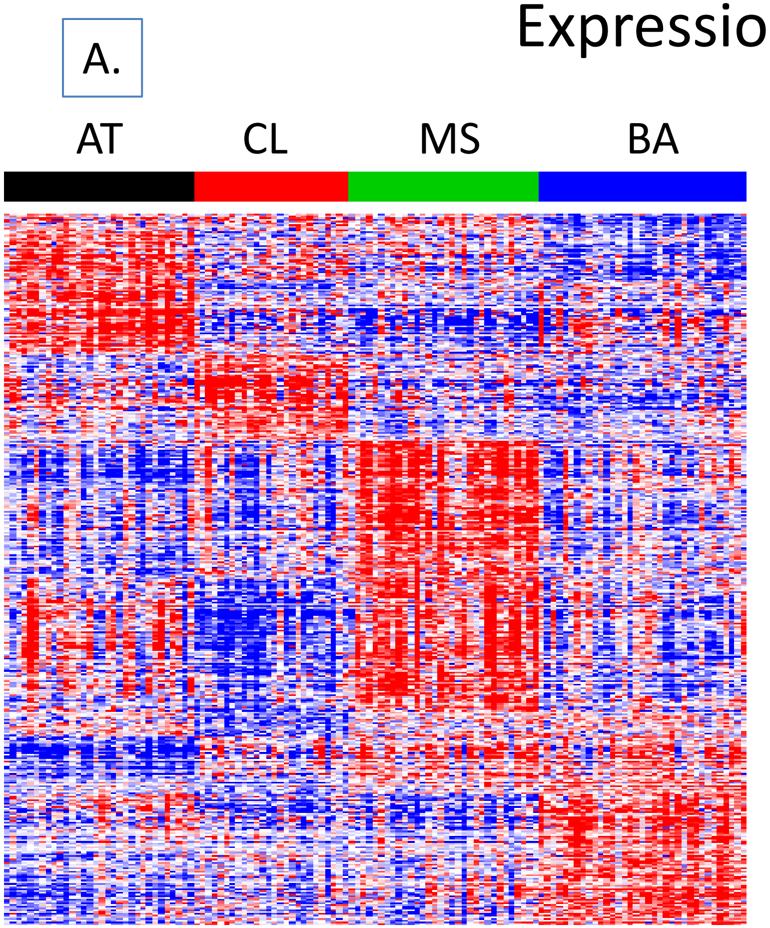


TCGA LUSC, 2012



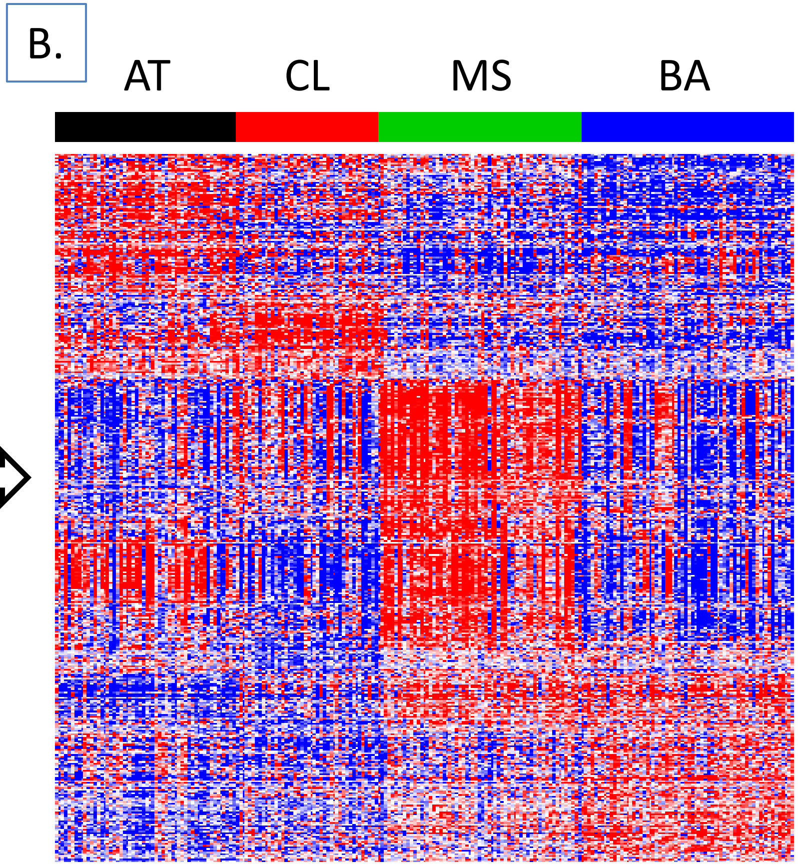
Wilkerson, 2010

Expression Profiling in HNSC

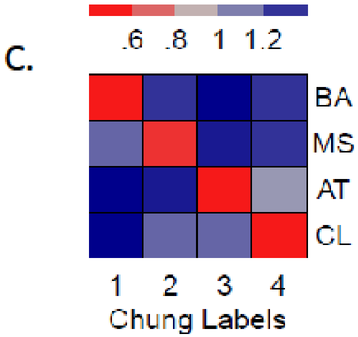


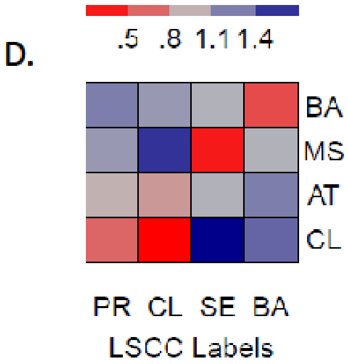
Walter, unpublished

840 gene classifier

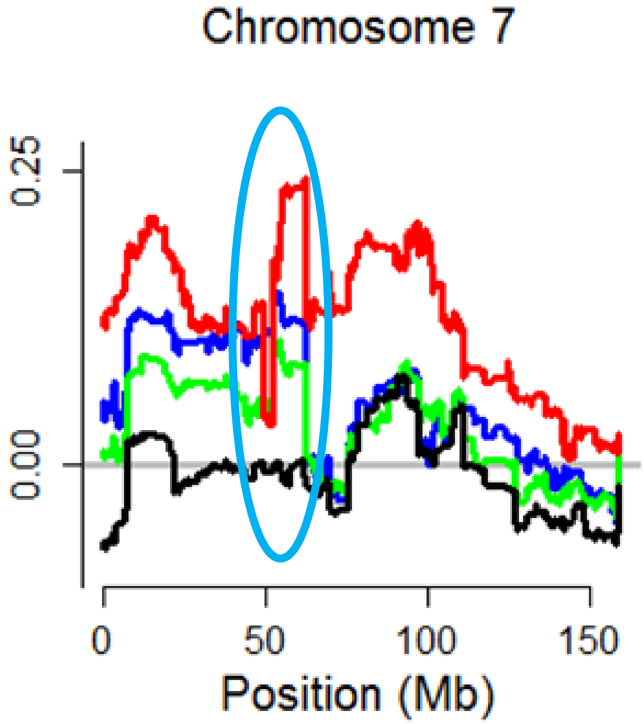


TCGA HNSC, unpublished

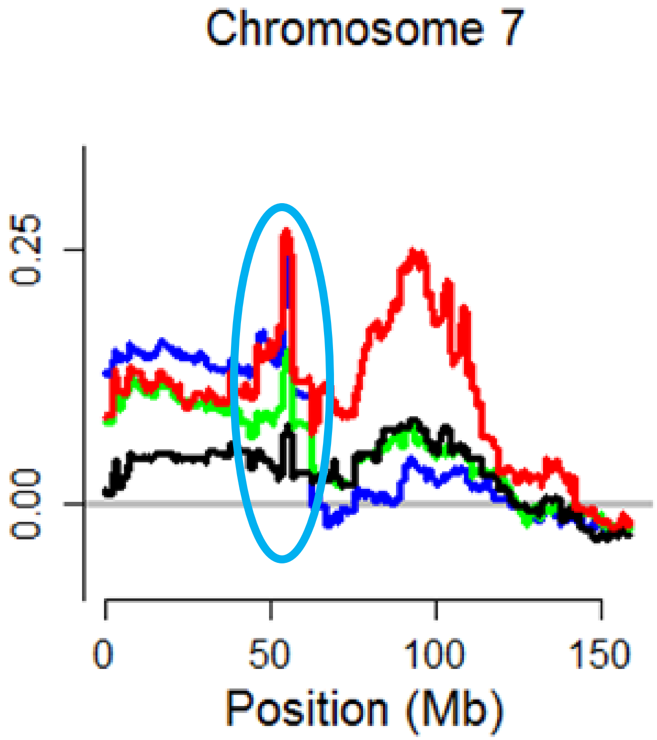




Expression subtypes reflect structural rearrangements

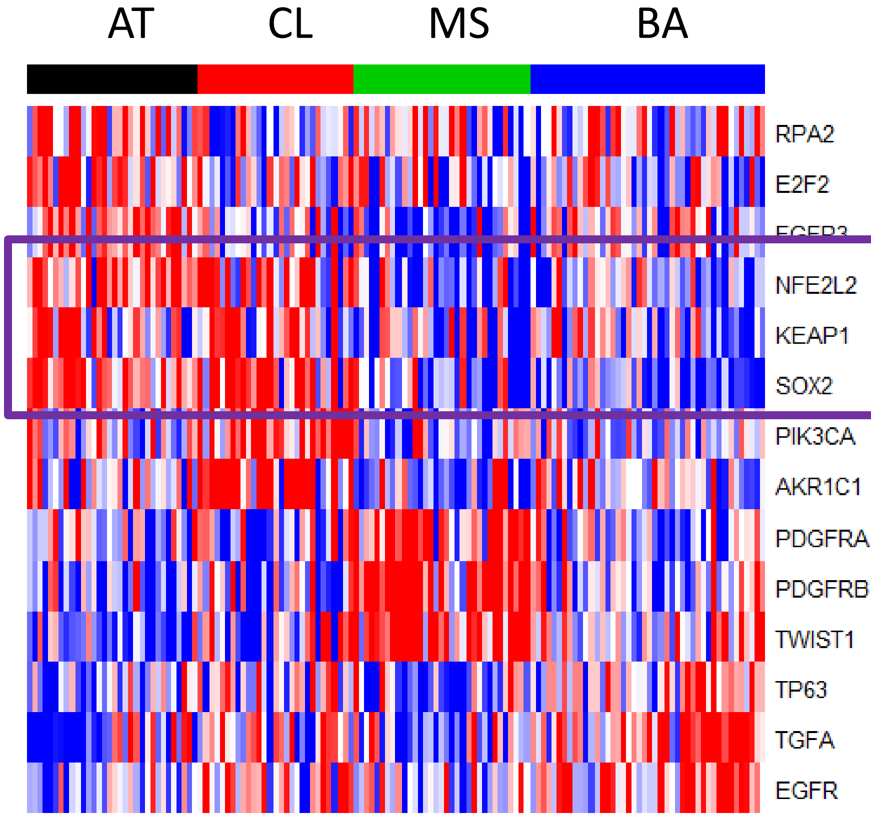


UNC, unpublished

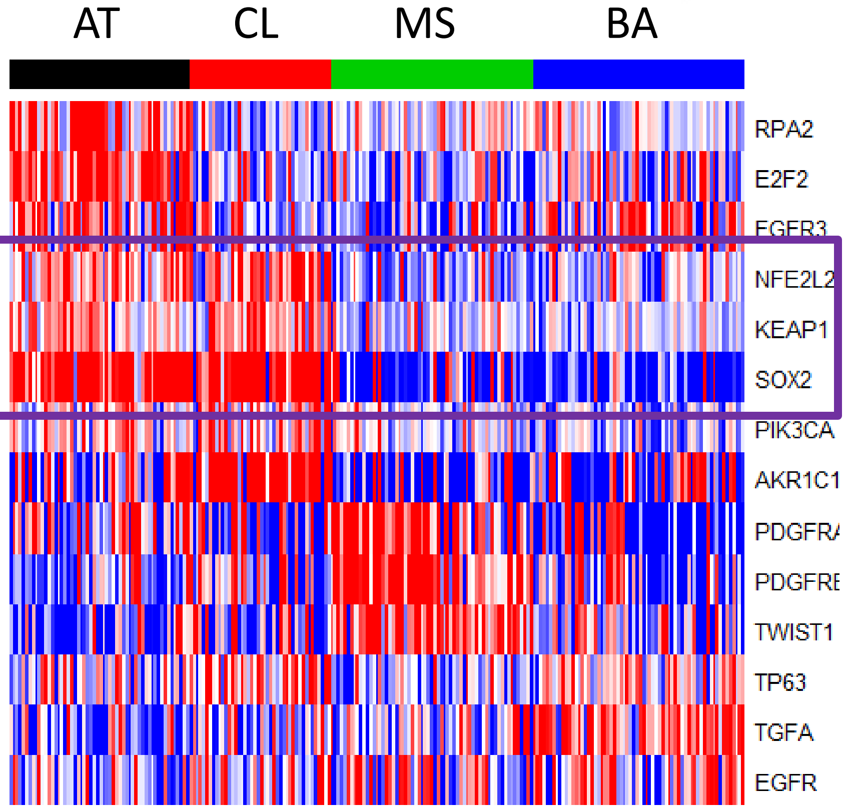


TCGA HNSC, unpublished

Expression Profiling in HNSC

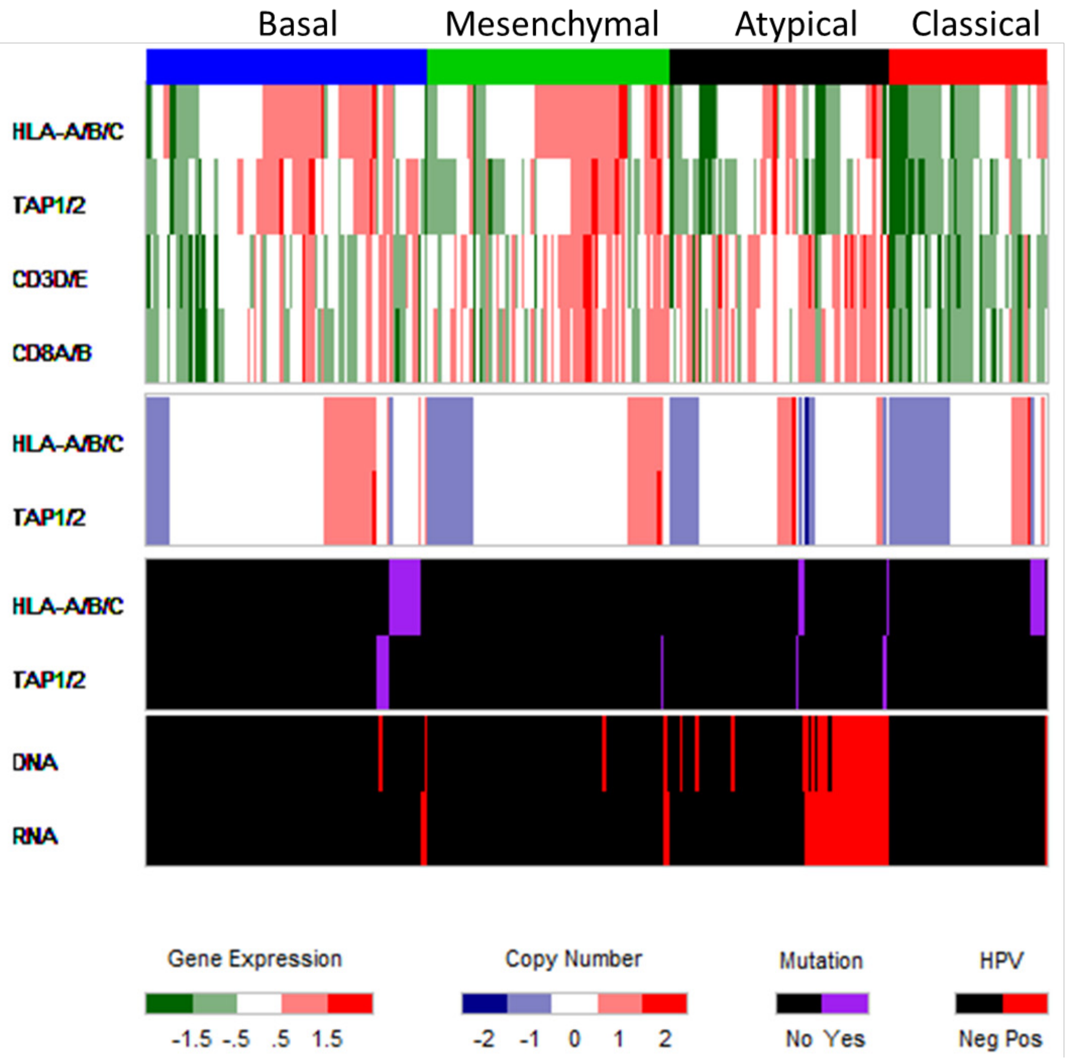


Walter, unpublished

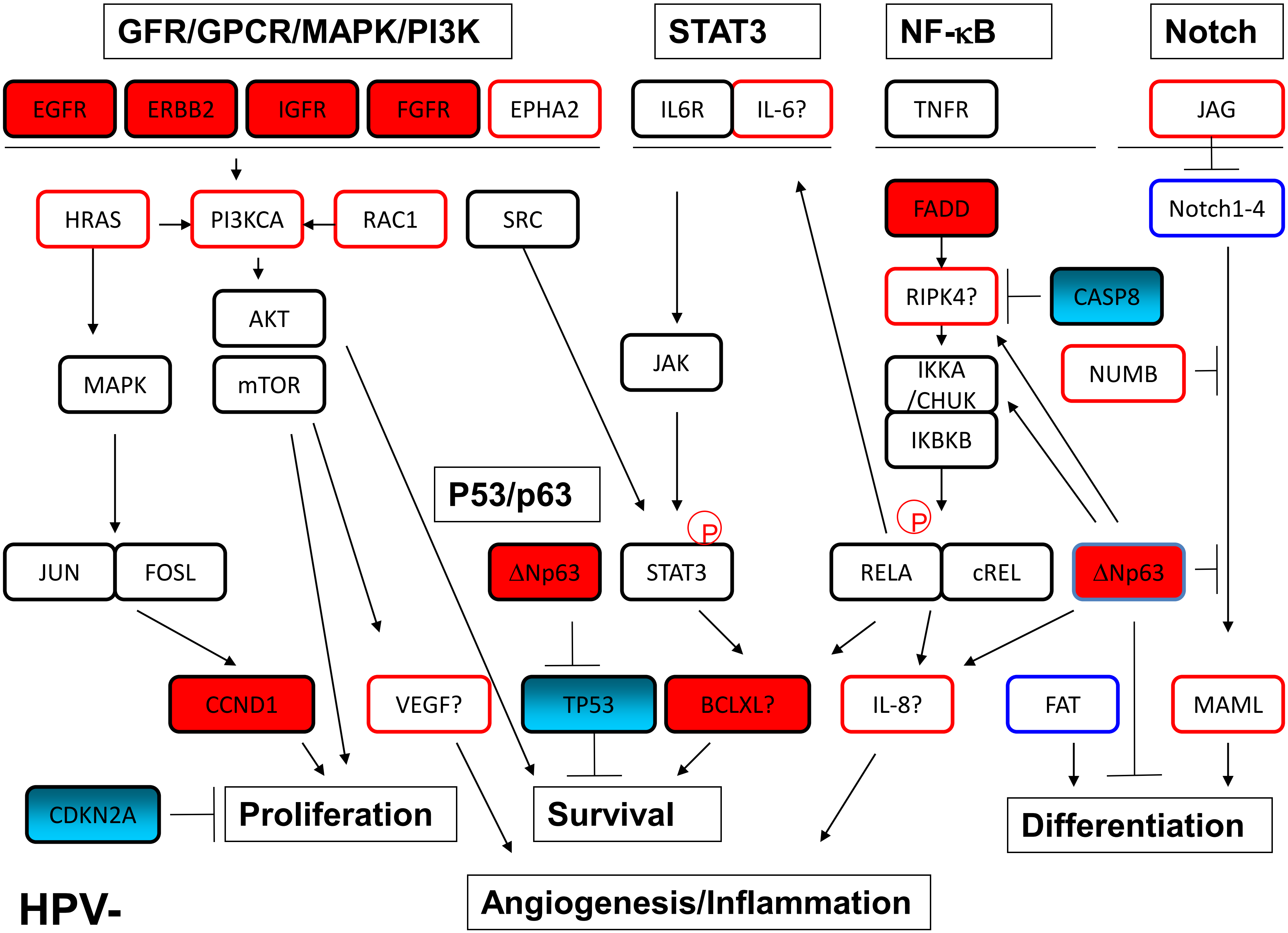


TCGA HNSC, unpublished

Subtypes to evaluated marker genes



The Cancer Genome Atlas



GFR/GPCR/MAPK/PI3K

EGFR
ERBB2
IGFR
FGFR
EPHA2
HRAS
PI3KCA
RAC1
SRC
AKT
mTOR
MAPK
JUN
FOSL
CCND1
VEGF?
CDKN2A

STAT3

IL6R
IL-6?
JAK
P
STAT3
BCLXL?

NF-κB

TNFR
FADD
RIPK4?
CASP8
IKKA /CHUK
IKBKB
P
RELA
cREL
IL-8?

Notch

JAG
Notch1-4
NUMB
ΔNp63
FAT
MAML

P53/p63

ΔNp63
TP53

Proliferation

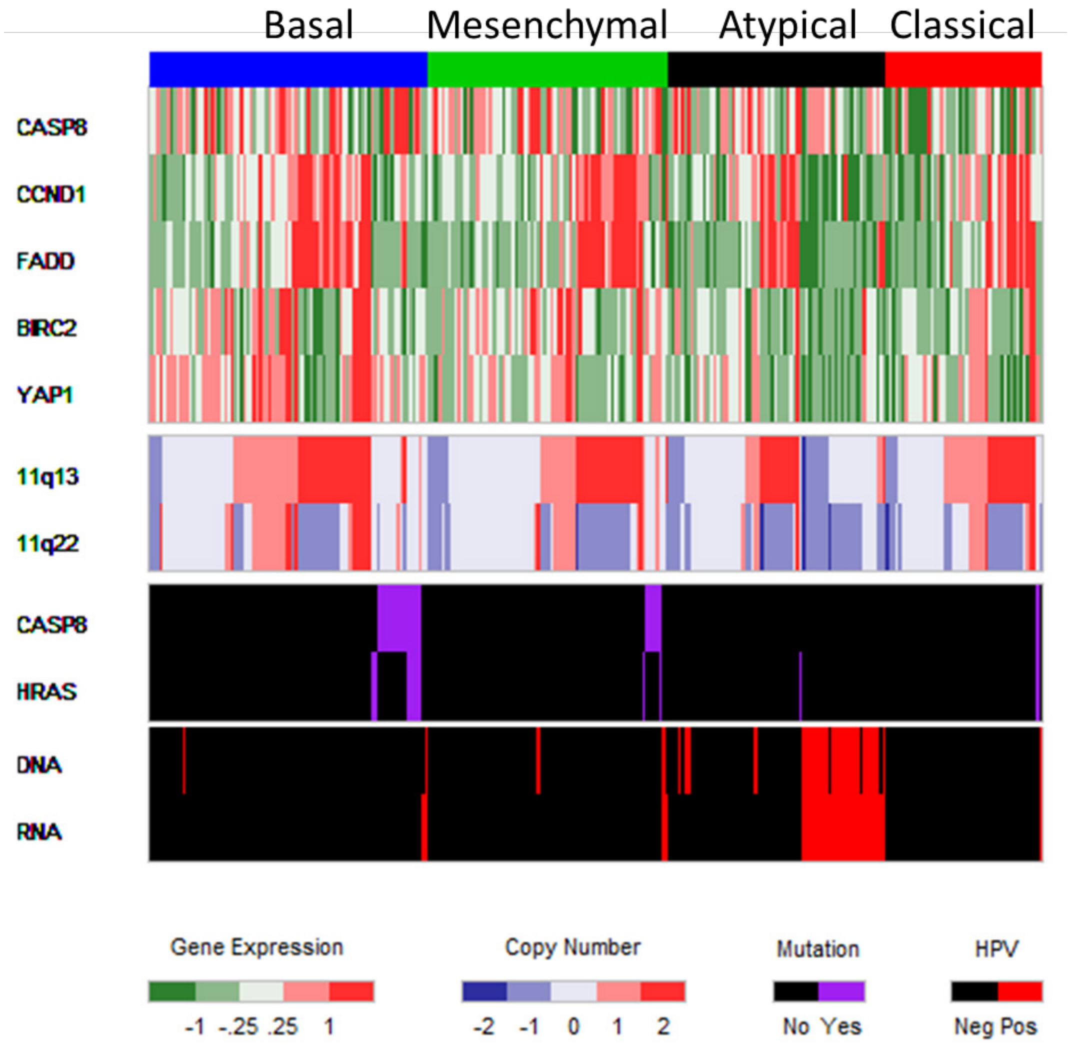
Survival

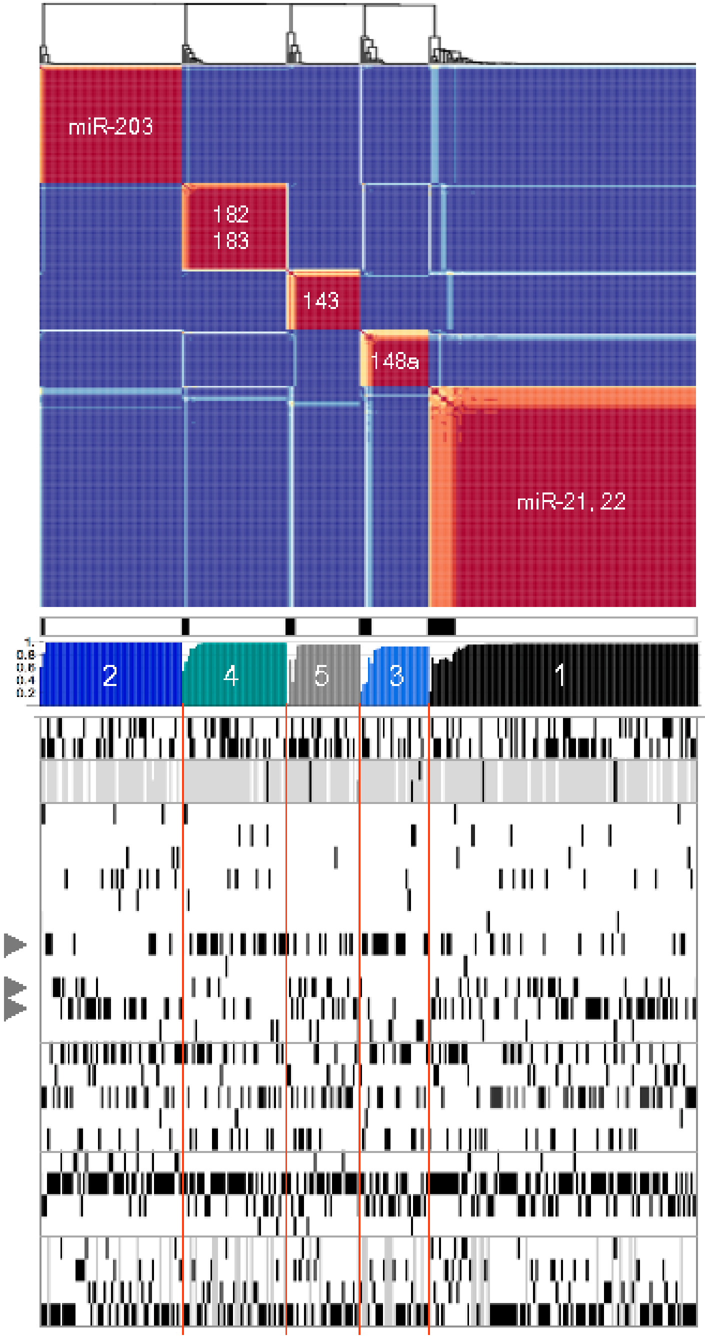
Differentiation

Angiogenesis/Inflammation

HPV-

Subtypes to evaluated pathways:Cell Death/Apoptosis

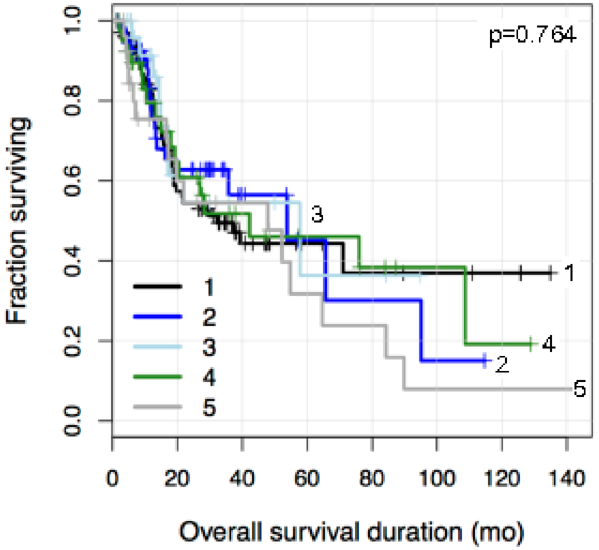




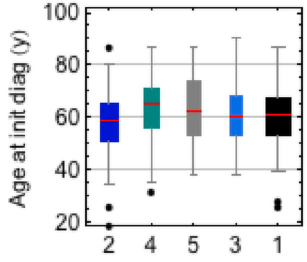
F	gender	F=70,M=189
DEC	vital status	L=156,D=103
ISH	HPV	P=5,N=41
P16		P=6,N=41
alveolar ridge	anatomic location	6
base of tongue		9
buccal mucosa		7
floor of mouth		23
hard palate		4
hypopharynx		3
larynx		68
lip		2
oral cavity		42
oral tongue		77
tonsil		18
N0	lymph node spread	82
N1		27
N2,2a,b,c		86
N3		4
NX		47
G1	grade	18
G2		169
G3		67
GX		5
I	stage	14
II		35
III		35
IVA,B		140

P N NA

HNSC Public Clinical Data Sep-17-2012.xlsx



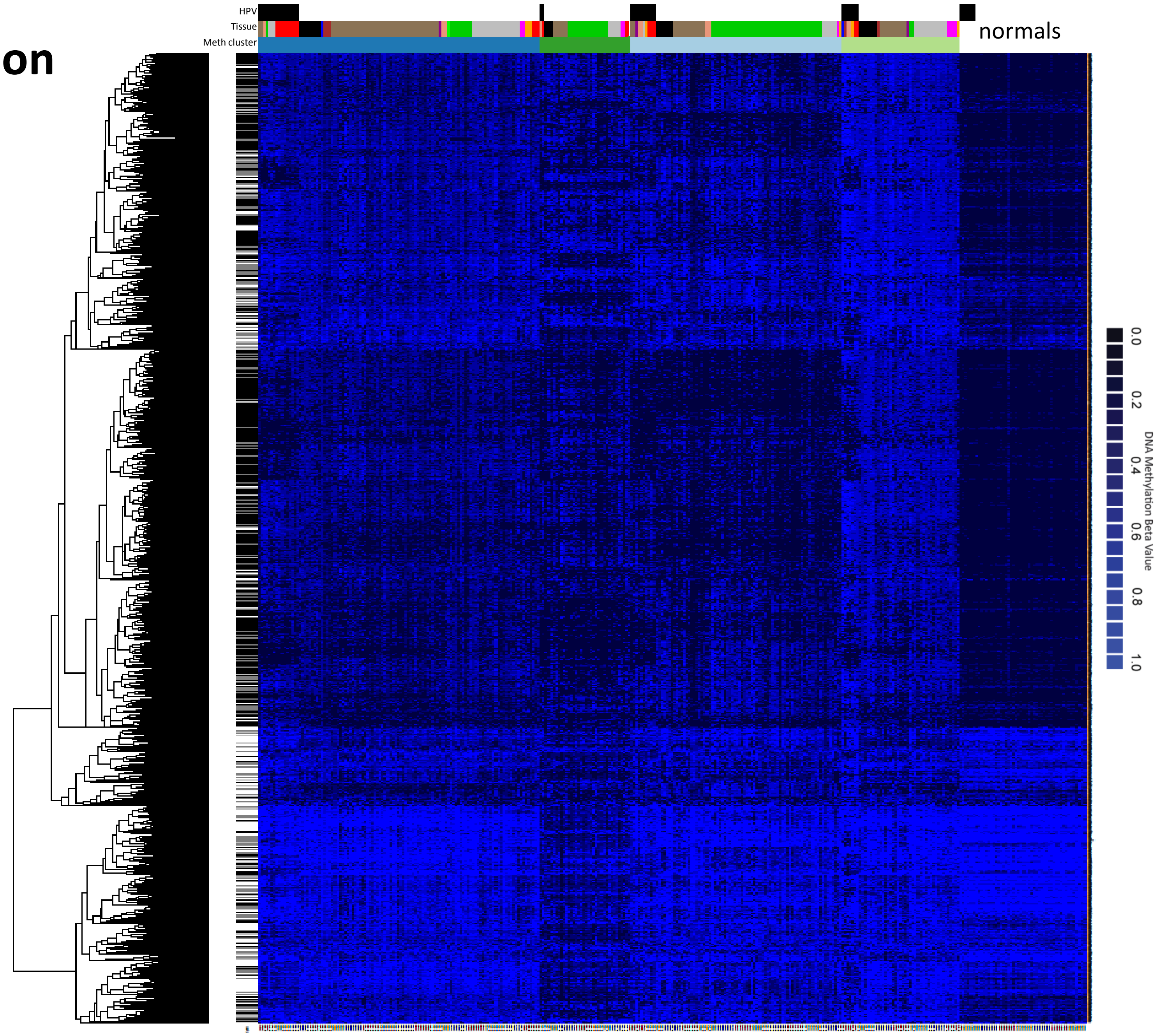
c	2	4	5	3	1	all
n	56	41	29	27	106	259
w	0.98	0.95	0.89	0.84	0.94	0.93



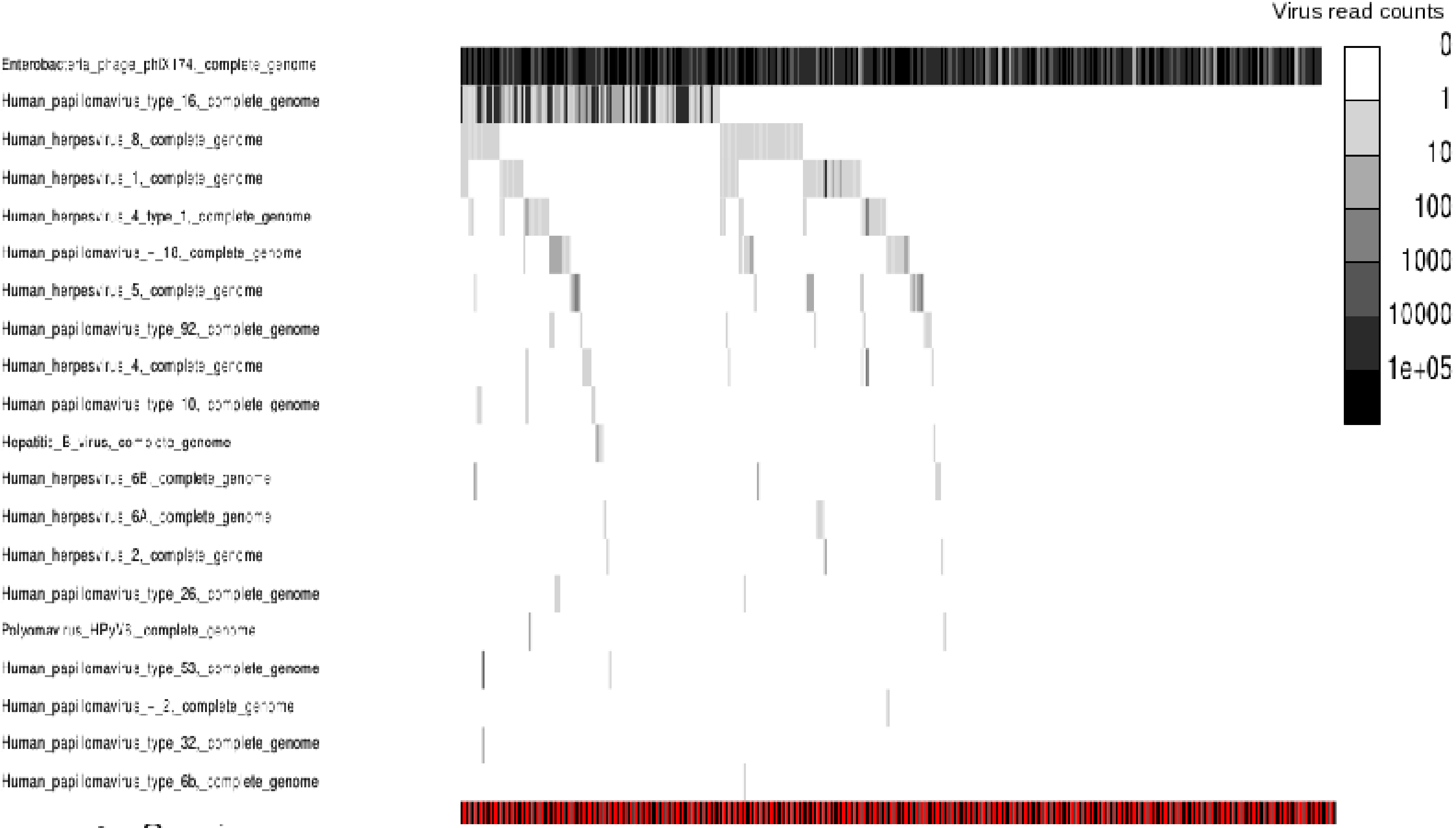
DNA Methylation Subtyping

■ Alveolar Ridge
■ Base of Tongue
■ Buccal Mucosa
■ Floor of Mouth
■ Hard Palate
■ Hypopharynx
■ Larynx
■ Lip
■ Oral Cavity
■ Oral Tongue
■ Oropharynx
■ Tonsil

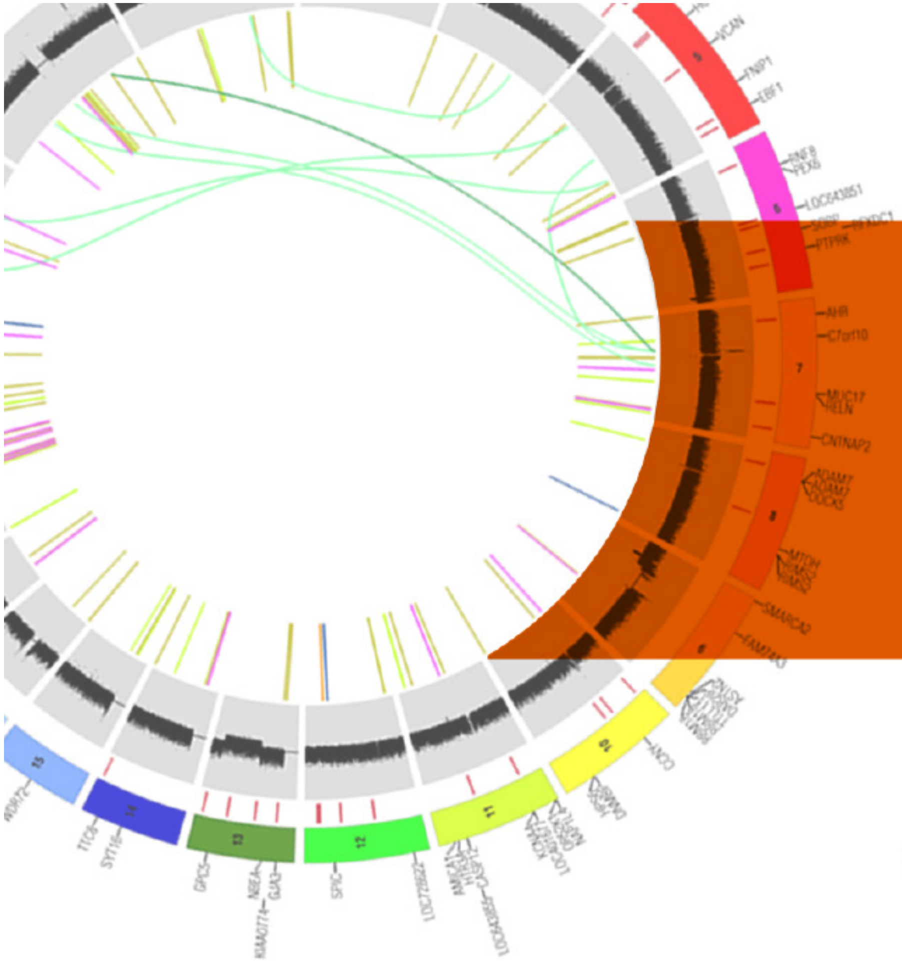
■ Island
■ Shore
■ Shelf



Do HNSCC present with Viral Transcripts?



- Species:
  - HPV (16, 18, 92, 10, 26, 53, 32, 6b)
  - Herpes (One high level HSV-1)
  - Rare and low level HepB and Polyoma



The Cancer Genome Atlas

Low Pass Whole Genome Head and Neck Sequencing: Detection of Viral Sequences

19-20 September, 2012

Harvard GCC Team

HNSCC Analysis Working Group