8q24: Prostate Cancer

Matthew Freedman

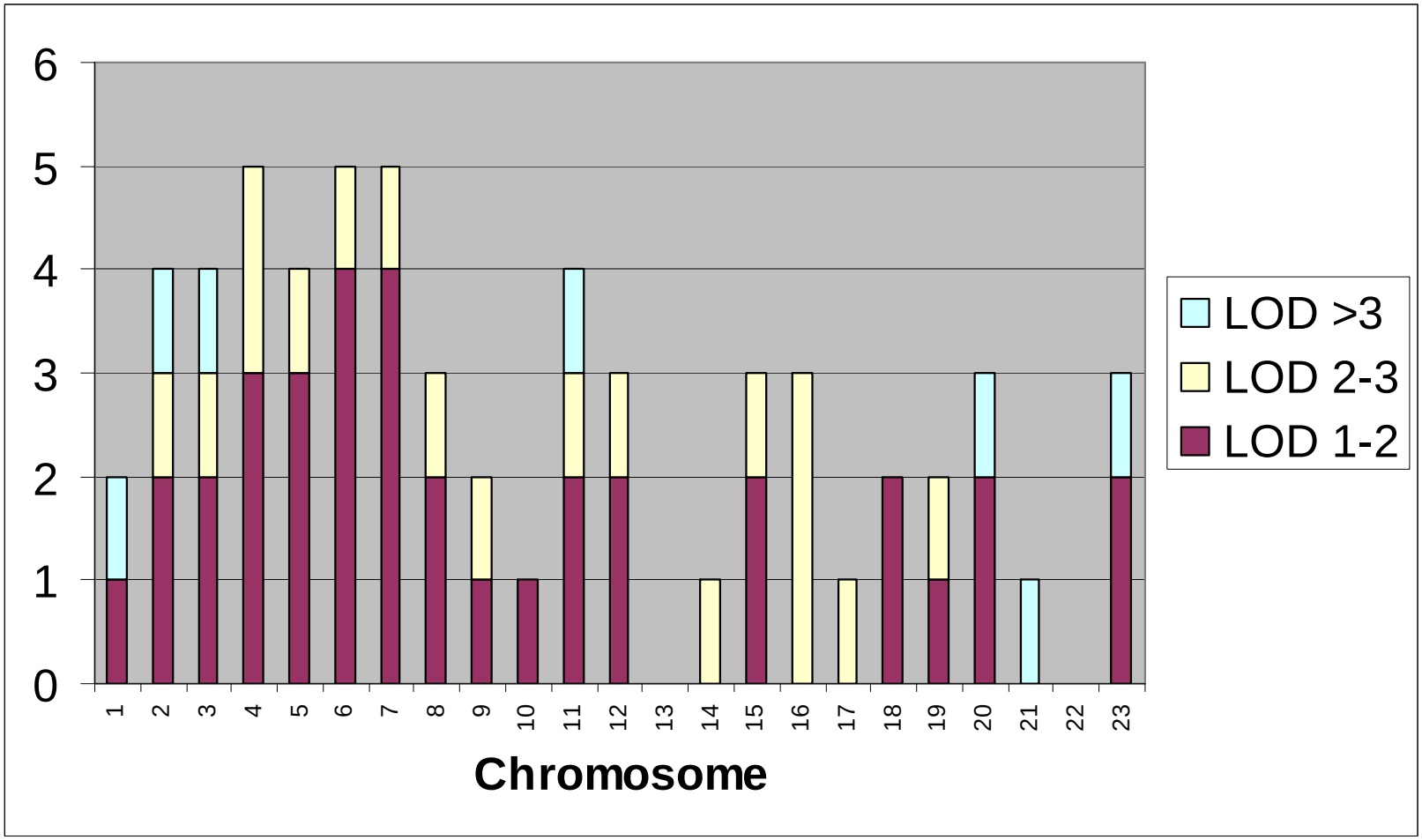
GAIN II

October 18, 2007

DANA-FARBER CANCER INSTITUTE

BROAD INSTITUTE

Summary of LOD scores for 11 CaP linkage scans



Adapted from
Schaid
Hum Molec Genet 13:R103

History of prostate cancer genetics

**Where Are the Prostate Cancer Genes?—
A Summary of Eight Genome Wide Searches**

Prostate. 2003 Dec 1;57(4):261-9.

Prostate cancer susceptibility genes: Many studies, many results, no answers

Cancer Metastasis Rev. 2001;20(3-4):155-64.

REVIEW ARTICLE
Genetics of Prostate Cancer: Too Many Loci, Too Few Genes

Am J Hum Genet. 2000 Dec;67(6):1367-75.

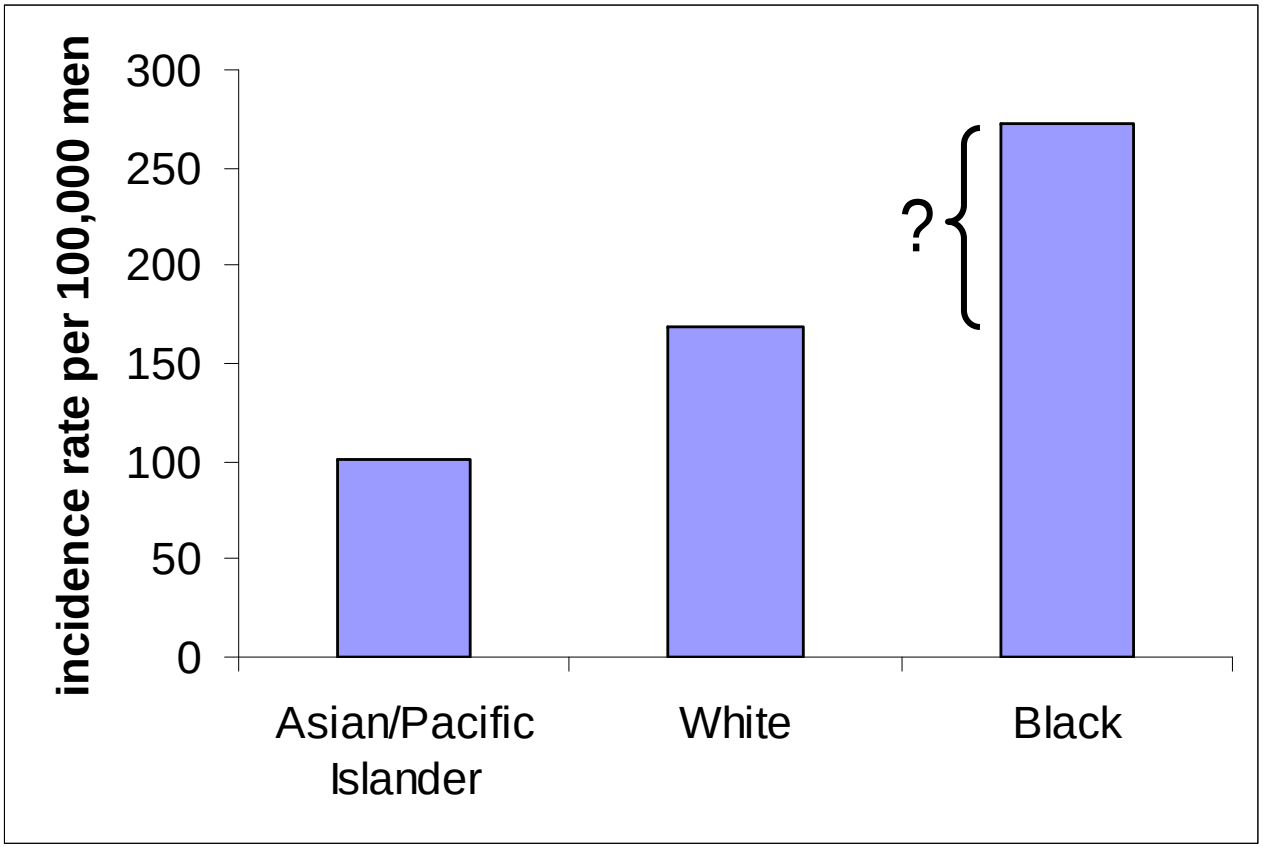
Outline

- Whole genome admixture scan
- Fine mapping
- Work in progress

Established risk factors for prostate cancer

- Age
- Family history
- Ethnicity

Prostate cancer: epidemiology



SEER data – 1998-2002
age adjusted

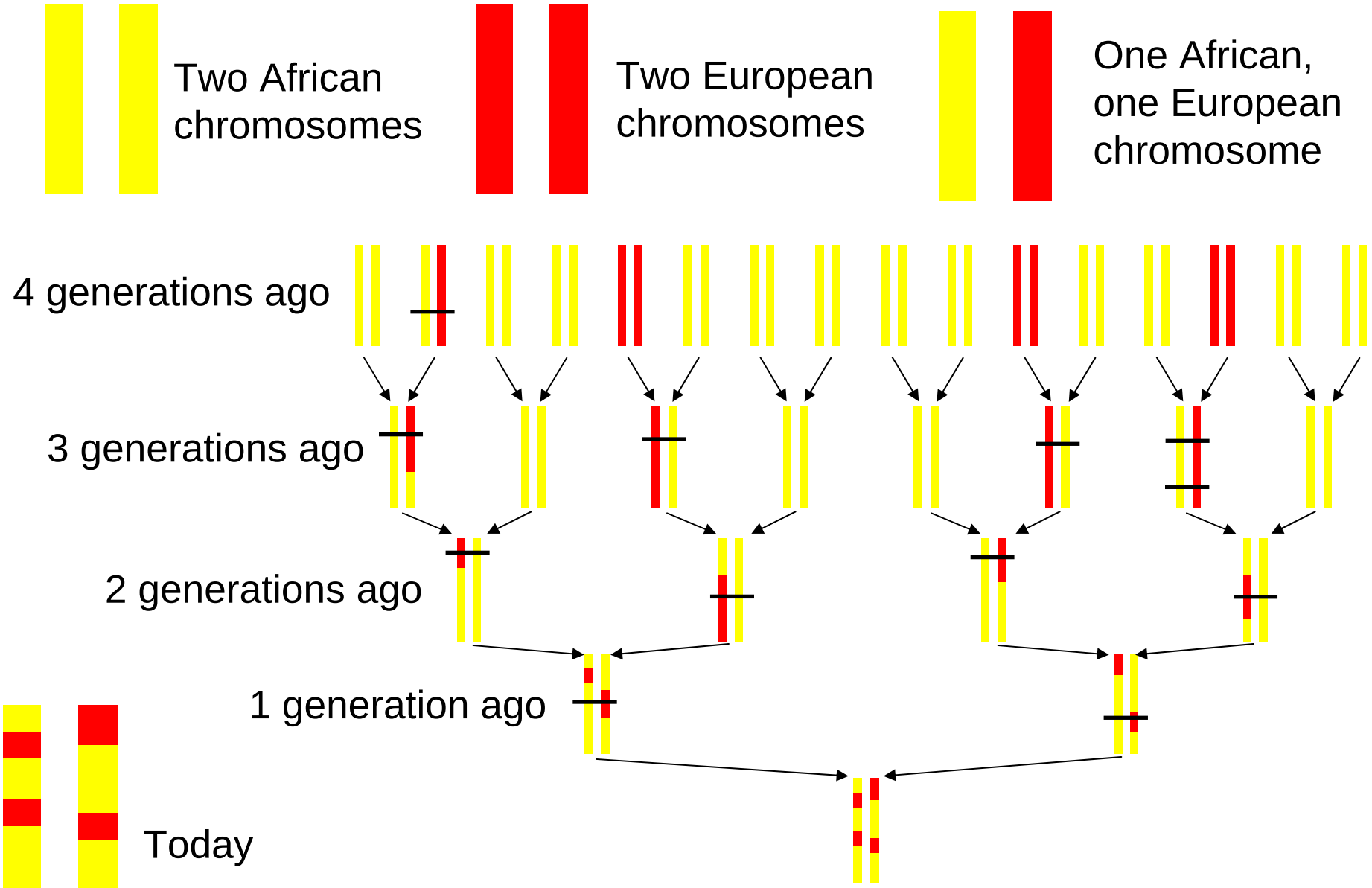
Whole genome admixture scan

- Started 3 years ago
- Risk allele must be differentially distributed between ancestral populations
- Can scan the genome with many fewer markers than for non-admixed pops

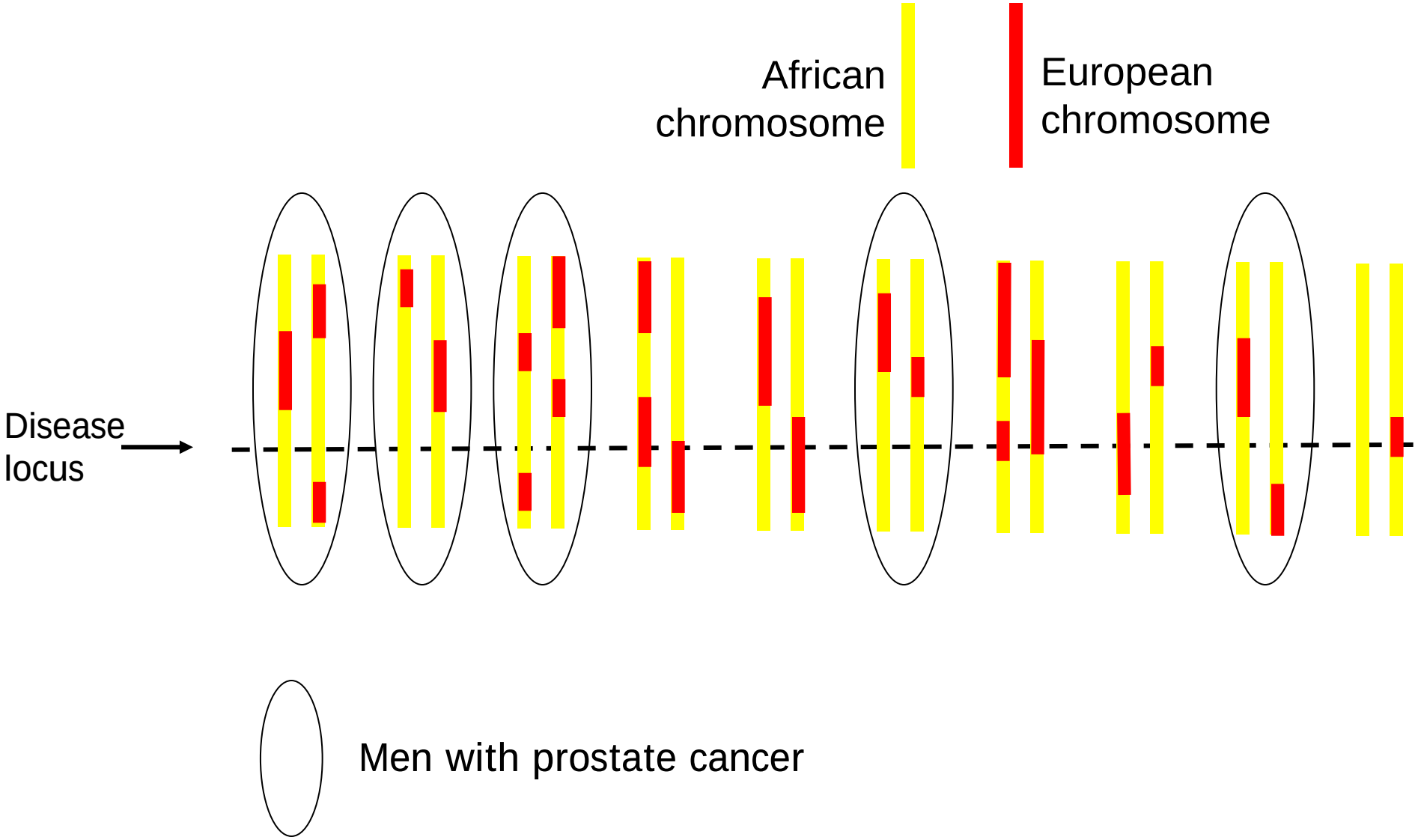
Prostate cancer is a strong candidate disease for admixture mapping

- Incidence rates in African-American men ~1.6 fold higher than European-Am men
- Epidemiologic evidence suggests that prostate cancer is even higher in African men
- Prostate cancer has one of the highest heritabilities out of all epithelial cancers
- No gene has been consistently identified

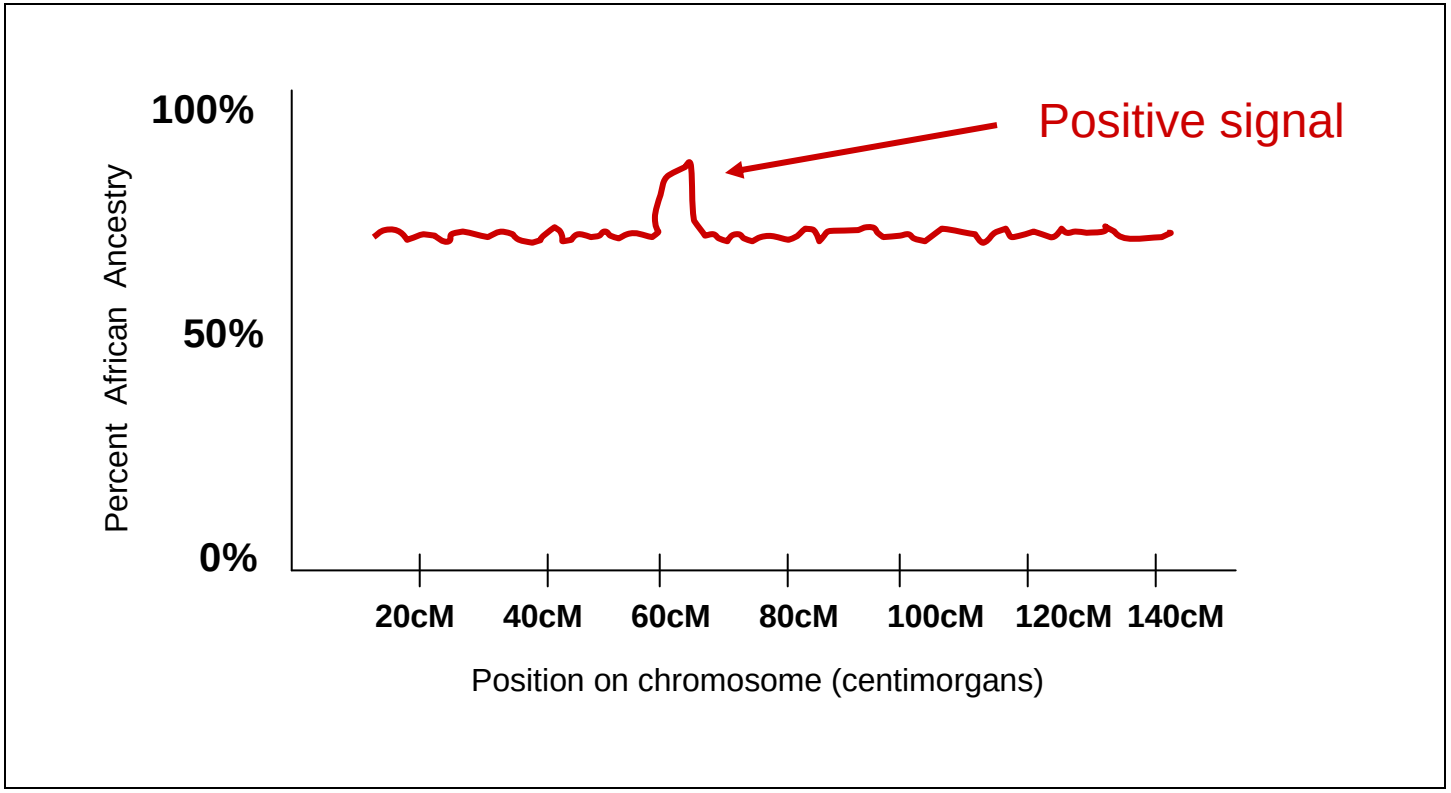
Admixture creates a mosaic



How does admixture mapping work?



The signal of admixture association

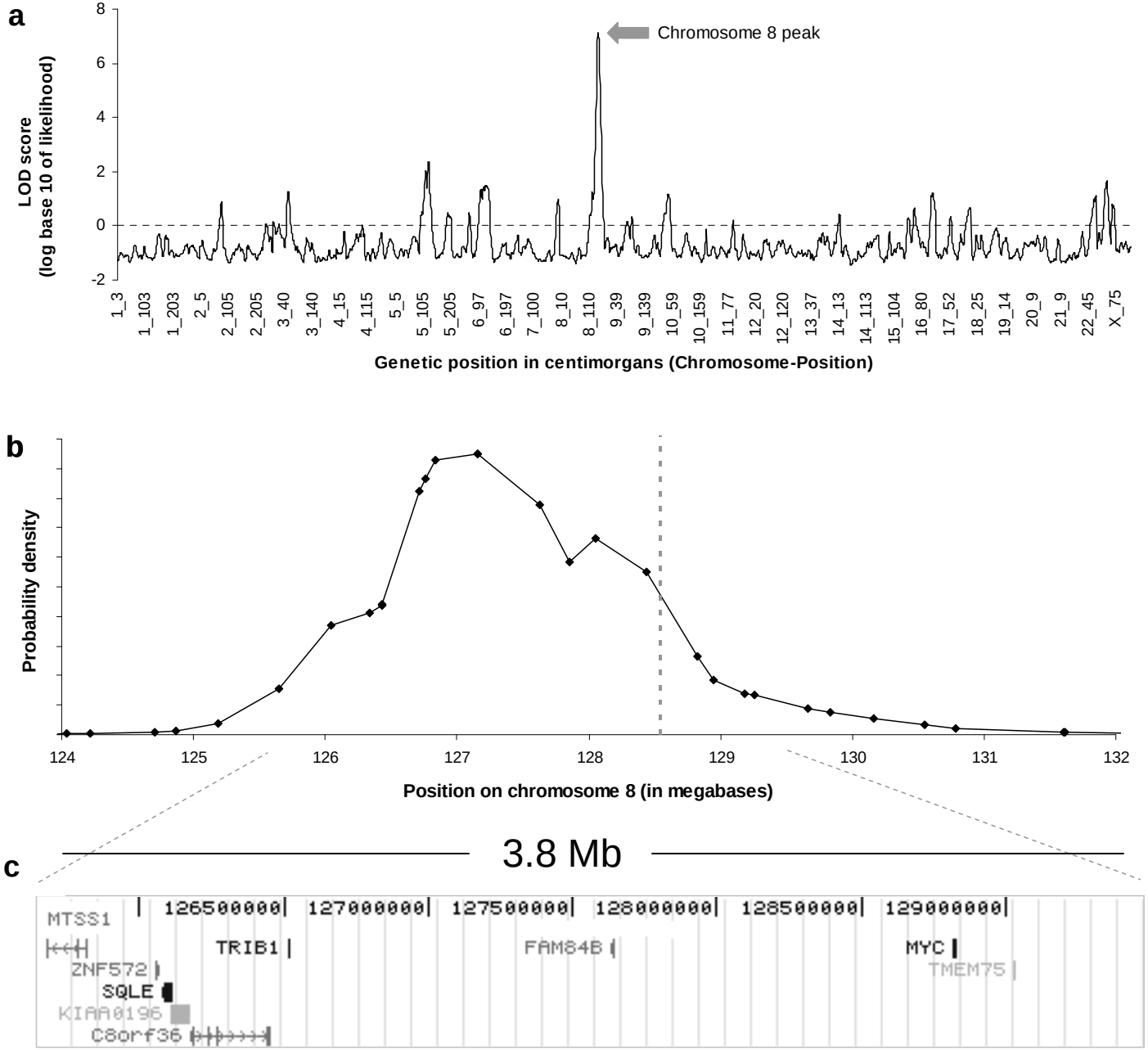


Admixture mapping identifies 8q24 as a prostate cancer risk locus in African-American men

Matthew L. Freedman[a,b,c], Christopher A. Haiman[c,d], Nick Patterson[b,c], Gavin J. McDonald[b,e], Arti Tandon[b,e], Alicja Waliszewska[b,e,f], Kathryn Penney[b], Robert G. Steen[e,g], Kristin Ardlie[b,h], Esther M. John[i,j], Ingrid Oakley-Girvan[i,j], Alice S. Whittemore[j], Kathleen A. Cooney[k,l], Sue A. Ingles[d], David Altshuler[b,e,m,n], Brian E. Henderson[d], and David Reich[b,e,o]

	Location	Cases	Controls	Cases % European ± 1 standard err.	Controls % European ±1 standard err.	mean age diagnosis (range)	% with Gleason score >7	% with non-local tumors	% with prostate cancer in a first degree relative	* Decrease in peak LOD if these samples are removed
Multiethnic Cohort	CA & HI	810	730	23.57 ± 0.50%	25.42 ± 0.57%	68 (46-85)	18%	15%	12%	2.58
L.A. County Men's Health Study	CA	366	107	22.34 ± 0.83%	26.37 ± 2.13%	63 (42-88)	28%	39%	21%	1.37
Study Early Onset Pros. Cancer	CA	104	-	20.89 ± 1.37%	-	60 (45-65)	31%	49%	14%	1.01
PCGP	MI	103	-	19.50 ± 1.01%	-	55 (40-86)	11%	29%	39%	1.15
Flint Men's Health Study	MI	85	-	18.05 ± 1.21%	-	65 (47-77)	12%	28%	15%	0.06
Bay Area Men's Health Study	CA	82	36	19.06 ± 1.52%	20.13 ± 2.15%	64 (44-78)	25%	94%	28%	1.16
Genomics Collaborative	All U.S.	47	-	16.16 ± 1.51%	-	62 (39-81)	14%	38%	28%	0.57
Combined samples		1,597	873	22.11 ± 0.36%	25.32 ± 0.55%	65 (39-88)	21%	29%	18%	7.14

Freedman et al., PNAS :14068 (2006)

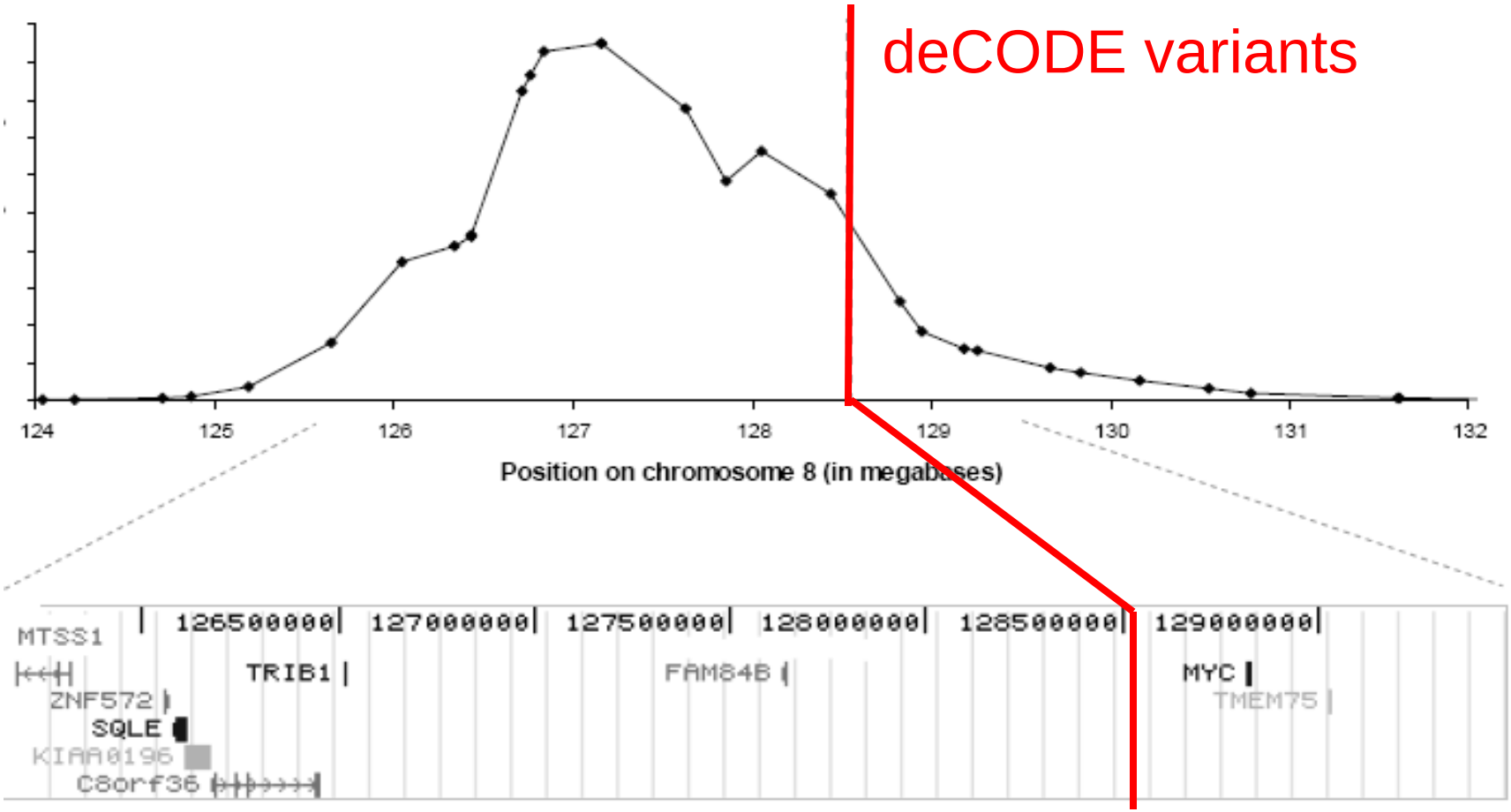


Freedman et al., PNAS :14068 (2006)

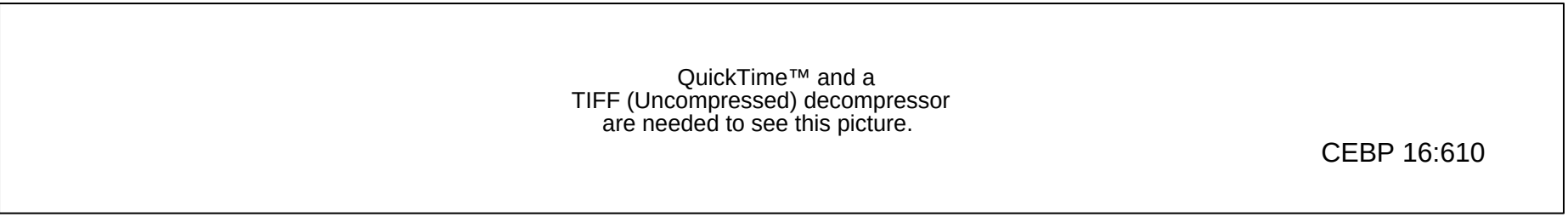
A common variant associated with prostate cancer in European and African populations

Laufey T Amundadottir[1,12], Patrick Sulem[1,12], Julius Gudmundsson[1,12], Agnar Helgason[1], Adam Baker[1], Bjarni A Agnarsson[2], Asgeir Sigurdsson[1], Kristrun R Benediktsdottir[2], Jean-Baptiste Cazier[1], Jesus Sainz[1], Margret Jakobsdottir[1], Jelena Kostic[1], Droplaug N Magnusdottir[1], Shyamali Ghosh[1], Kari Agnarsson[1], Birgitta Birgisdottir[1], Louise Le Roux[1], Adalheidur Olafsdottir[1], Thorarinn Blondal[1], Margret Andresdottir[1], Olafia Svandis Gretarsdottir[1], Jon T Bergthorsson[1], Daniel Gudbjartsson[1], Arnaldur Gylfason[1], Gudmar Thorleifsson[1], Andrei Manolescu[1], Kristleifur Kristjansson[1], Gudmundur Geirsson[3], Helgi Isaksson[2], Julie Douglas[4], Jan-Erik Johansson[5], Katarina Bälter[6], Fredrik Wiklund[6], James E Montie[7], Xiaoying Yu[8], Brian K Suarez[9], Carole Ober[10], Kathleen A Cooney[7,11], Henrik Gronberg[6], William J Catalona[8], Gudmundur V Einarsson[3], Rosa B Barkardottir[2], Jeffrey R Gulcher[1], Augustine Kong[1], Unnur Thorsteinsdottir[1] & Kari Stefansson[1]

Nature Genetics **38**, 652 - 658 (2006)



Convergence of independent methods and data



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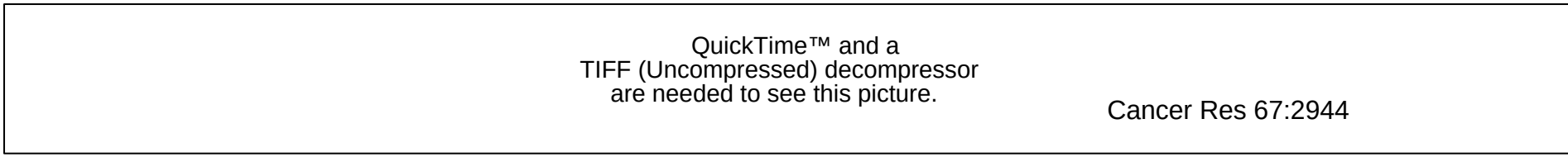
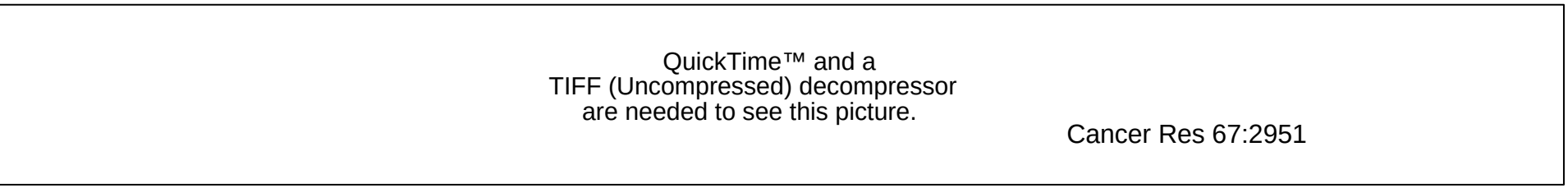
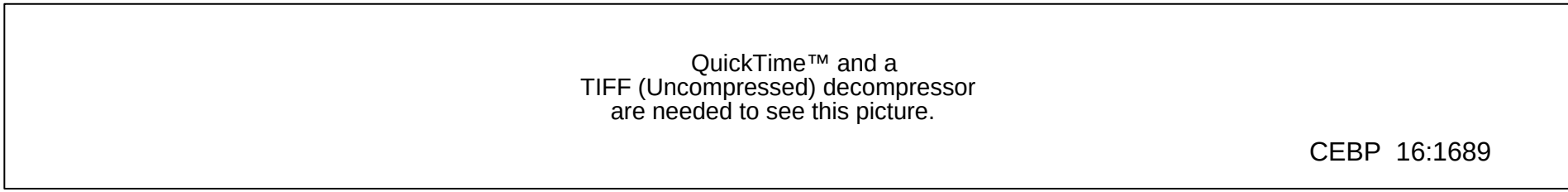


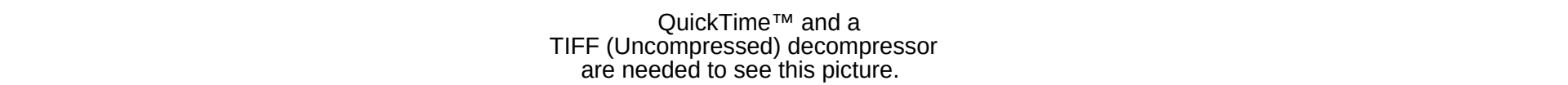
Cancer Res 67:2944



Cancer Res 67:2951



CEBP 16:1689

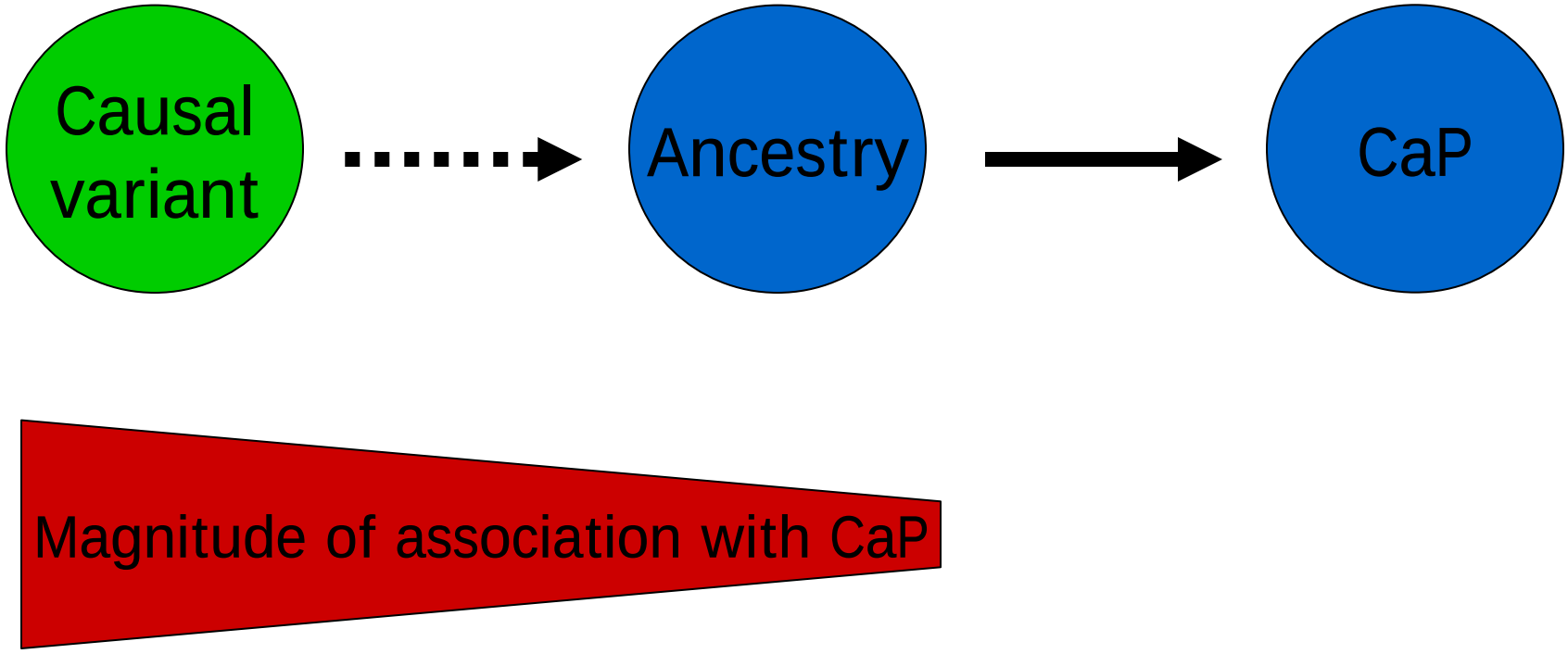


JNCI 99:1525

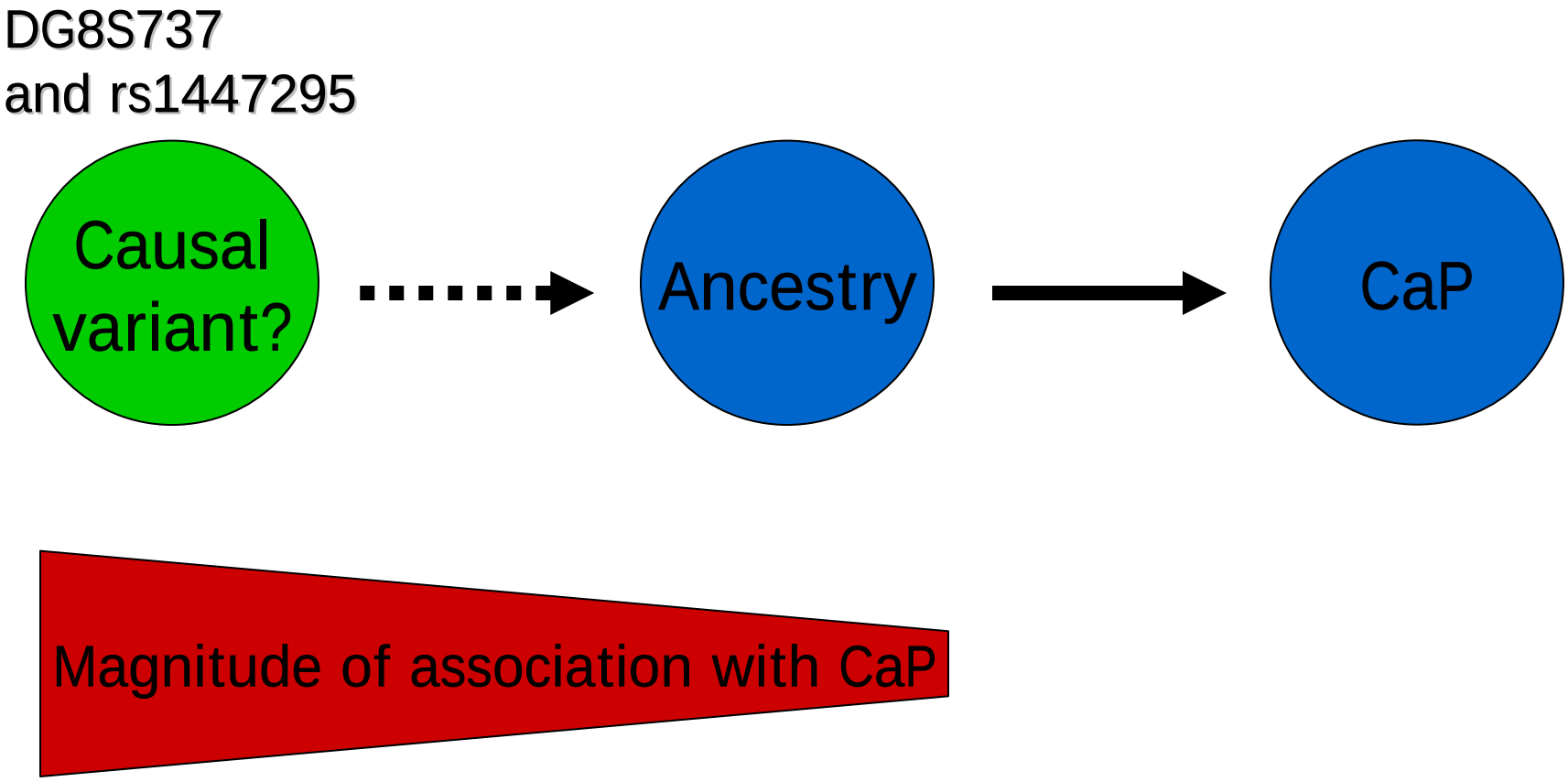
Outline

- Whole genome admixture scan
- Fine mapping
- Work in progress

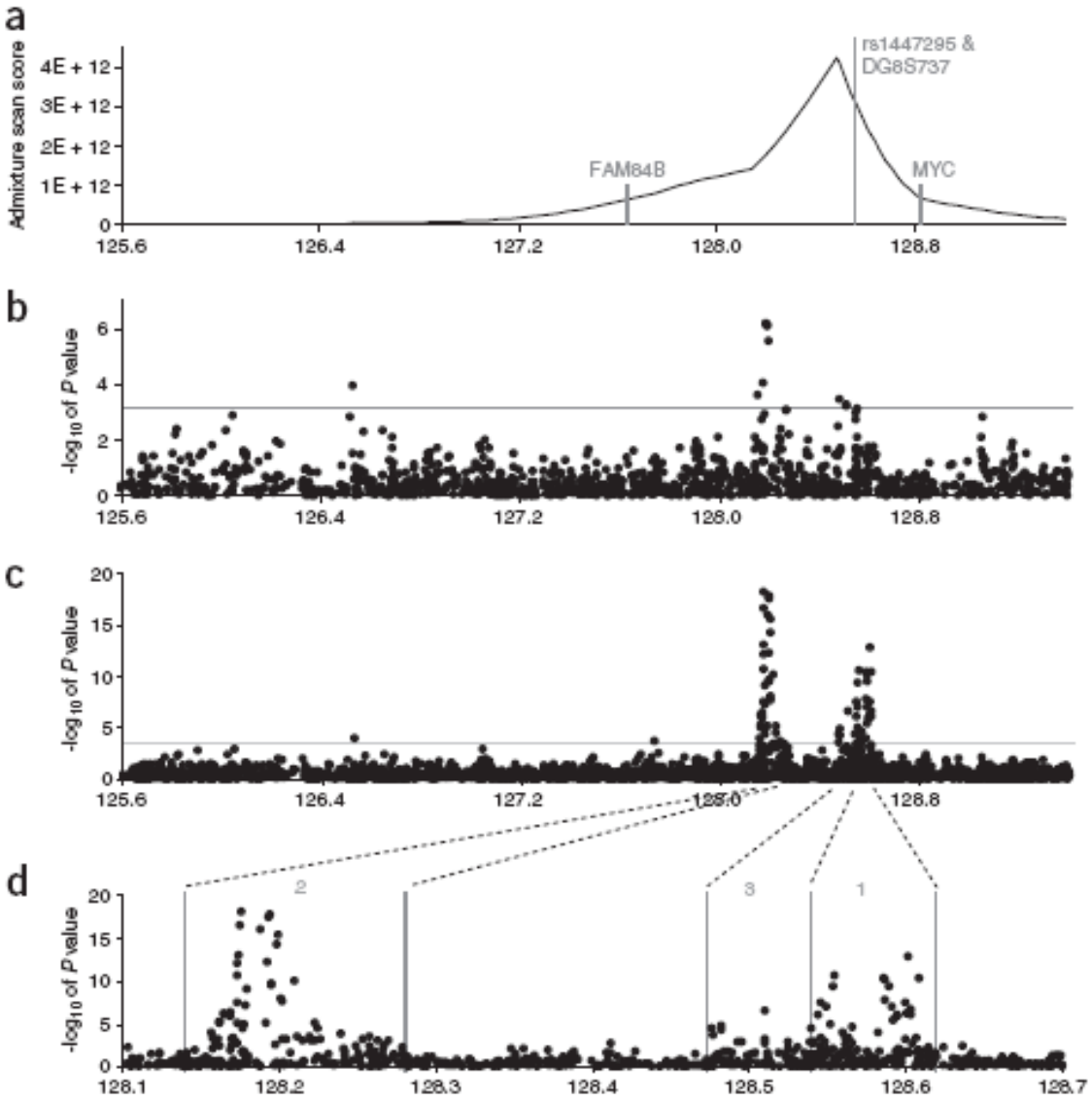
Ancestry is a proxy for the causal variant



Do deCODE variants fully explain ancestry risk in African American men?



Fine mapping identifies 3 regions contributing to PCa risk



7 alleles associated with CaP in a multiethnic cohort

Marker	African American	Japanese American	Native Hawaiians	Latinos	European Americans	Pooled OR
rs13254738	1.24 (1.09-1.42)	1.57 (1.33-1.83)	1.46 (1.00-2.12)	1.25 (1.07-1.46)	1.11 (0.97-1.26)	1.26 (1.18-1.36)
rs6983561	1.34 (1.18-1.53)	1.78 (1.47-2.15)	3.17 (1.87-5.36)	1.99 (1.34-2.96)	1.16 (0.86-1.58)	1.51 (1.37-1.67)
Broad11934905	2.45 (1.65-3.62)	-	-	-	-	2.45 (1.65-3.62)
rs6983267	1.43 (1.17-1.75)	1.22 (1.05-1.42)	1.29 (0.88-1.89)	1.05 (0.89-1.24)	1.13 (0.99-1.28)	1.18 (1.09-1.27)
rs7000448	1.33 (1.12-1.58)	1.23 (1.04-1.46)	1.38 (0.89-2.14)	1.29 (1.07-1.56)	1.14 (0.93-1.40)	1.26 (1.15-1.38)
DG8S737-8	1.25 (1.06-1.49)	1.48 (1.16-1.88)	2.55 (1.33-4.89)	1.46 (1.05-2.02)	1.45 (0.96-2.19)	1.39 (1.23-1.57)
rs10090154	1.11 (0.94-1.32)	1.49 (1.23-1.81)	2.54 (1.61-4.02)	1.98 (1.49-2.61)	1.44 (1.17-1.76)	1.43 (1.30-1.58)

Genome-wide association study identifies a second prostate cancer susceptibility variant at 8q24

Julius Gudmundsson[1,17], Patrick Sulem[1,17], Andrei Manolescu[1,17], Laufey T Amundadottir[1,17],
Dani
Adar
Thor
Jona
Marj
Seba
Kath
Gudr
Willi
Augu

Genome-wide association study of prostate cancer identifies a second risk locus at 8q24

Meredith Yeager[1,2], Nick Orr[3], Richard B Hayes[2], Kevin B Jacobs[4], Peter Kraft[5], Sholom Wacholder[2],
Ma
Bri
De
Wa
Ed
Da

Multiple regions within 8q24 independently affect risk for prostate cancer

Christopher A Haiman[1], Nick Patterson[2], Matthew L Freedman[2,3], Simon R Myers[2], Malcolm C Pike[1], Alicja Waliszewska[2,4,5], Julie Neubauer[2,4], Arti Tandon[2,4], Christine Schirmer[2,4], Gavin J McDonald[2,4], Steven C Greenway[4], Daniel O Stram[1], Loic Le Marchand[6], Laurence N Kolonel[6], Melissa Frasco[1], David Wong[1], Loreall C Pooler[1], Kristin Ardlie[2,7], Ingrid Oakley-Girvan[8,9], Alice S Whittemore[9], Kathleen A Cooney[10,11], Esther M John[8,9], Sue A Ingles[1], David Altshuler[2,4,12,13], Brian E Henderson[1] & David Reich[2,4]

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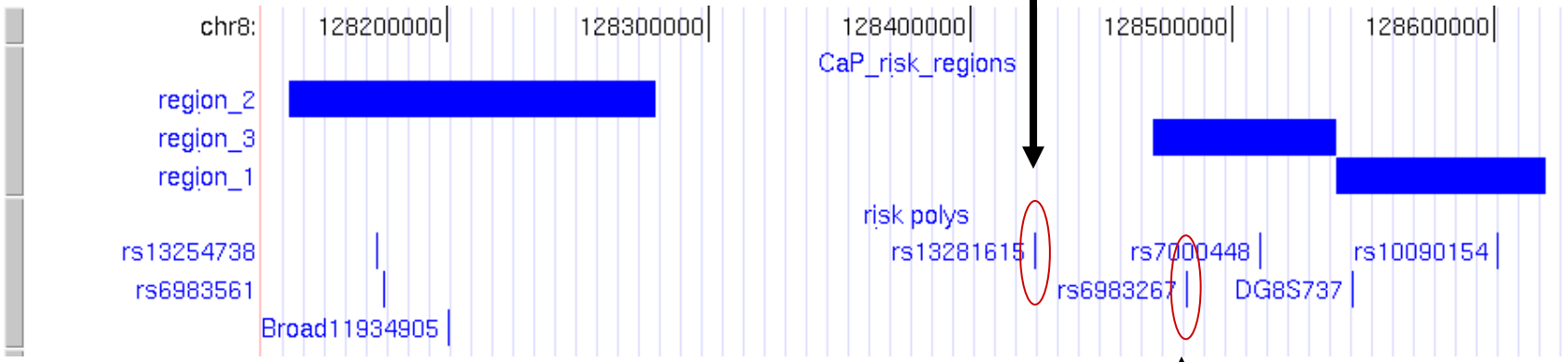
Summary: Fine mapping

- Multiple alleles contributing risk in a noncoding region
  - Population attributable risk is large across populations
- Power of studying multiple ethnicities
- Most risk alleles are shared across populations (although this is also what we are most powered for)
- MYC is closest gene

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Nature 447:1087



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TIFF (Uncompressed) decompressor
are needed to see this picture.

Nature Genetics 39:989

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Nature Genetics 39:984

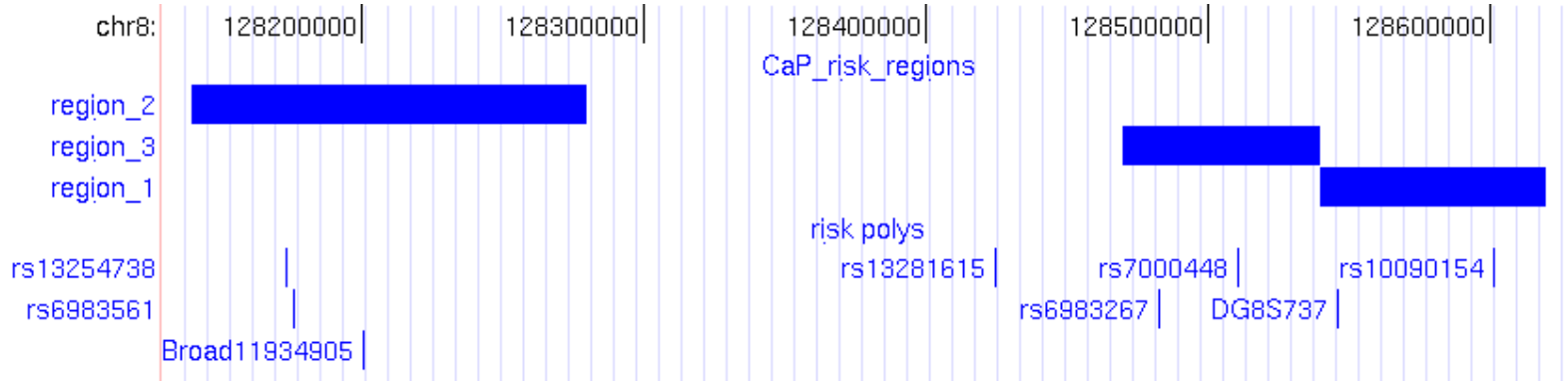
QuickTime™ and a
TIFF (Uncompressed) decompressor

Nature Genetics 39:984

Outline

- Whole genome admixture scan
- Fine mapping
- Work in progress

Now what??



Sequence

Function

Sequencing - ascertain all variation across three regions

- N=48 - across 3 ethnic groups

- Newly discovered and poorly tagged variants will be tested in larger multiethnic cohort

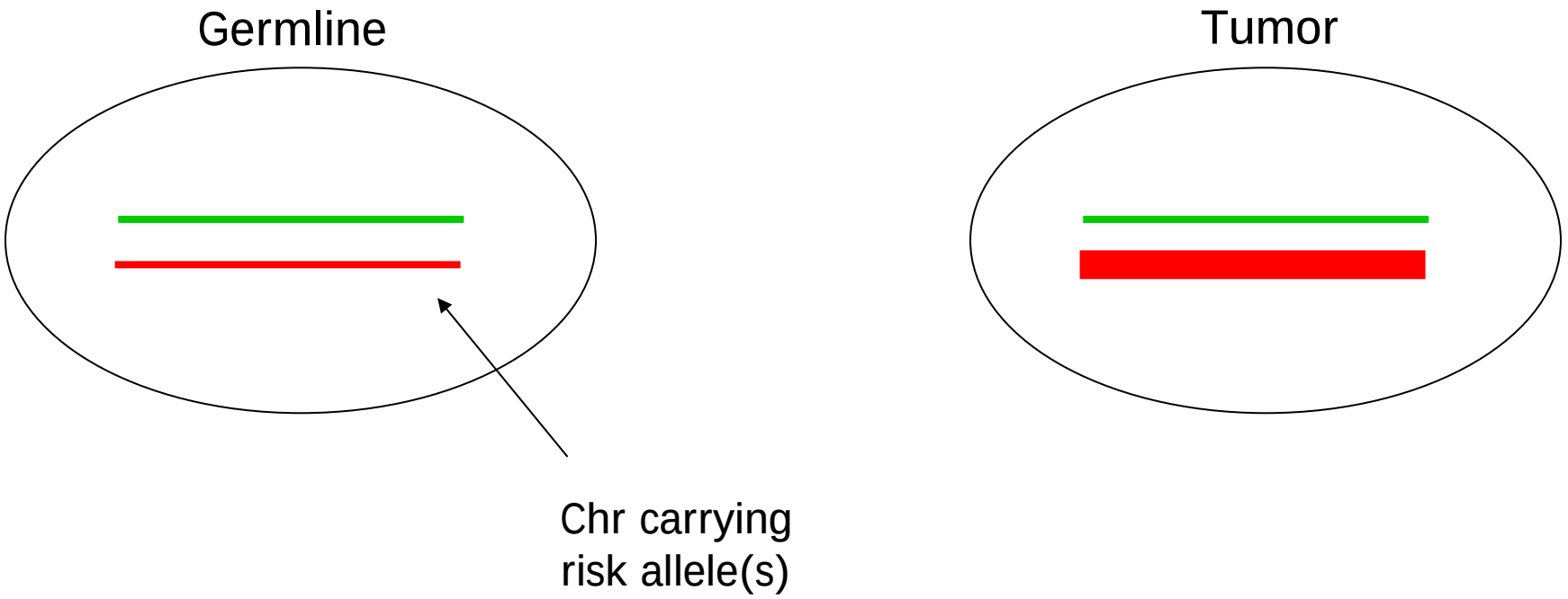
What is the mechanism of risk?

- Possible hypotheses
  - structurally unstable
  - unannotated transcript
    - tiling arrays
  - promoter/enhancer
    - tiling arrays
    - chromatin markers

Hypothesis: structural instability

Mark Pomerantz

Are the 8q24 risk alleles associated with somatic 8q amplification?



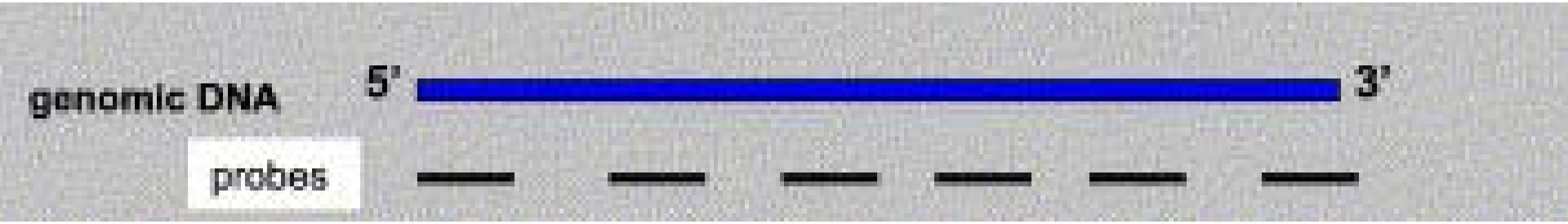
Being performed on 140 paired normal/tumor samples from the DFCI/Gelb center

Tiling arrays

USC - Ben Berman,Chris Haiman, Gerry Coetzee
DFCI - Jerome Eekhoute, Mathieu Lapien, Mark Pomerantz, Myles Brown

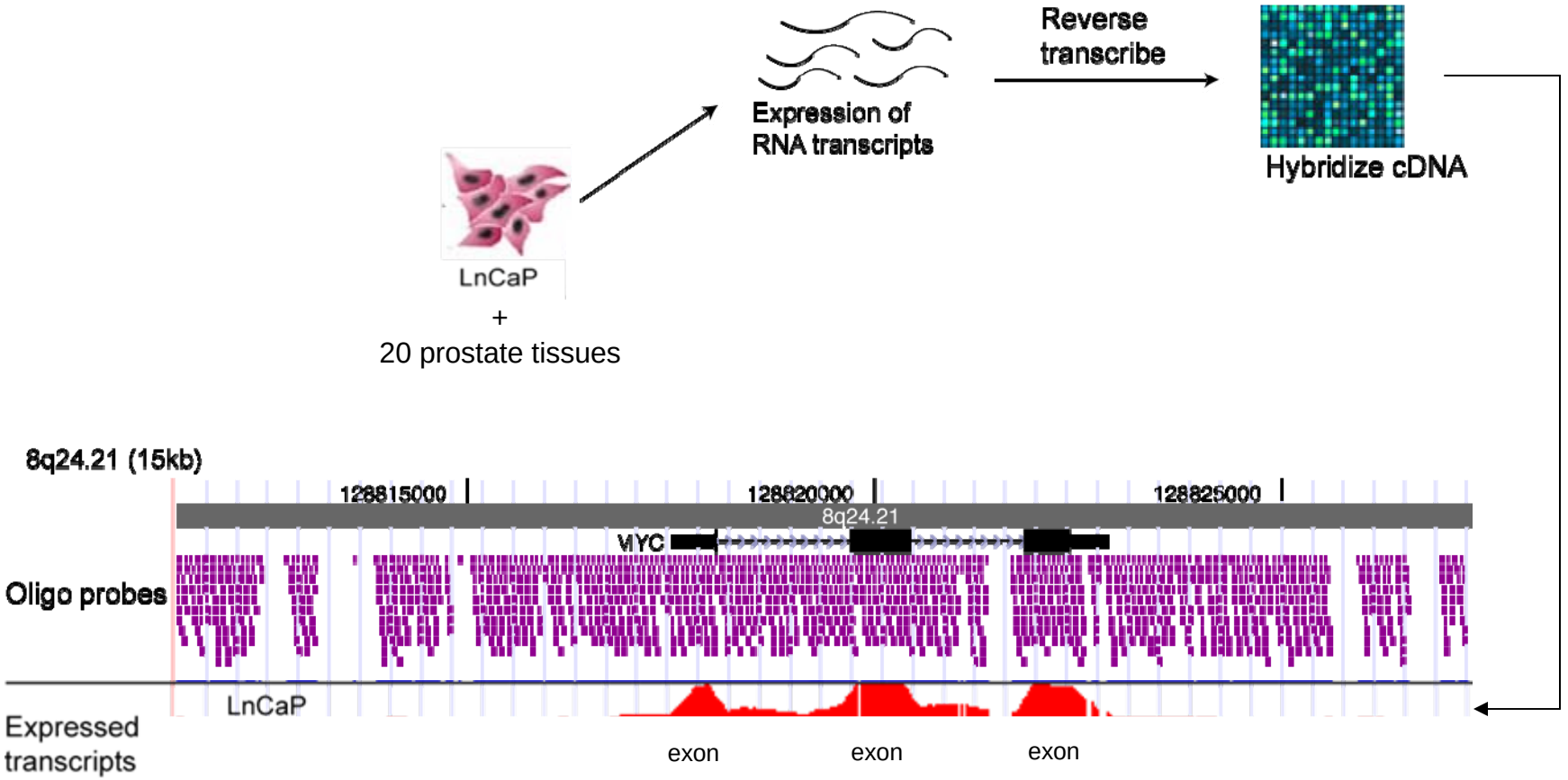
array that “tiles” oligos across a given region so that it can be interrogated at ultra-high resolution

Genomics 85:1

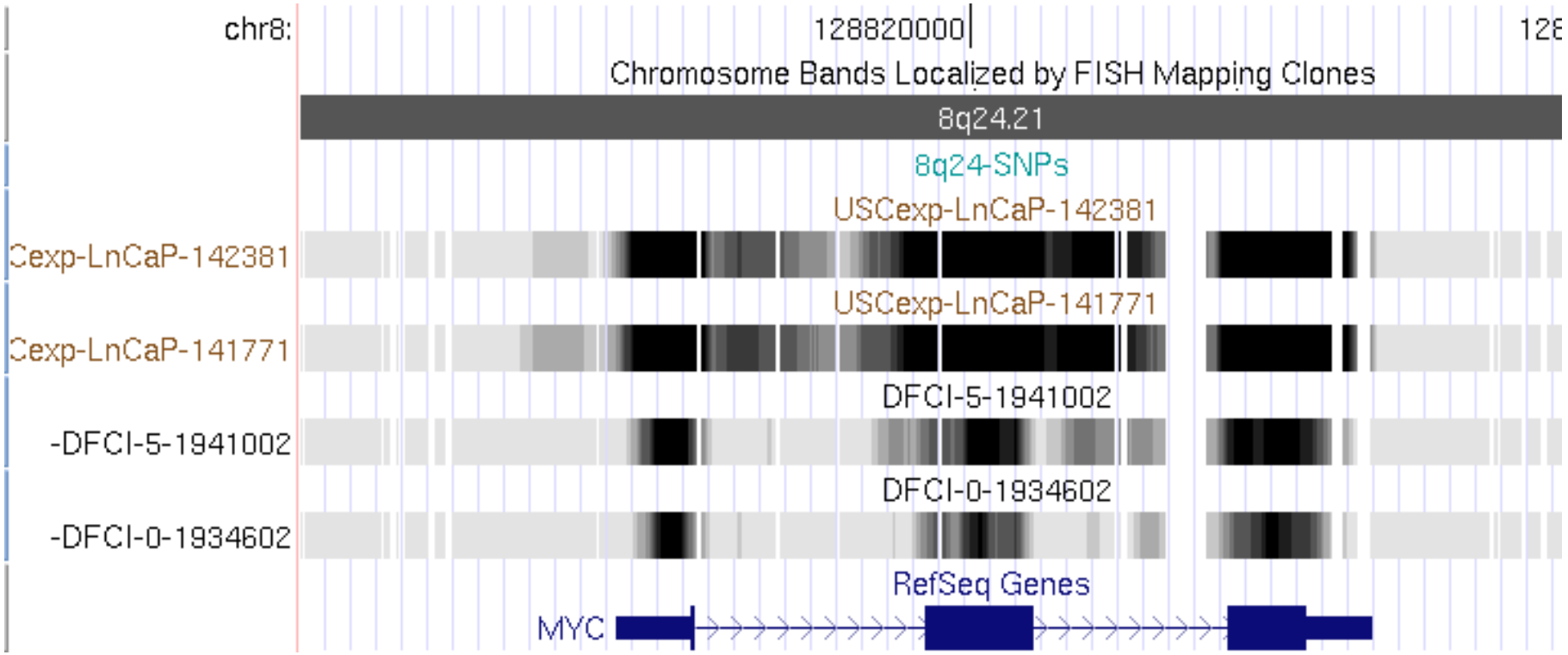


we tiled a 5 megabase region at 8q24 with a mean probe spacing of 8bp

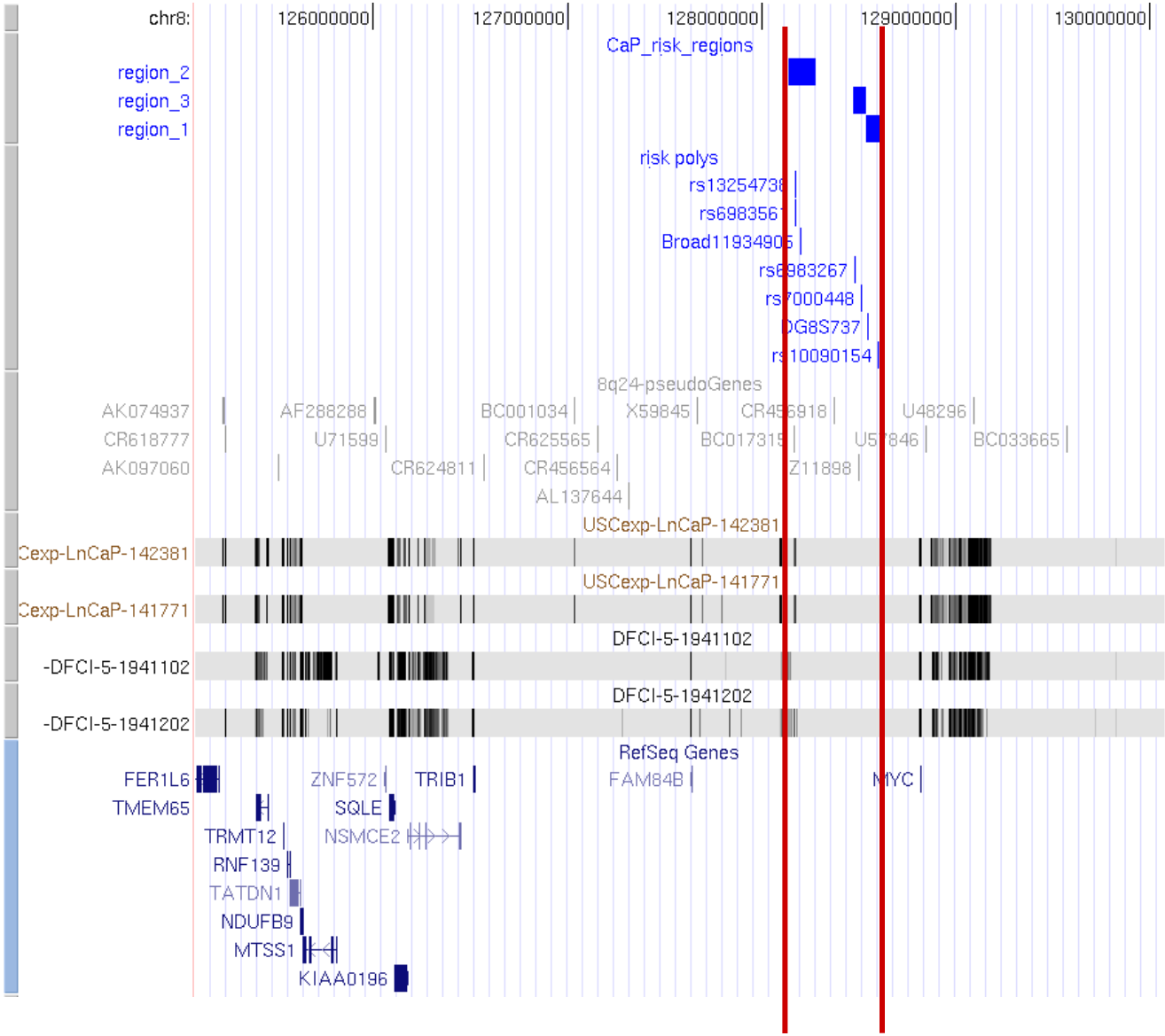
Analyzed cDNA and acetylation


Reverse
transcribe
Expression of
RNA transcripts
Hybridize cDNA
LnCaP
+
20 prostate tissues
8q24.21 (15kb)
128815000
128820000
128825000
8q24.21
MYC
Oligo probes
LnCaP
Expressed
transcripts
exon
exon
exon

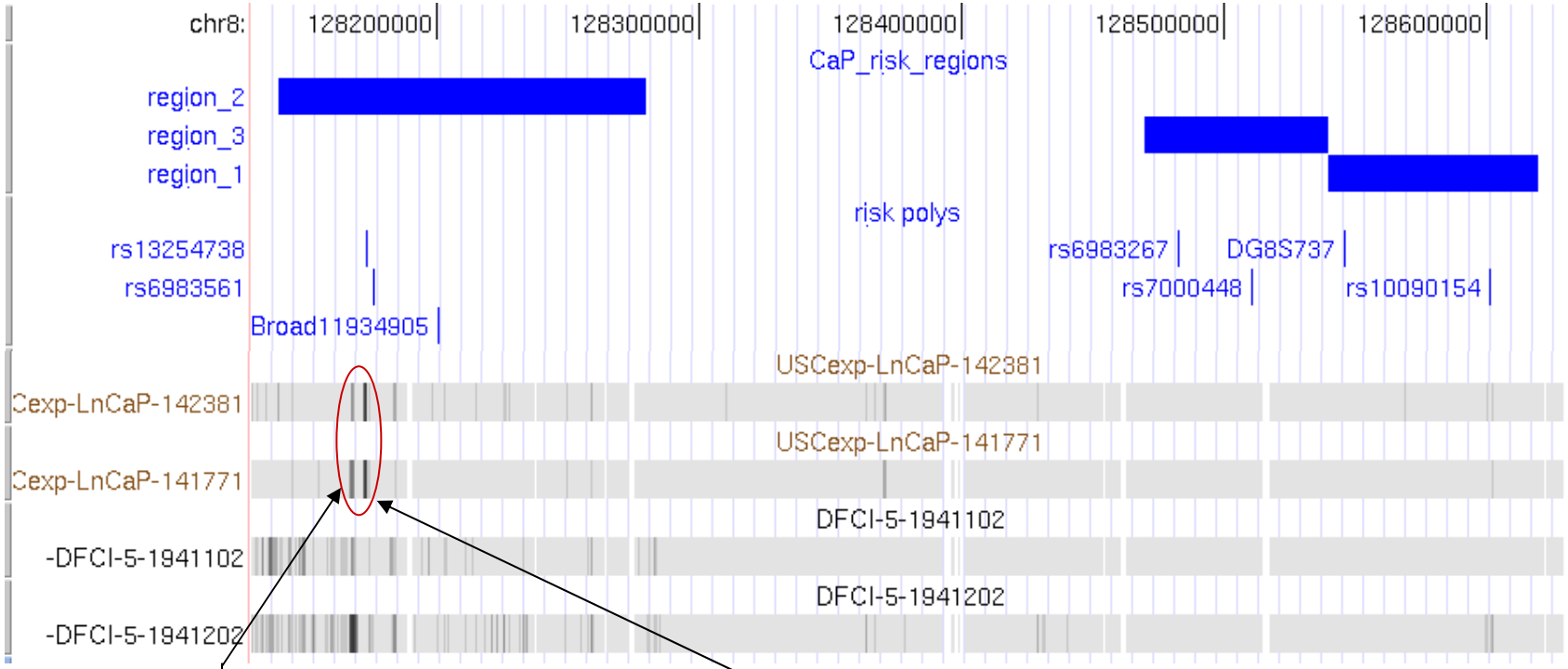
Blow-up of region - MYC



Transcriptional landscape of 8q24



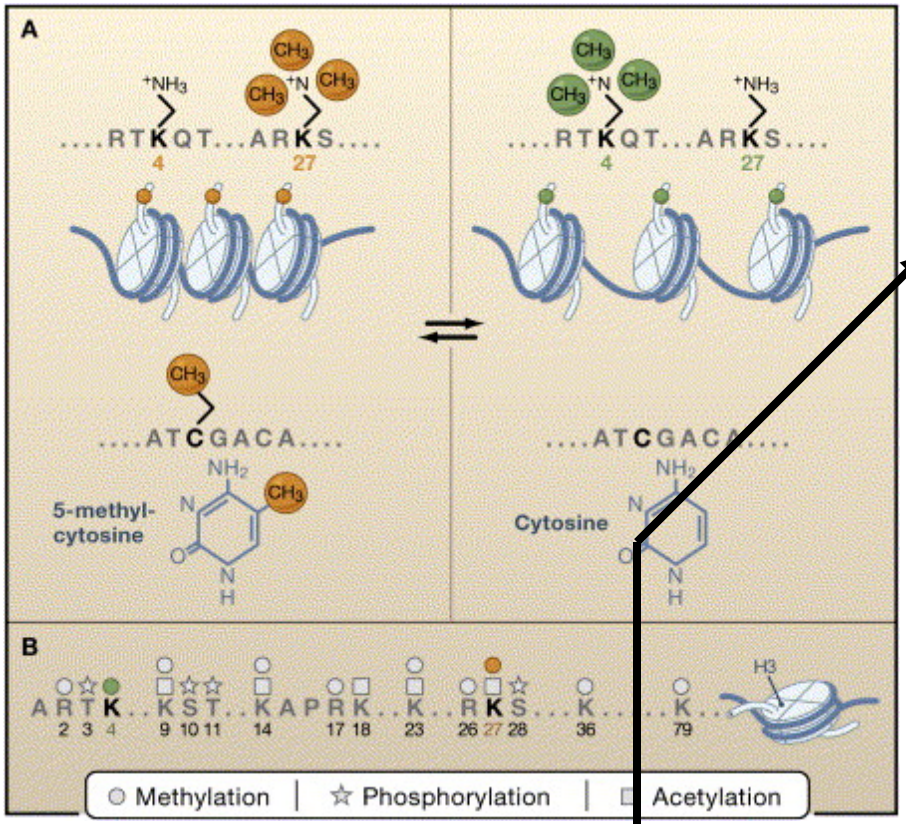
New transcripts in risk region



Confirmed by RT-PCR in prostate tissue
Currently performing 5' RACE to fully characterize

Pseudogene

Chromatin markers:epigenetics



Cell 128:669

Table 1 | Chromatin modifications

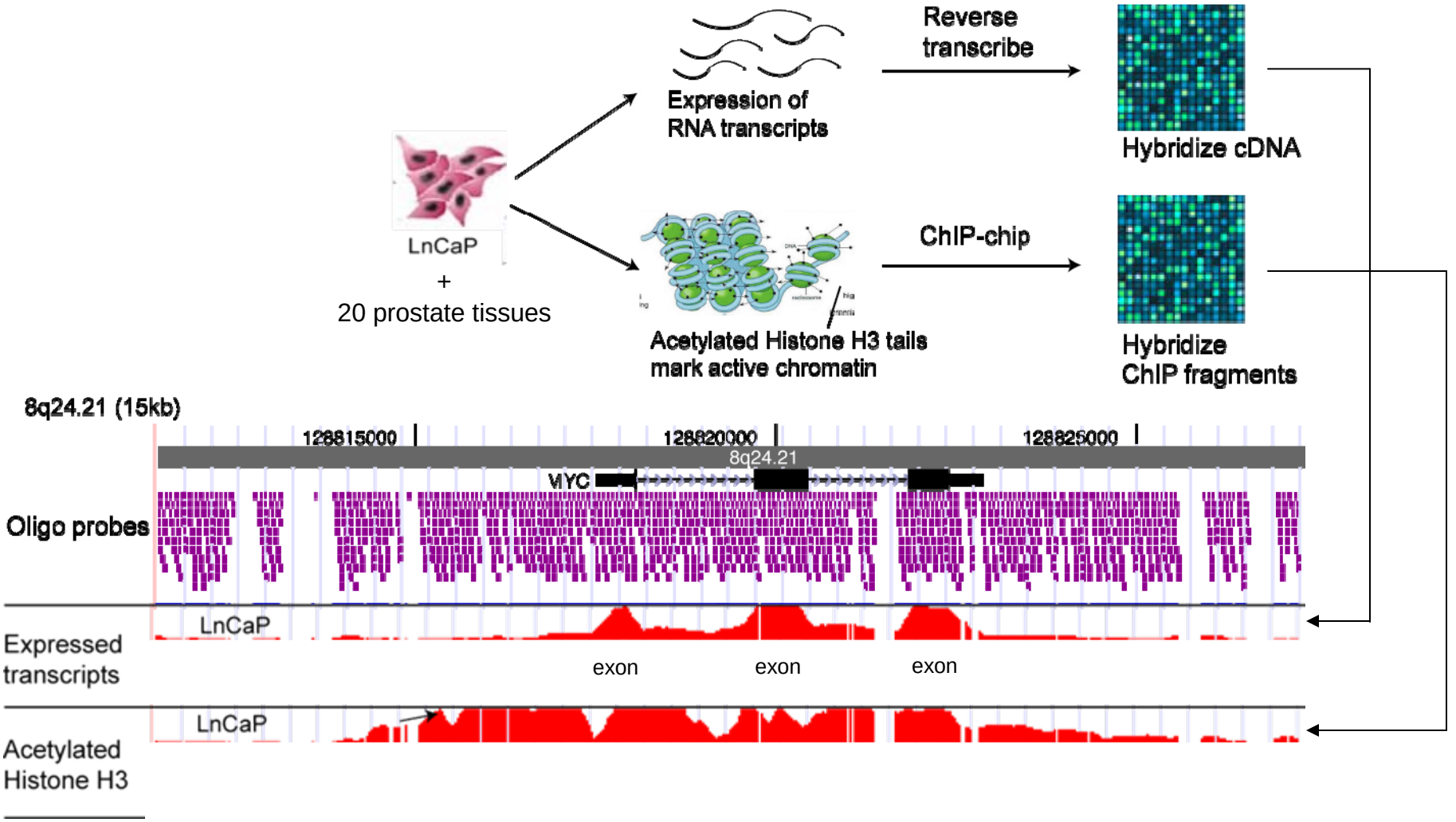
Mark*	Transcriptionally relevant sites†	Transcriptional role‡
DNA methylation		
Methylated cytosine (meC)	CpG islands	Repression
Histone PTMs		
Acetylated lysine (Kac)	H3 (9, 14, 18, 56), H4 (5, 8, 13, 16), H2A, H2B	Activation
Phosphorylated serine/threonine (S/Tph)	H3 (3, 10, 28), H2A, H2B	Activation
Methylated arginine (Rme)	H3 (17, 23), H4 (3)	Activation
Methylated lysine (Kme)	H3 (4, 36, 79) H3 (9, 27), H4 (20)	Activation Repression
Ubiquitylated lysine (Kub)	H2B (123§/120¶) H2A (119¶)	Activation Repression
Sumoylated lysine (Ksu)	H2B (6/7), H2A (126)	Repression
Isomerized proline (Pisom)	H3 (30–38)	Activation/repression

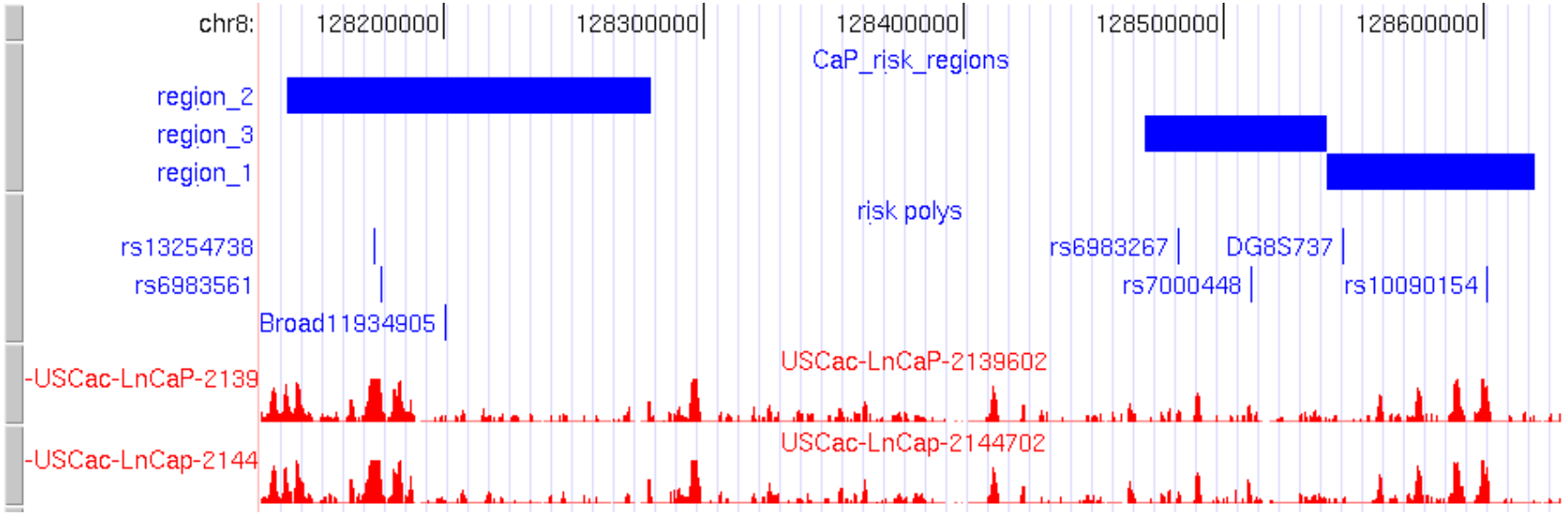
*The modification on either DNA or a histone.
†Well-characterized sites with regard to genomic location for DNA methylation or residues within histones for PTMs.
‡Whether the epigenetic mark is associated with activation or repression.
§Yeast (*Saccharomyces cerevisiae*).
¶Mammals.

Nature 447:407

Active chromatin

Promoter/enhancer


Reverse transcribe
Expression of RNA transcripts
Hybridize cDNA
LnCaP
+
20 prostate tissues
ChIP-chip
Acetylated Histone H3 tails mark active chromatin
Hybridize ChIP fragments
8q24.21 (15kb)
128815000
128820000
128825000
8q24.21
MYC
Oligo probes
LnCaP
Expressed transcripts
exon
exon
exon
LnCaP
Acetylated Histone H3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128300000
128400000
128500000
128600000
CaP_risk_regions
region_2
region_3
region_1
risk polys
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rs6983561
Broad11934905
rs6983267
DG8S737
rs7000448
rs10090154
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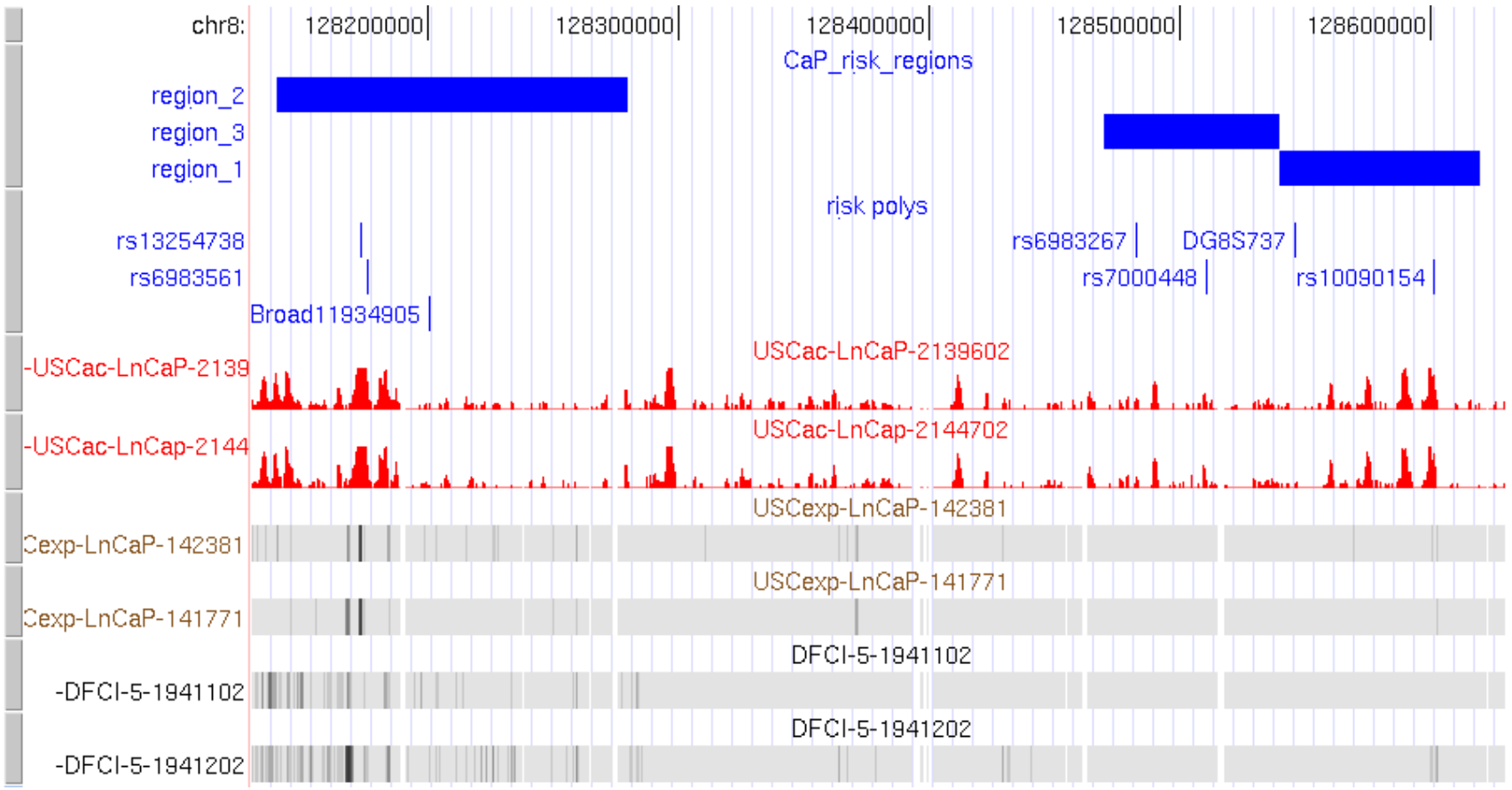
USCac-LnCaP-2139602
-USCac-LnCap-2144
USCac-LnCap-2144702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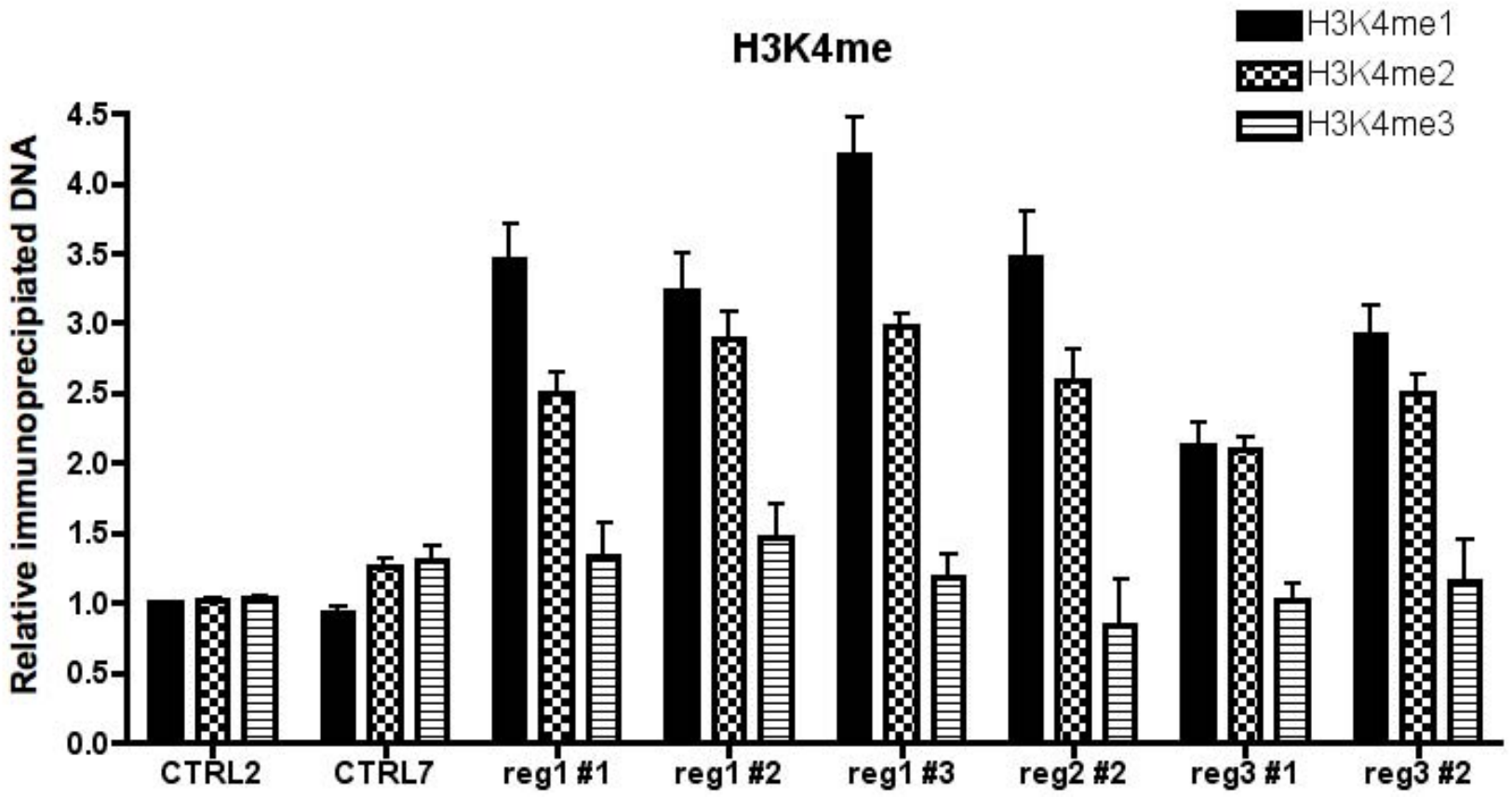
128300000
128400000
128500000
128600000
CaP_risk_regions
region_2
region_3
region_1
risk polys
rs13254738
rs6983561
Broad11934905
rs6983267
DG8S737
rs7000448
rs10090154
-USCac-LnCaP-2139
USCac-LnCaP-2139602
-USCac-LnCap-2144
USCac-LnCap-2144702
Cexp-LnCaP-142381
USCexp-LnCaP-142381
Cexp-LnCaP-141771
USCexp-LnCaP-141771
-DFCI-5-1941102
DFCI-5-1941102
-DFCI-5-1941202
DFCI-5-1941202

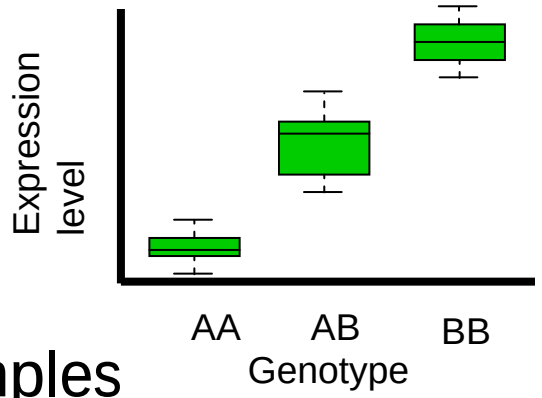
8q24 regions are bear marks of enhancers

Jerome Eekhoute and Mathieu Lupien



Future directions: what is the region enhancing?

- How is 8q24 influencing expression?
  - Directed
    - 12 transcripts
    - 150 histologically normal prostate samples
  - Unbiased
    - Chromosome Conformation Capture



QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

Science 312:269

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8q24 variants and clinical parameters

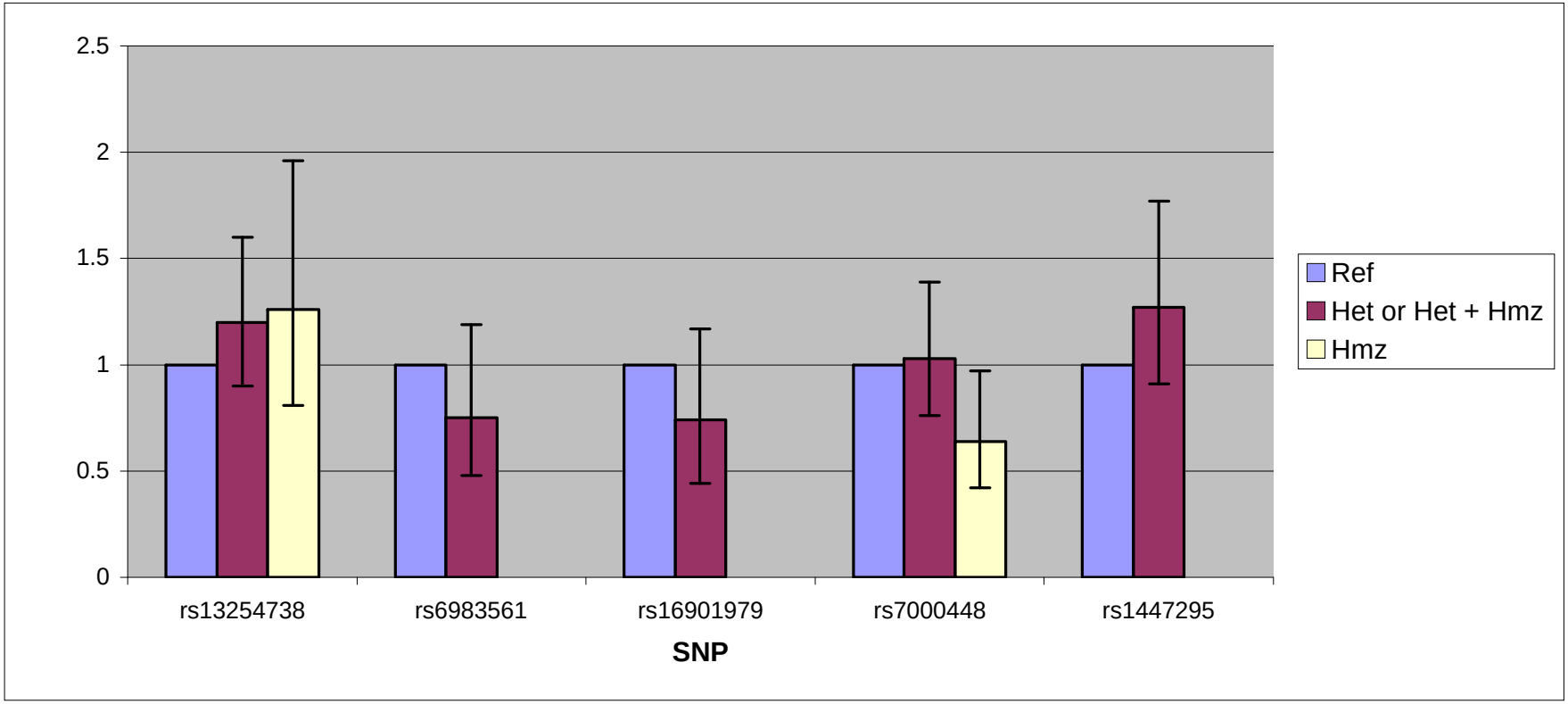
Kathryn Penney and Mark Pomerantz

	PHS (n=598)	DFCI aggressive (n=762)	DFCI RP (n=500)
Age at diagnosis (mean)	(n=598) 68.5	(n=734) 62.2	(n=459) 56.7
PCa deaths/long term survivors	156/396	277/168	---
Gleason score **<7** **7** **>7**	(n=490) 51.4 32.9 15.7	(n=684) 17.8 32.7 49.4	(n=460) 41.5 51.1 7.4
PSA at diagnosis* (median)	(n=221) 9.1	(n=414) 11.0	(n=426) 5.0
Pathologic stage **T1-T2** **T3-T4**	---	---	(n=454) 85.9 14.1

*PSA at diagnosis does not include individuals who were diagnosed with metastases

8q24 and PCa mortality

Kathryn Penney and Mark Pomerantz



N=433 PCa deaths and N=564 > 10 year survivors
Adjusted for age at diagnosis and cohort