# Illumina Arrays

## Data Evaluation

*uwe.menzel@genpat.uu.se*

human610-quad beadchip

Genotyping & CNV analysis

*http://www.illumina.com/pages.ilmn?ID=248*

| HUMAN610-QUAD V1 CONTENT | |
|---|---|
| Number of Markers per Sample | 620,901 |
| Number of Samples per BeadChip | 4 |
| DNA Input Requirement (per sample) | 200 ng |
| **Genomic Coverage** | |
| CEU (Mean/Median/$r^2$ > 0.8) | 0.93/1.0/0.89 |
| CHB+JPT | 0.91/1.0/0.86 |
| YRI | 0.75/0.88/0.58 |
| **Minor Allele Frequency*** | |
| CEU (Mean/Median) | 0.23/0.23 |
| CHB+JPT | 0.21/0.20 |
| YRI | 0.22/0.20 |
| **Spacing (kb)** | |
| (Mean/Median) | 4.7/2.7 |
| 90th %ile Largest Gap | 11.0 |
| **Marker Categories** | |
| Markers Within 10kb of a RefSeq Gene | 309,978 |
| Non-Synonymous SNPs** | 7,577 |
| MHC†/ADME†/Indel SNPs | 5,728/8,189/0 |
| Sex Chromosome (X/Y/PAR Loci) | 17,681/2,160/452 |
| Mitochondrial SNPs | 138 |
| **CNV Coverage** | |
| Number of DGV§ Regions Represented | 3,938 |
| Number of Markers in DGV Regions | 184,064 |
| Average Markers per Region | 37.7 |
| Targets Novel CNV Regions (~9K) | Yes |

# human610-quad beadchip

- 610,000 rationally selected tag SNPs and markers per sample
- captures the majority of known variations (haplotypes) (based on HapMap[1] release 23)
- detection of both known and novel CNV regions

[1] *http://www.hapmap.org/whatishapmap.html.en*

# Illumina GenomeStudio
(replaces BeadStudio)

**Software: GenomeStudio - Genotyping Module**

The GenomeStudio Genotyping (GT) Module supports the analysis of Infinium and GoldenGate Genotyping Assay data collected by the iScan System and BeadXpress Reader. This module enables efficient genotyping data normalization, genotype calling, clustering, data intensity analysis, loss of heterozygosity (LOH) calculation, and copy number variation (CNV) analysis. Fully integrated with Infinium LIMS server, the GT Module allows you to directly access data and manage projects from within GenomeStudio.

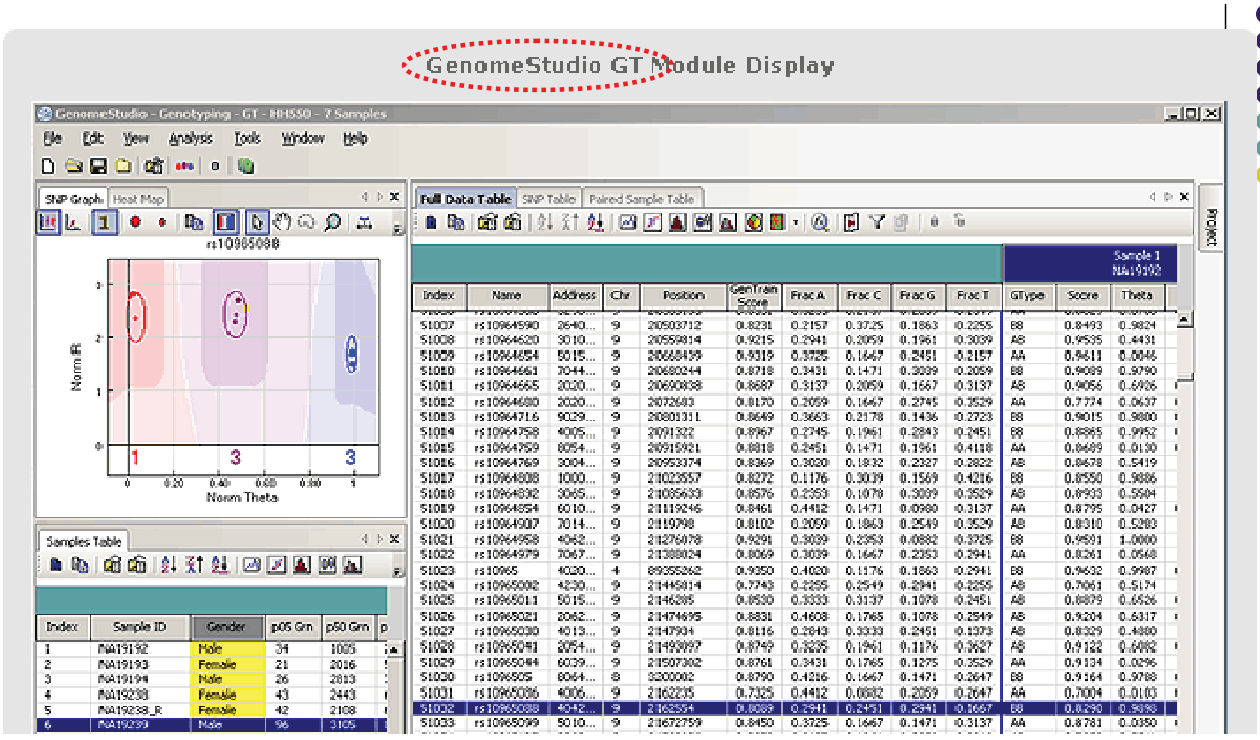
As in all GenomeStudio modules, the GenomeStudio Framework displays data output in tabular form and enables you to quickly and easily visualize your results using the Illumina Genome Viewer and Illumina Chromosome Browser graphical tools.

*http://www.illumina.com/pages.ilmn?ID=309*

# GenomeStudio – Genotyping Module

Structural variation is identified using the same markers as genotyping and intensity-only probes with algorithms to calculate loss of heterozygosity (LOH), and abnormal copy numbers (CNVs). Identified structural variants can be bookmarked

*see also: GenomeStudioTM Data Analysis Software.pdf* page 3




"Export genotype data to various third party applications; access multiple CNV algorithms and copy number variation analysis tools"

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# From GenomeStudio:

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | **Name** | **Chr** | **Position** | **X.GType** | **X.Log R Ratio** | **X.B Allele Freq** |
| 2 | rs12354060 | 1 | 10004 | BB | 0.05767579 | 1 |
| 3 | rs2691310 | 1 | 46844 | NC | -0.1525835 | 0.5361379 |
| 4 | rs2531266 | 1 | 59415 | NC | 0.2049716 | 0.3973282 |
| 5 | rs4124251 | 1 | 97215 | NC | -0.09452707 | 0.09473621 |
| 6 | rs8179466 | 1 | 224176 | NC | -0.02189994 | 1 |
| 7 | rs6603779 | 1 | 227744 | NC | 0.0836625 | 0.6220879 |
| 8 | cnvi0007379 | 1 | 311662 | NC | -0.1188801 | 1 |
| 9 | cnvi0019140 | 1 | 314893 | NC | -0.1406044 | 0 |
| 10 | cnvi0007389 | 1 | 318309 | NC | -0.1599025 | 0 |
| 11 | | | | | | |

*Tomas Axelsson, ETJ1 data*

# CBS script on anaconda:

Usage: run_CBS OPTIONS

-f S1.txt : Illumina SNP-CGH file for one sample
-lcol 5 : Number of the column containing the log2 intensity ratios
-minsnp 10 : Minimum number of markers in an aberration
-minlength 50k : Minimum length of an aberration

*EXAMPLE:* run_CBS -f Sample1.txt -lcol 5 -minsnp 10 -minlength 50k

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# *run_CBS* runs *DNAcopy* from the Bioconductor package (R)

CBS

## DNAcopy

### DNA copy number data analysis

Segments DNA copy number data using circular binary segmentation to detect regions with abnormal copy number

| | |
|---|---|
| Author | Venkatraman E. Seshan, Adam Olshen |
| Maintainer | Venkatraman E. Seshan |

To install this package, start R and enter:

```
source("http://bioconductor.org/biocLite.R")
biocLite("DNAcopy")
```

*http://www.bioconductor.org/packages/2.3/bioc/html/DNAcopy.html*

*Biostatistics* (2004), **5**, 4, *pp.* 557–572
doi: 10.1093/biostatistics/kxh008

# Circular binary segmentation for the analysis of array-based DNA copy number data

ADAM B. OLSHEN, E. S. VENKATRAMAN
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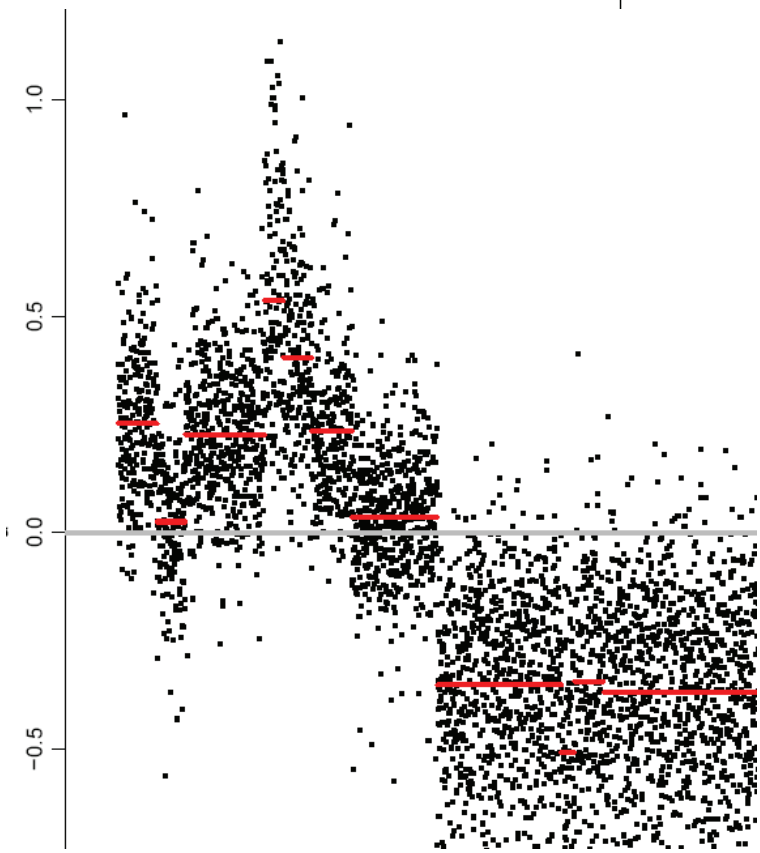
ROBERT LUCITO, MICHAEL WIGLER
*Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724, USA*

SUMMARY

DNA sequence copy number is the number of copies of DNA at a region of a genome. Cancer progression often involves alterations in DNA copy number. Newly developed microarray technologies enable simultaneous measurement of copy number at thousands of sites in a genome. We have developed a modification of binary segmentation, which we call *circular binary segmentation*, to translate noisy intensity measurements into regions of equal copy number. The method is evaluated by simulation and is demonstrated on cell line data with known copy number alterations and on a breast cancer cell line data set.

# Change-point method

- looks for changes in the distribution of data
- uses log-ratios of normalized intensities




# Binary Segmentation
## (Sen & Srivastava, 1975)

Likelihood ratio statistics

$H_0$: no change of the (normal-) distribution

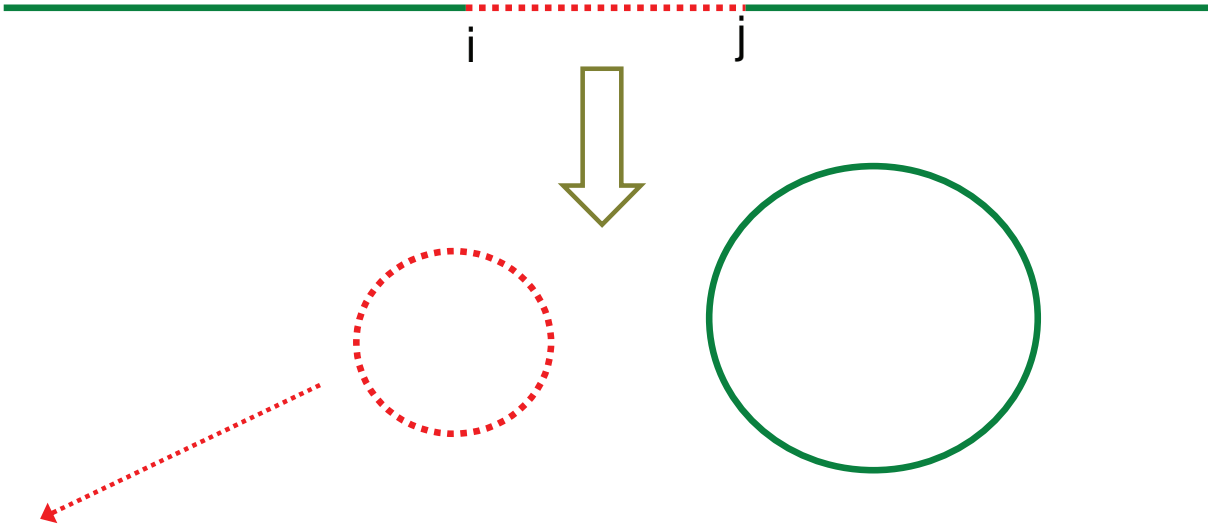
$$S_i = X_1 + \cdots + X_i, 1 \leqslant i \leqslant n$$

partial sums

$$Z_i = \{1/i + 1/(n-i)\}^{-1/2}\{S_i/i - (S_n - S_i)/(n-i)\}$$

$H_0$ is rejected if $Z_i$ gets too large → change point at i (break point)

# Circular Binary Segmentation




$$Z_{ij} = \{1/(j-i) + 1/(n-j+i)\}^{-1/2}\{(S_j - S_i)/(j-i) - (S_n - S_j + S_i)/(n-j+i)\}$$

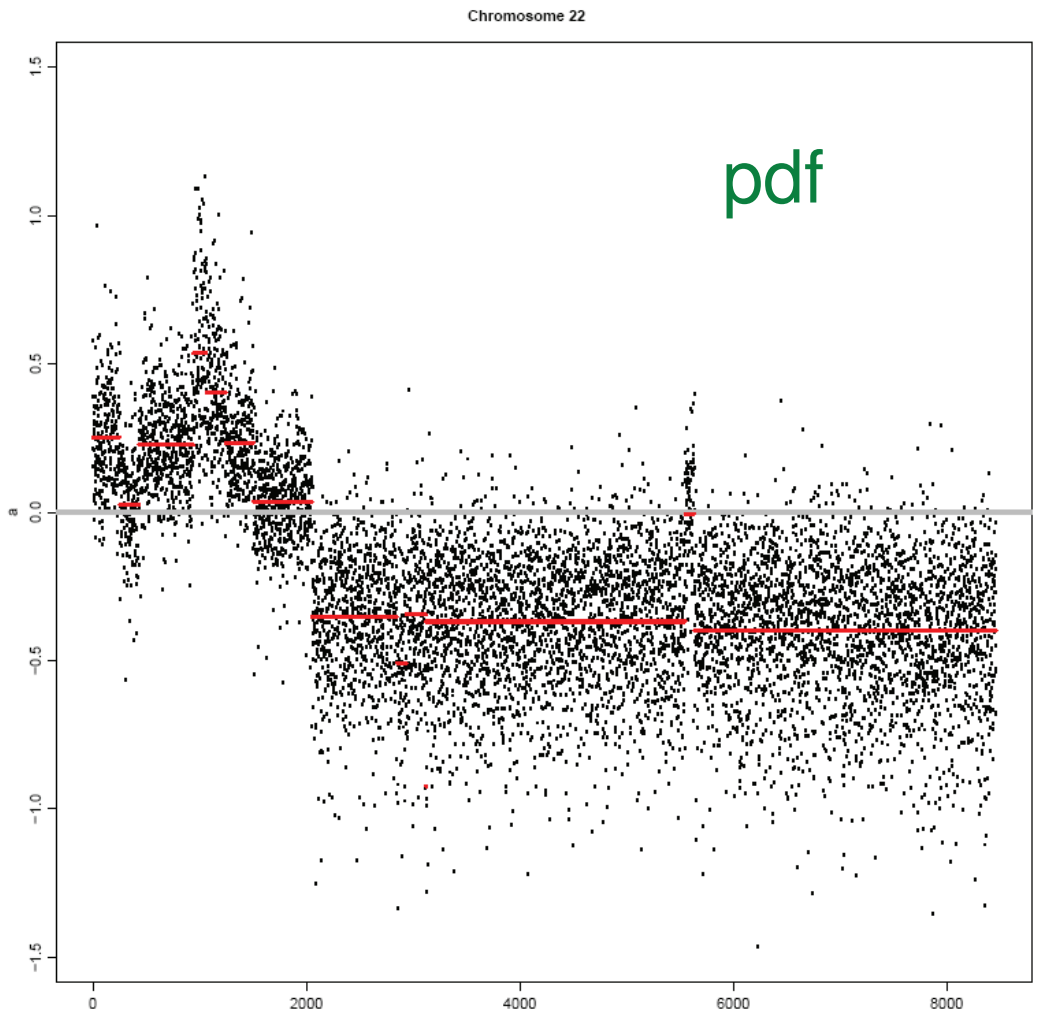
Tests if the arc from i to j has a mean which is different from the mean of the **complement.**

# Practical example

- anaconda:/nfs/1d/menzel/RUN_CBS >
  - run_CBS -f *Sample1.txt* -lcol *5* -minsnp *10* -minlength *50k*
  - 620312 rows in Sample1.txt
  - ≤ 45 minutes
- Output:
  - Sample1_CBS.pdf
  - Sample1_CBS.txt
  - Sample1_CBS.gff

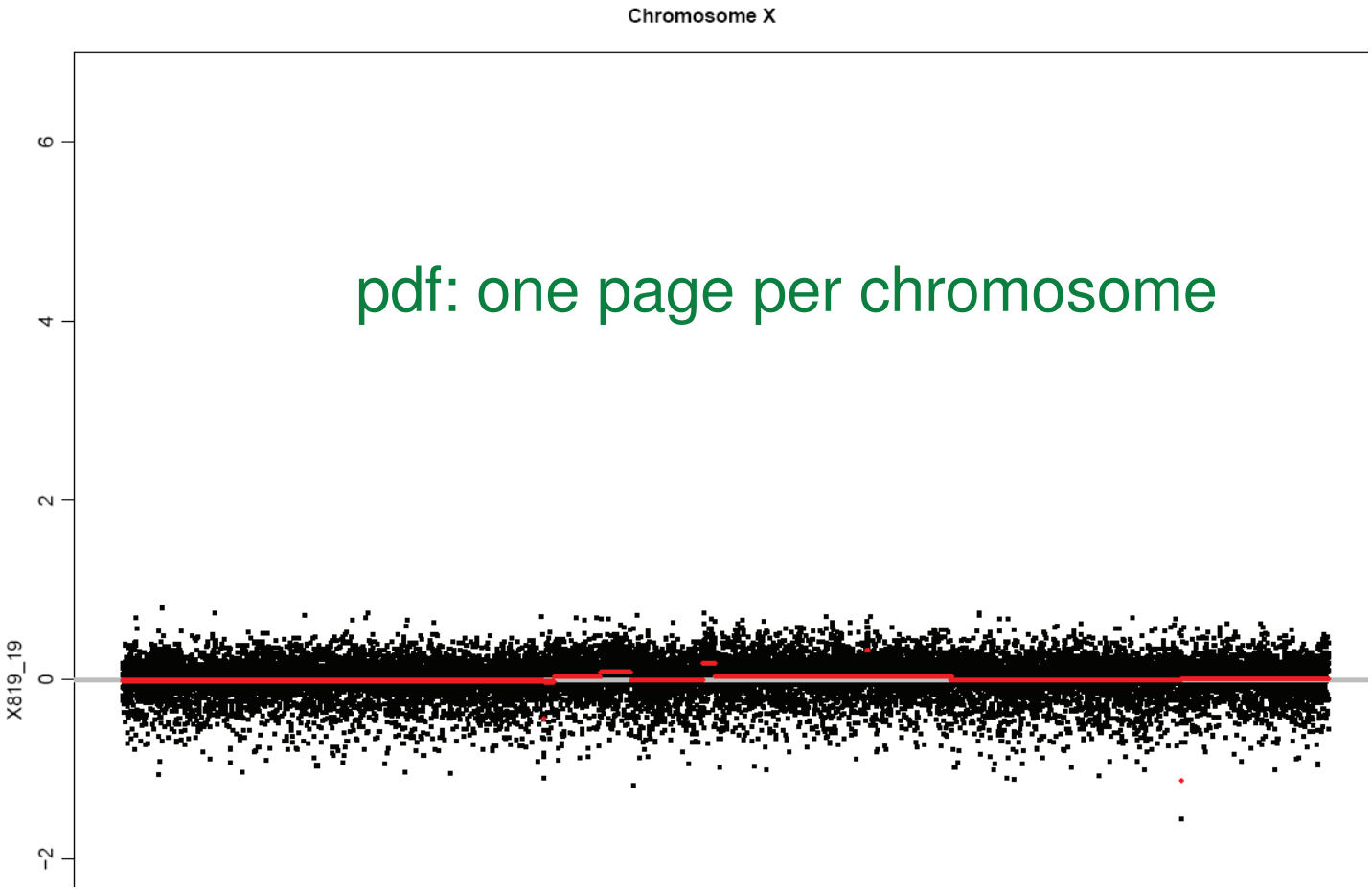
} next 3 slides

*uwe.menzel@genpat.uu.se*




pdf

from Devins data:
chr22, Tumor 5
**run_DNAcopy.R**
21/10/08
anaconda




pdf: one page per chromosome

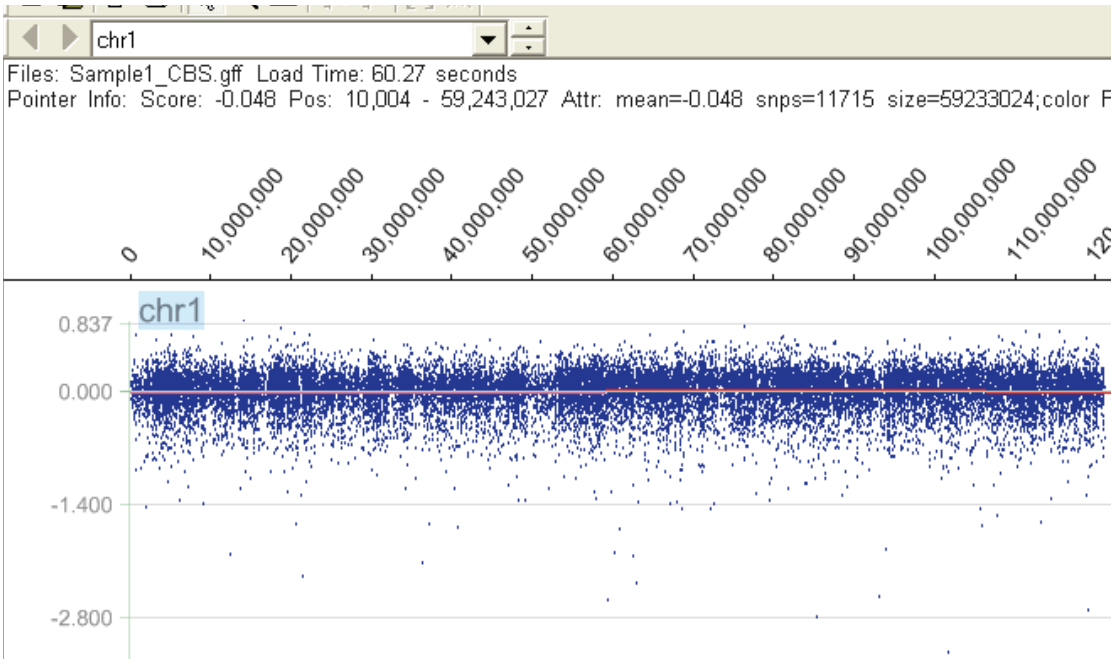
# Textual output of DNAcopy

txt

| ID | chrom | loc.start | loc.end | num.mark | seg.mean |
|---|---|---|---|---|---|
| X819_19 | 1 | 10004 | 59243027 | 11715 | -0.048 |
| X819_19 | 1 | 59244985 | 106543804 | 9983 | -0.0292 |
| X819_19 | 1 | 106552132 | 167468191 | 8082 | -0.0514 |
| X819_19 | 1 | 167495768 | 167505182 | 3 | -5.8199 |
| X819_19 | 1 | 167524930 | 247185943 | 17298 | -0.0439 |
| X819_19 | 10 | 59083 | 135372744 | 32059 | -0.0385 |
| X819_19 | 11 | 123495 | 36481331 | 9235 | -0.0429 |
| X819_19 | 11 | 36481704 | 42432739 | 1057 | 0.0072 |
| X819_19 | 11 | 42445071 | 55124465 | 1596 | -0.0446 |
| X819_19 | 11 | 55127597 | 55165276 | 21 | -0.5282 |
| X819_19 | 11 | 55171592 | 89354301 | 6805 | -0.0473 |
| X819_19 | 11 | 89362241 | 111723307 | 4856 | -0.2925 |
| X819_19 | 11 | 111725592 | 125065390 | 3311 | -0.2358 |
| X819_19 | 11 | 125067925 | 134445626 | 2801 | -0.0465 |
| X819_19 | 12 | 21054 | 44185663 | 10725 | -0.0424 |
| X819_19 | 12 | 44194223 | 44211231 | 3 | -0.9389 |
| X819_19 | 12 | 44217885 | 132288869 | 18582 | -0.0417 |
| X819_19 | 13 | 17922259 | 56654503 | 9313 | -0.0374 |
| X819_19 | 13 | 56659471 | 56680301 | 6 | -0.8908 |
| X819_19 | 13 | 56690719 | 114123122 | 13367 | -0.0235 |
| X819_19 | 14 | 18070422 | 40502100 | 5283 | -0.0314 |
| X819_19 | 14 | 40503056 | 48154747 | 1449 | 0.0102 |

# The gff-file can be loaded to SignalMap (NimbleGen)

gff

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# CBS (DNAcopy)

- ... does not make ”calls”

# CGHcall

BIOINFORMATICS APPLICATIONS NOTE

Vol. 23 no. 7 2007, pages 892–894
doi:10.1093/bioinformatics/btm030

*Genome analysis*

## CGHcall: calling aberrations for array CGH tumor profiles

Mark A. van de Wiel[1,2,3,*], Kyung In Kim[4], Sjoerd J. Vosse[1], Wessel N. van Wieringen[3], Saskia M. Wilting[1] and Bauke Ylstra[1]

[1]Department of Pathology and [2]Department of Biostatistics, VU University Medical Center, PO Box 7057, 1007MB Amsterdam, [3]Department of Mathematics, Vrije Universiteit, Amsterdam and [4]Department of Mathematics, Technische Universiteit, Eindhoven, The Netherlands

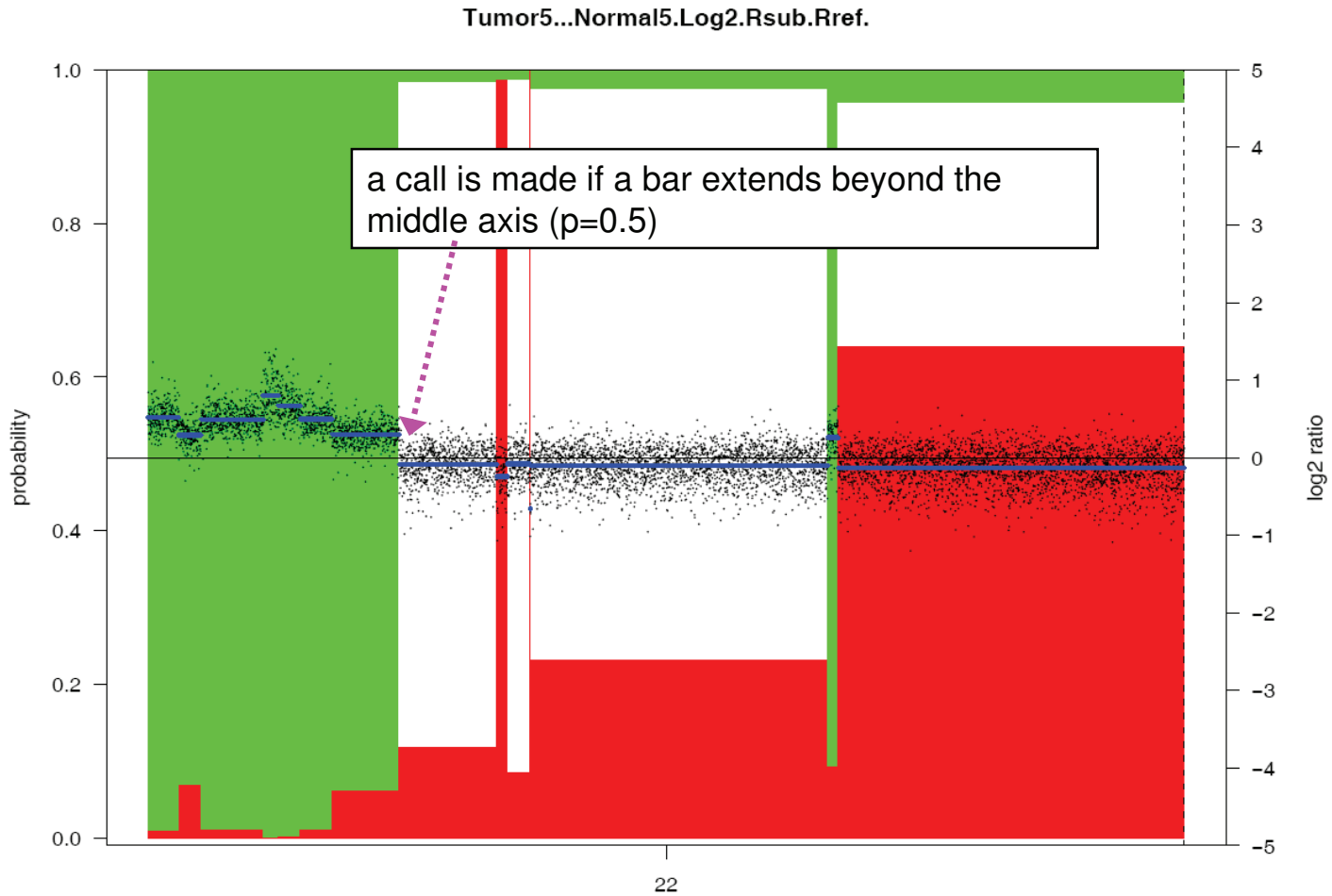
Received on December 15, 2006; revised on January 23, 2007; accepted on January 23, 2007

# CGHcall

Our algorithm, named CGHcall, combines strong concepts of previously developed methods. First, we used the segmentation results of DNAcopy (also known as CBS) (Olshen *et al.*, 2004), which was shown to be one of the strongest segmentation algorithms (Willenbrock and Fridlyand, 2005). Secondly, one cannot expect loss, normal and gain levels to be uniform over all data, so we allow fluctuations by using random effects (Engler *et al.*, 2006). Finally, as in (Picard *et al.*, 2005), we combine the segmentation results with a mixture model to obtain the most likely classification per segment rather than per individual clone.

"calls"

*run_CGHcall.R*




*anaconda:/nfs/1d/menzel/TEST_DNAcopy/Sample_550K_Paired_LogR_chr22_nos4.txt.sample4.pdf*

# Textual output of CGHcall

| Name | Chr | Position | Tumor1 | T2 | T3 | T5 | T6 |
|---|---|---|---|---|---|---|---|
| rs4911642 | 22 | 14884399 | 0 | 0 | 0 | 1 | 0 |
| rs2027653 | 22 | 15298335 | 0 | 0 | 0 | 1 | 0 |
| rs5747620 | 22 | 15412698 | 0 | 0 | 0 | 1 | 0 |
| rs9605903 | 22 | 15434720 | 0 | 0 | 0 | 1 | 0 |
| rs5747968 | 22 | 15447504 | 0 | 0 | 0 | 1 | 0 |
| rs2236639 | 22 | 15452483 | 0 | 0 | 0 | 1 | 0 |
| rs5747999 | 22 | 15455353 | 0 | 0 | 0 | 1 | 0 |
| rs11089263 | 22 | 15467656 | 0 | 0 | 0 | 1 | 0 |
| rs2096537 | 22 | 15474749 | 0 | 0 | 0 | 1 | 0 |
| rs9604959 | 22 | 15479107 | 0 | 0 | 0 | 1 | 0 |
| rs9604967 | 22 | 15492342 | 0 | 0 | 0 | 1 | 0 |
| rs4819849 | 22 | 15532611 | 0 | 0 | 0 | 1 | 0 |
| rs9605028 | 22 | 15534984 | 0 | 0 | 0 | 1 | 0 |
| rs1892844 | 22 | 15535383 | 0 | 0 | 0 | 1 | 0 |

*anaconda:/nfs/1d/menzel/TEST_DNAcopy/CGHcall_results.txt*

0,1,-1

# Comparison Studies

BIOINFORMATICS ORIGINAL PAPER Vol. 21 no. 22 2005, pages 4084–4091
doi:10.1093/bioinformatics/bti677

*Genome analysis*

## A comparison study: applying segmentation to array CGH data for downstream analyses

Hanni Willenbrock[1] and Jane Fridlyand[2,*]

[1]Center for Biological Sequence Analysis, Department of Biotechnology, Building 208, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark and [2]Department of Epidemiology and Biostatistics, University of California at San Francisco, 2340 Sutter Street, N224, San Francisco, CA 94143, USA

Received on July 5, 2005; revised on September 8, 2005; accepted on September 12, 2005
Advance Access publication September 13, 2005

frequently cited by BioDiscovery ( Nexus)

# Willenbrock, Fridlyand:

Our results have indicated that segmentation by any of the three methods aids downstream analyses of array CGH data. Of the methods under comparison, DNAcopy has the best operational characteristics in terms of its sensitivity and FDR for breakpoint detection. However, it should be noted that it is not able to identify single clone aberrations. While our comparison was limited to only three methods, albeit widely used, our study sets an example as a

DNAcopy
HMM (Fridlyand)
GLAD (Hupe)

BIOINFORMATICS ORIGINAL PAPER
Vol. 22 no. 23 2006, pages 2910–2917
doi:10.1093/bioinformatics/btl502

*Gene expression*

## Evaluating the performance of microarray segmentation algorithms

Antti Lehmussola*, Pekka Ruusuvuori and Olli Yli-Harja
Institute of Signal Processing, Tampere University of Technology, PO Box 553, 33101 Tampere, Finland

Received on July 21, 2006; revised on September 13, 2006; accepted on September 30, 2006

| Algorithm | Description |
|---|---|
| Fixed circle (FC) (Eisen, 1999) | Circular mask with constant radius |
| Adaptive circle (AC) (Buhler *et al.*, 2000) | Circular mask with independently estimated radius for each spot |
| Seeded region growing (SRG) (Yang *et al.*, 2002) | Segmentation with seeded region growing segmentation algorithm |
| Mann–Whitney (MW) (Chen *et al.*, 1997) | Computing segmentation threshold iteratively with Mann–Whitney test |
| *k*-means (KM) (Bozinov and Rahnenführer, 2002) | *k*-means clustering of pixels |
| Hybrid *k*-means (HKM) (Rahnenführer and Bozinov, 2004) | *k*-means clustering of pixels and removing outliers with mask matching |
| Markov random field (MRF) (Demirkaya *et al.*, 2005) | MRF modeling of pixels |
| Model-based segmentation (MBS) (Li *et al.*, 2005) | Model-based clustering of pixels and extraction of connected components |
| Matarray (MA) (Wang *et al.*, 2001) | Iterative modification of target mask based on spatial and intensity information |

k-means algorithm best (simulated data)

BIOINFORMATICS ORIGINAL PAPER

Vol. 21 no. 19 2005, pages 3763–3770
doi:10.1093/bioinformatics/bti611

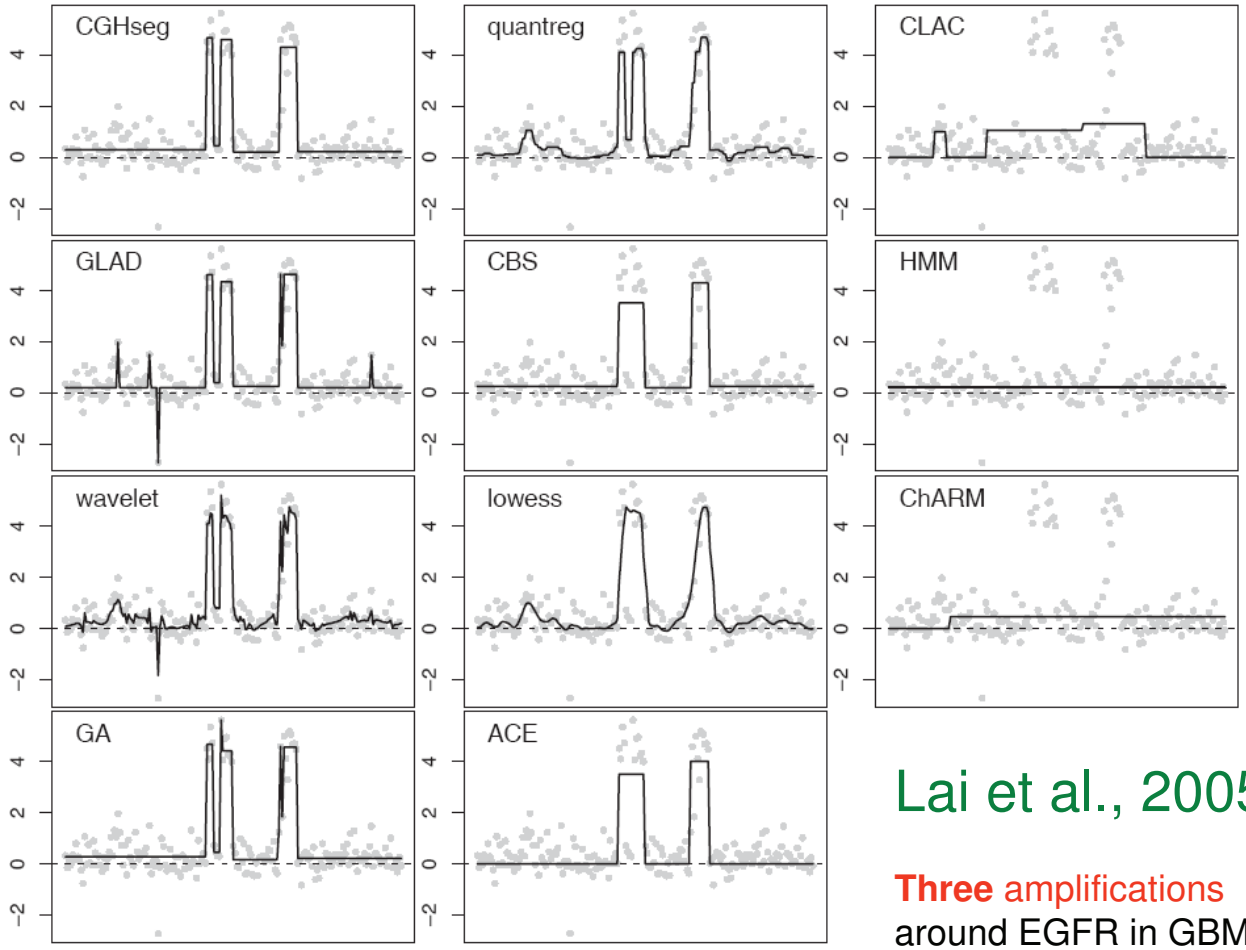
*Genetics and population analysis*

# Comparative analysis of algorithms for identifying amplifications and deletions in array CGH data

Weil R. Lai[1], Mark D. Johnson[2], Raju Kucherlapati[1] and Peter J. Park[1,3,*]

[1]Harvard-Partners Center for Genetics and Genomics, 77 Avenue Louis Pasteur, Boston, MA 02115, USA, [2]Department of Neurological Surgery, Brigham and Women's Hospital and Harvard Medical School, 75 Francis Street, Boston, MA 02115, USA and [3]Children's Hospital Informatics Program, 300 Longwood Ave, Boston, MA 02115, USA

| Name | Reference | Method | Software |
|---|---|---|---|
| CGHseg | Picard *et al.* (2005) | CGH Segmentation | CGHseg, Nov, 2004 (MATLAB) |
| Quantreg | Eilers and de Menezes (2005) | Quantile Smoothing | quantreg, v3.76 (R)* |
| CLAC | Wang *et al.* (2005) | Clustering Along Chromosomes | CLAC, v0.1-1 (R) |
| GLAD | Hupe *et al.* (2004) | Adaptive Weights Smoothing | GLAD, v1.0.2 (R) |
| CBS | Olshen *et al.* (2004) | Circular Binary Segmentation | DNAcopy, v1.1.1 (R) |
| HMM | Fridlyand *et al.* (2004) | Hidden Markov Model | aCGH, v1.1.4 (R) |
| Wavelet | Hsu *et al.* (2005) | Maximal Overlap Discrete Wavelet Transform | waveslim, v1.4 (R)* |
| Lowess | | Locally Weighted Regression | stats, v2.0.1 (R)* |
| ChARM | Myers *et al.* (2004) | Chromosomal Aberration Region Miner | ChARM, v1.6 (JAVA) |
| GA | Jong *et al.* (2003) | Genetic Local Search | aCGHSmooth, Nov, 2004 (exec) |
| ACE | Lingjaerde *et al.* (2005) | Analysis of Copy Errors | CGH-Explorer, v2.3 (JAVA) |




Lai et al., 2005

**Three** amplifications around EGFR in GBM29

# Bioconductor Task View: DNACopyNumber

## Subview of

- Microarray

## Packages in view

| Package | Maintainer | Title |
|---|---|---|
| aCGH | Jane Fridlyand | Classes and functions for Array Comparative Genomic Hybridization data. |
| beadarraySNP | Jan Oosting | Normalization and reporting of Illumina SNP bead arrays |
| CGHbase | Sjoerd Vosse | CGHbase: Base functions and classes for arrayCGH data analysis. |
| CGHcall | Sjoerd Vosse | Calling aberrations for array CGH tumor profiles. |
| CGHregions | Mark van de Wiel | Dimension Reduction for Array CGH Data with Minimal Information Loss. |
| DNAcopy | Venkatraman E. Seshan | DNA copy number data analysis |
| GLAD | Philippe Hupe | Gain and Loss Analysis of DNA |
| ITALICS | Guillem Rigaill | ITALICS |
| KCsmart | Jorma de Ronde | Multi sample aCGH analysis package using kernel convolution |
| MANOR | Pierre Neuvial | CGH Micro-Array NORmalization |
| quantsmooth | Jan Oosting | Quantile smoothing and genomic visualization of array data |
| reb | Karl J. Dykema | Regional Expression Biases |
| SIM | Marten Boetzer | Integrated Analysis of gene expression and copynumber data |
| SMAP | Robin Andersson | A Segmental Maximum A Posteriori Approach to Array-CGH Copy Number Profiling |
| snapCGH | Thomas Hardcastle | Segmentation, normalisation and processing of aCGH data. |
| SNPchip | Robert Scharpf | Classes and Methods for high throughput SNP chip data |
| VanillaICE | Robert Scharpf | Methods for fitting Hidden Markov Models to SNP chip data |

*http://bioconductor.org/packages/2.3/DNACopyNumber.html*

# ”SNP-CGH”

GENOME RESEARCH

## High-resolution genomic profiling of chromosomal aberrations using Infinium whole-genome genotyping

Daniel A. Peiffer, Jennie M. Le, Frank J. Steemers, *et al.*

*Genome Res.* 2006 16: 1136-1148; originally published online Aug 9, 2006;
Access the most recent version at doi:10.1101/gr.5402306

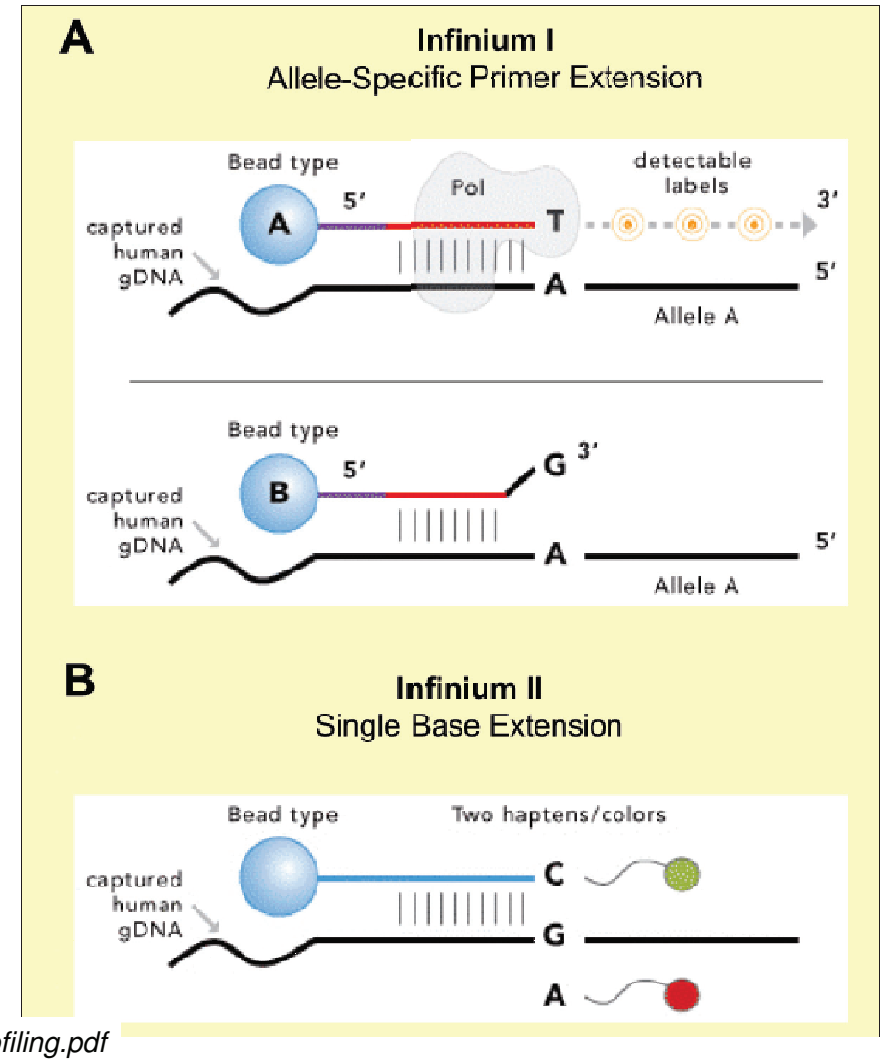
*High-resolution genomic profiling of chromosomal aberrations.pdf*

# SNP-CGH

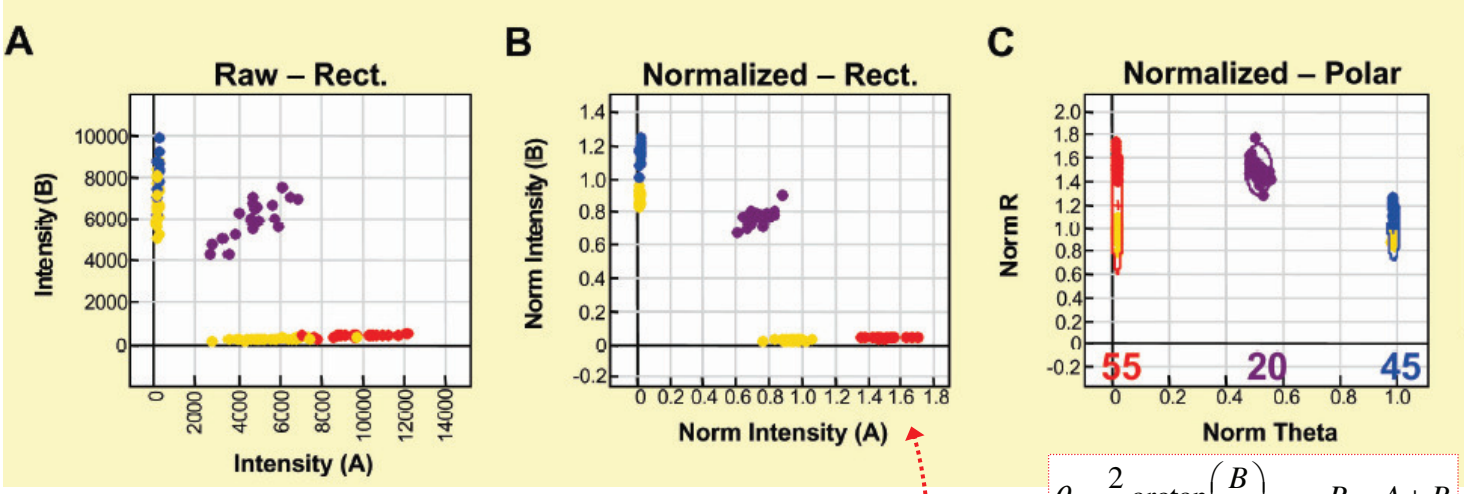
- simultaneous measurement of both signal intensity and allelic composition
  - **(BAF = B-Allele Frequency)**
- detect both copy number changes and copy-neutral loss-of-heterozygosity (LOH)
- Infinium whole-genome genotyping (WGG) BeadChips

*uwe.menzel@genpat.uu.se*

# What B-allele frequency



# Calculation of the BAF




$$\theta = \frac{2}{\pi}\arctan\left(\frac{B}{A}\right) \qquad R = A + B$$

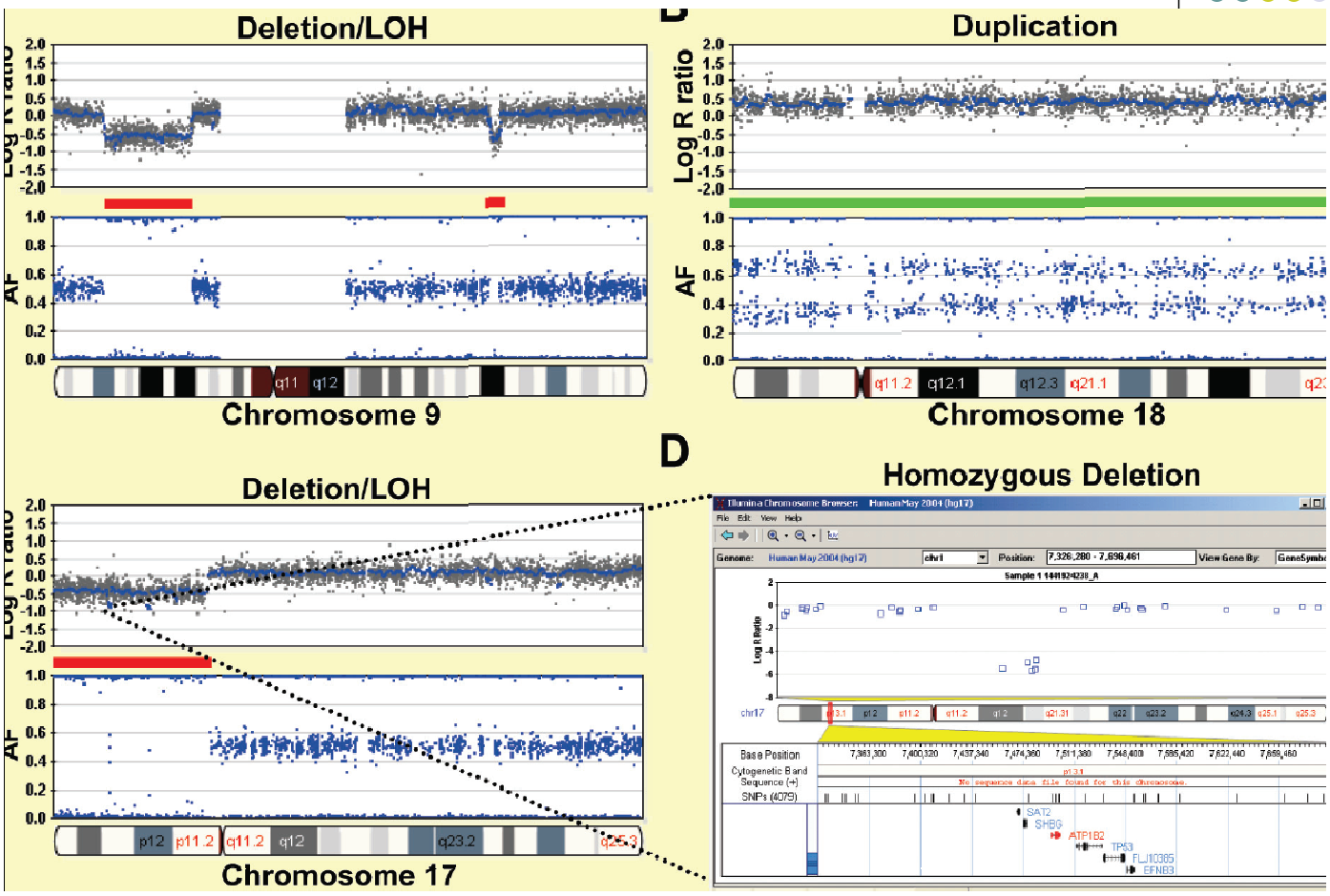
chrX, 120 normal individuals, one particular SNP
raw intensities: males in yellow, the others females
normalization is done using a ”proprietary” algorithm (B)
conversion to ”*polar coordinates*” (C)

| B/A | θ |
|---|---|
| 0 | 0 |
| 1 | 0.5 |
| inf | 1 |

”*canonical clusters*”

*SNP-CGH technologies for genomic profiling.pdf*

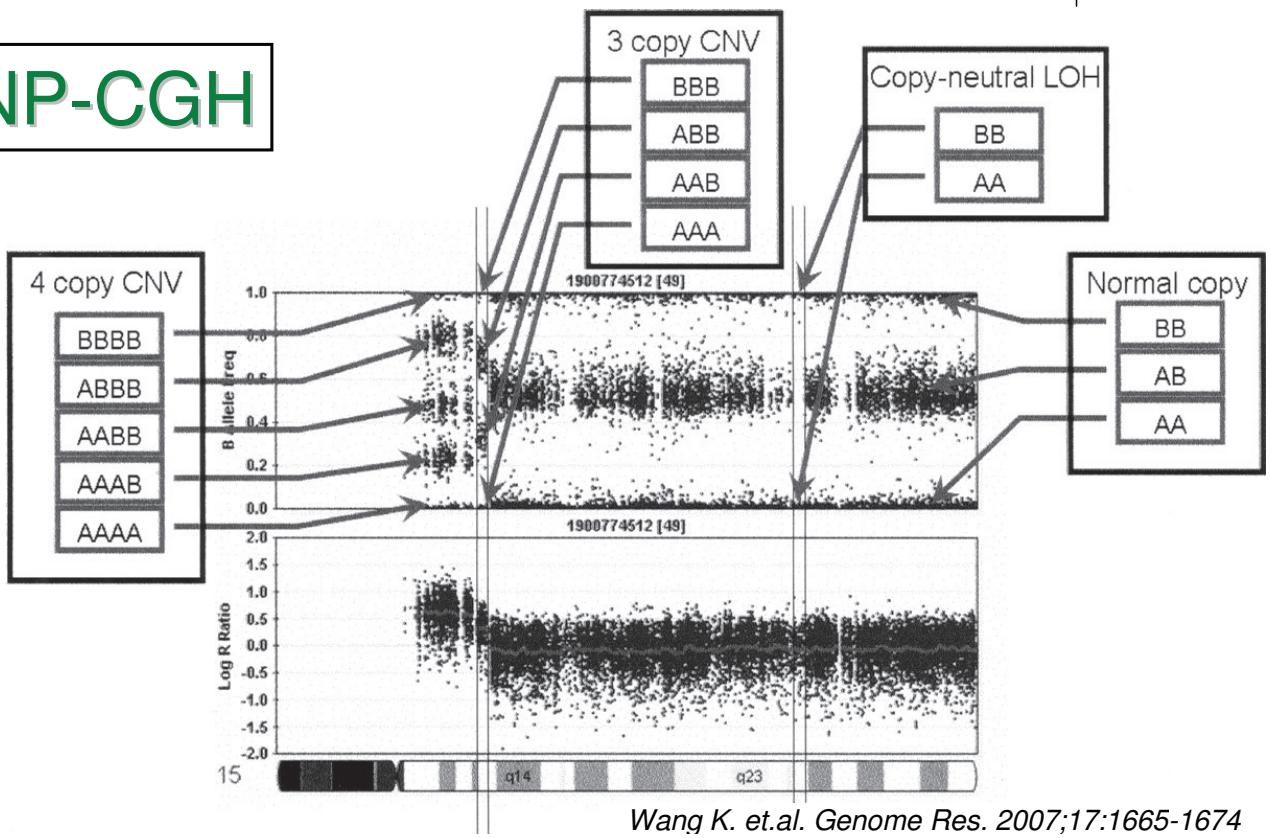
# LRR and BAF illustrated



# Both LRR[1] and BAF[2] can be used to determine copy number

[1]LRR = Log R ratio
[2]BAF = B-allele frequency

SNP-CGH




*Wang K. et.al. Genome Res. 2007;17:1665-1674*

# Illumina academic partners

**University of Pennsylvania**
Department of Genetics
University of Pennsylvania
Philadelphia, PA 19104
Phone: 215.898.0021
Contact: Kai Wang
Email: kai@mail.med.upenn.edu
Web: www.neurogenome.org/cnv/penncnv/
Application Areas Supported: Copy Number Variation Analysis (CNV)

PennCNV

**The Wellcome Trust Centre for Human Genetics**
Roosevelt Drive
Oxford
OX3 7BN
United Kingdom
Phone: +44 (0)1865 287500
Contact: Iaonnis Ragoussis
Email: ioannisr@well.ox.ac.uk
Web: www.well.ox.ac.uk
Application Area Supported: Copy Number Variation (CNV) analysis

QuantiSNP

**PLINK**
Whole genome association analysis toolset
The link below describes how Illumina's WG arrays data from BeadStudio can be converted into a file input format for WGAS using PLINK.

**PLINK Support for BeadStudio data output**

**dChip**
Analysis and visualization of gene expression and SNP microarrays
Contact: Dr Cheng Li
Department of Biostatistics, Harvard School of Public Health and
Department of Biostatistics and Computational Biology
Dana-Farber Cancer Institute
375 Longwood Ave, 6th Floor
Boston, MA, 02215 Email: cli@hsph.harvard.edu
Web: http://biosun1.harvard.edu/complab/dchip/
Application Areas Supported: Copy Number Variation (CNV) analysis, Gene Expression (GEX)

dChip

# PennCNV script on anaconda:

Usage: run_PennCNV OPTIONS

-f S1.txt : Illumina SNP-CGH file for one sample
-minsnp 10 : Minimum number of markers in an aberration
-minlength 50k : Minimum length of an aberration
-gcmodel : Try reducing fluctuations caused by GC-content variation

EXAMPLE: run_PennCNV -f Sample1.txt -minsnp 10 -minlength 50k -gcmodel

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# PennCNV Input:

| | A | B | C | D | E | F |
|---|---|---|---|---|---|---|
| 1 | **Name** | **Chr** | **Position** | **X.GType** | **X.Log R Ratio** | **X.B Allele Freq** |
| 2 | rs12354060 | 1 | 10004 | BB | 0.05767579 | 1 |
| 3 | rs2691310 | 1 | 46844 | NC | -0.1525835 | 0.5361379 |
| 4 | rs2531266 | | 15 | NC | 0.2049716 | 0.3973282 |
| 5 | rs4124251 | 1 | | NC | -0.09452707 | 0.09473621 |
| 6 | rs8179466 | 1 | | NC | -0.02189994 | 1 |
| 7 | rs6603779 | 1 | 2271 | NC | 0.0836625 | 0.6220879 |
| 8 | cnvi0007379 | 1 | 311662 | | -0.1188801 | 1 |
| 9 | cnvi0019140 | 1 | 314893 | | -0.1406044 | 0 |
| 10 | cnvi0007389 | 1 | 318309 | N | 0.1599025 | 0 |
| 11 | | | | | | |

same as for run_CBS

*Tomas Axelsson, ETJ1 data*

# PennCNV Paper


**PennCNV: An integrated hidden Markov model designed for high-resolution copy number variation detection in whole-genome SNP genotyping data**

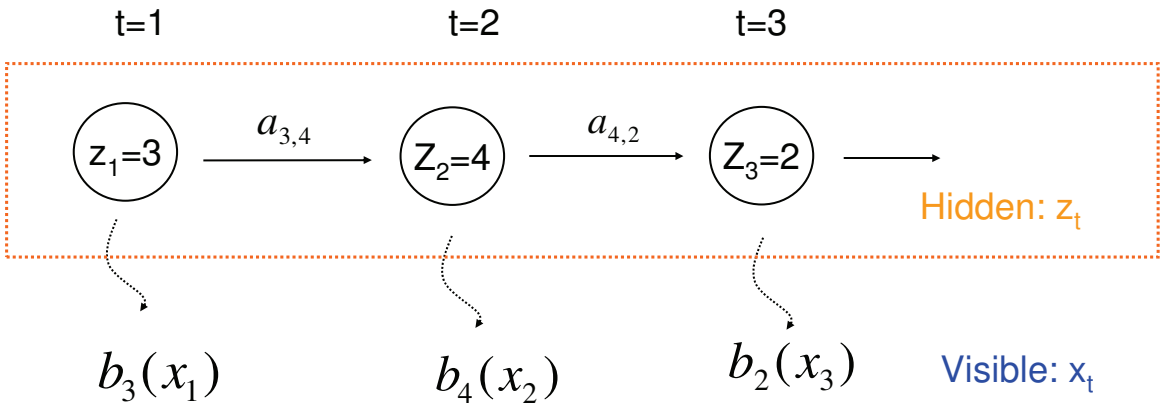
Kai Wang, Mingyao Li, Dexter Hadley, Rui Liu, Joseph Glessner, Struan F.A. Grant, Hakon Hakonarson and Maja Bucan

*Genome Res.* 2007 17: 1665-1674; originally published online Oct 5, 2007;
Access the most recent version at doi:10.1101/gr.6861907

# PennCNV

- Detection of CNVs from Illumina (*Infinium*) high-density SNP genotyping data using:
  - total signal intensity
  - allelic intensity ratio at each SNP marker (BAF)
  - pedigree information if available
- kilobase-resolution (~10 Kb)

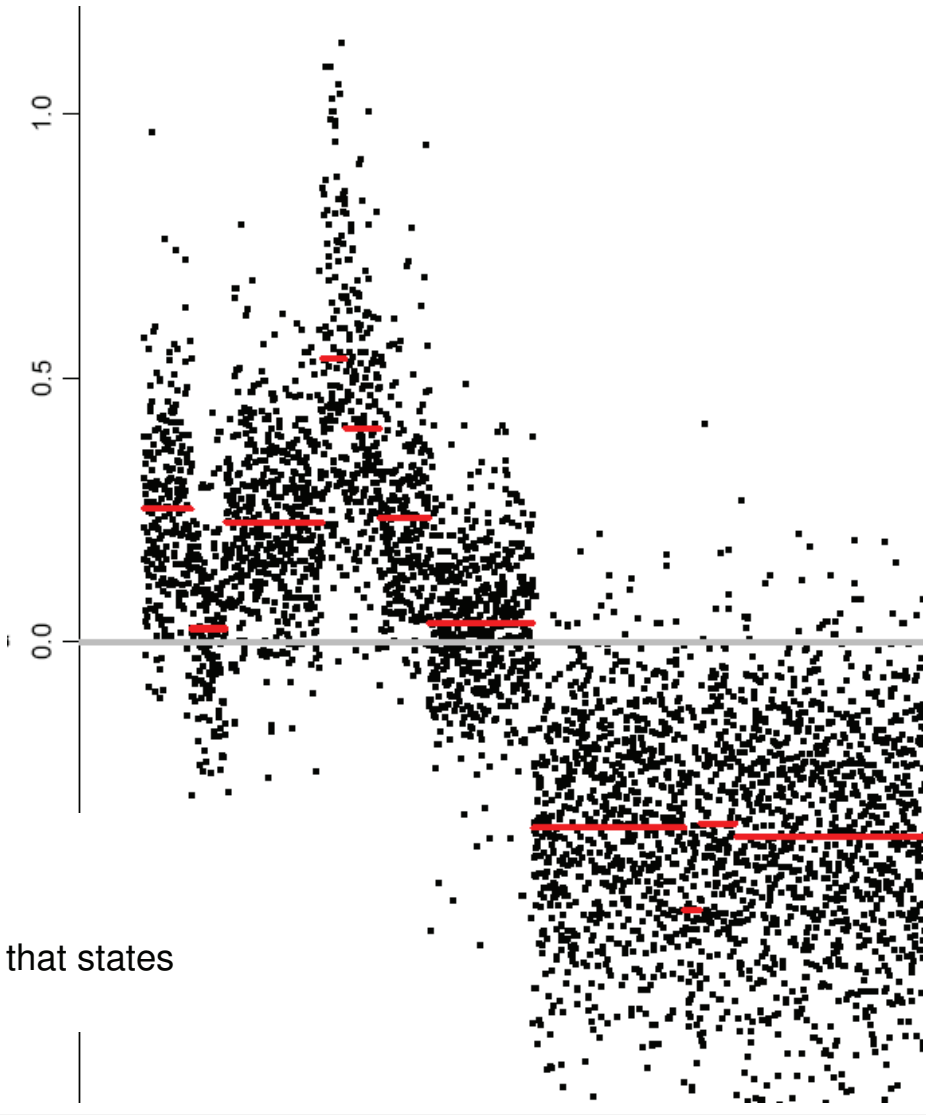
# Hidden Markov Model




$$P(x_1, x_2, ..., z_1, z_2, ... \mid \theta) = p(z_1) \cdot \quad b_{z_1}(x_1) \cdot a_{z_1, z_2} \cdot \quad b_{z_2}(x_2) \cdot a_{z_2, z_3} \cdot \quad ...$$

$$P(x, z \mid \theta) = p(z_1) \cdot \prod_{i=1}^{T} b_{z_i}(x_i) \cdot a_{z_i, z_{i+1}}$$

# HMM for CNV detection




states

. . . . emissions from that states

# PennCNV – states of the HMM

**Table 1.** Hidden states, copy numbers, and their descriptions

| Copy no. state | Total copy no. | Description (for autosome) | CNV genotypes |
|---|---|---|---|
| 1 | 0 | Deletion of two copies | Null |
| 2 | 1 | Deletion of one copy | A, B |
| 3 | 2 | Normal state | AA, AB, BB |
| 4 | 2 | Copy-neutral with LOH | AA, BB |
| 5 | 3 | Single copy duplication | AAA, AAB, ABB, BBB |
| 6 | 4 | Double copy duplication | AAAA, AAAB, AABB, ABBB, BBBB |

Allele frequency information included in the states of the HMM

# PennCNV implementation

run_PennCNV -f Sample1.txt -minsnp 10 -minlength 50k -gcmodel

same inputfile as for run_CBS

Perl-script: /usr/local/share/BIOSW/run_PennCNV.pl
runtime: a few minutes
output: Sample1.txt.log Sample1.txt.calls Sample1_PennCNV.gff

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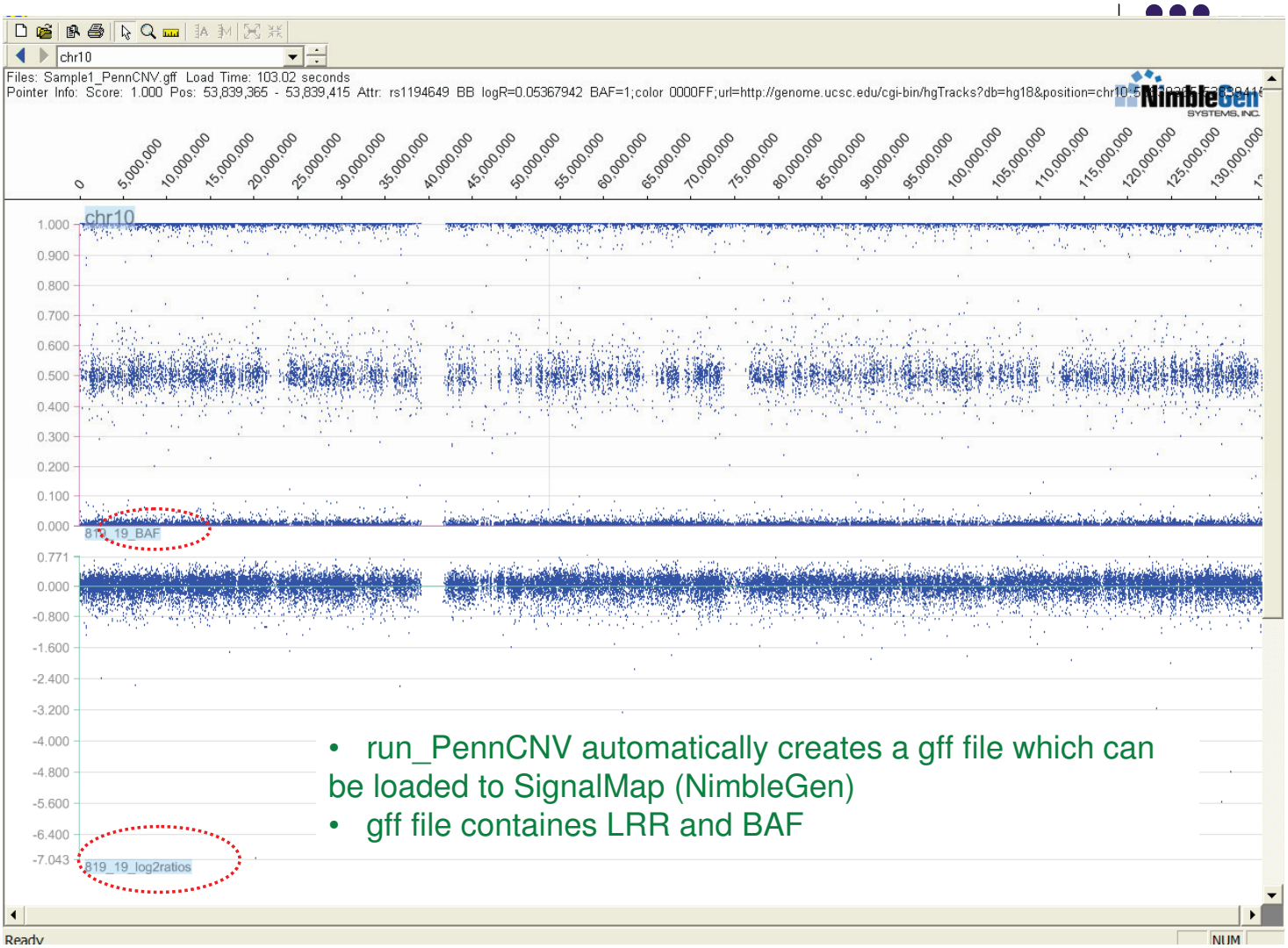
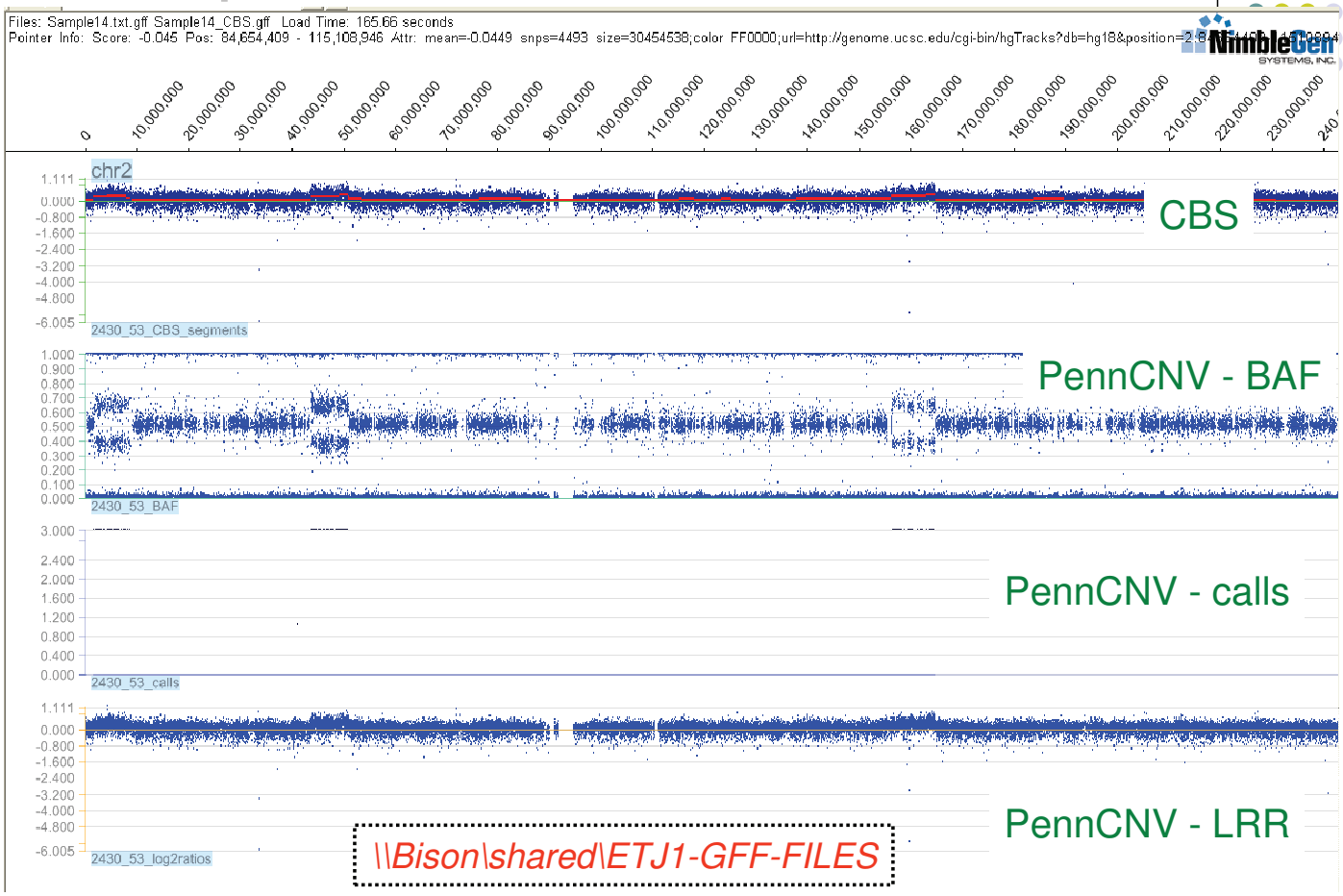
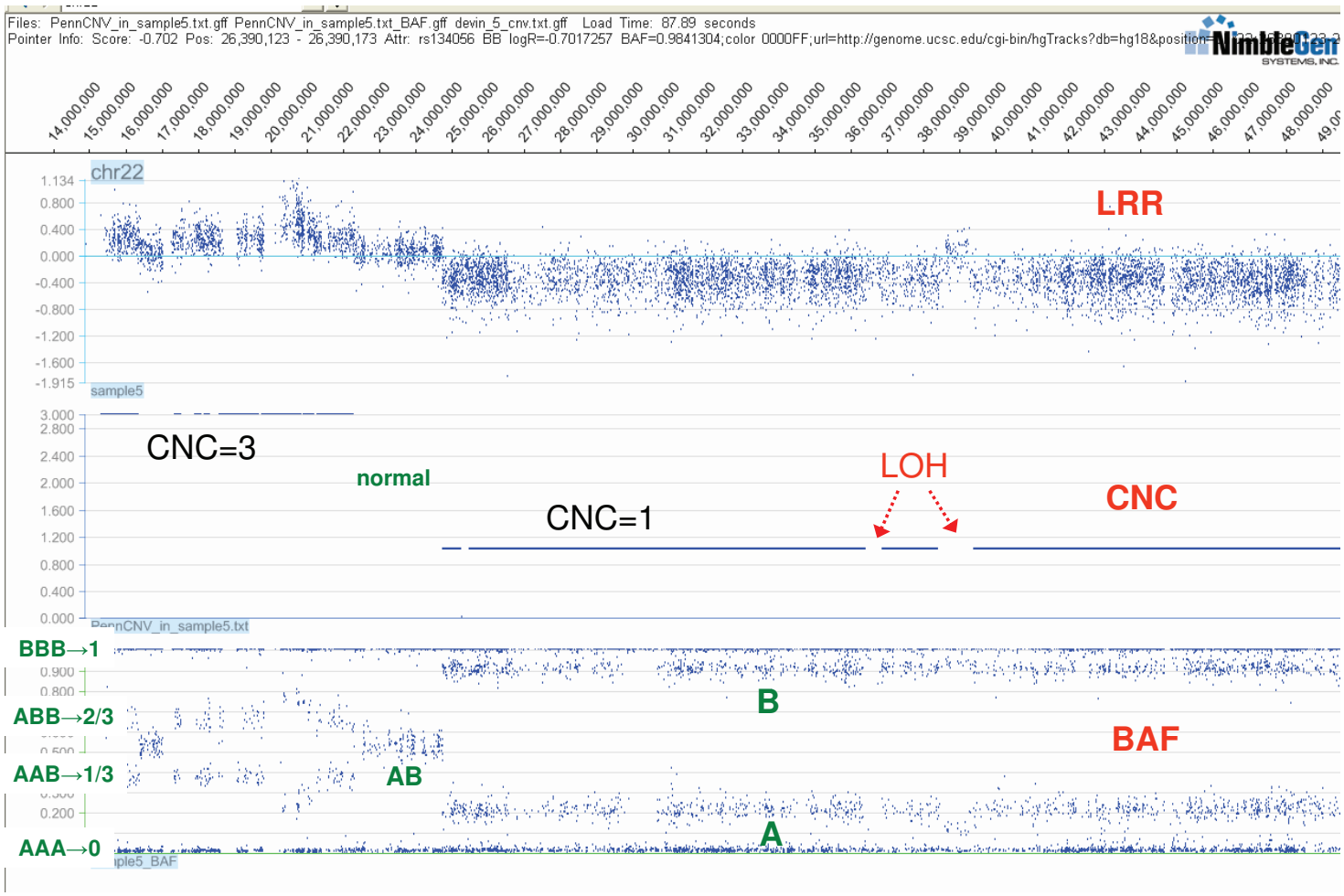


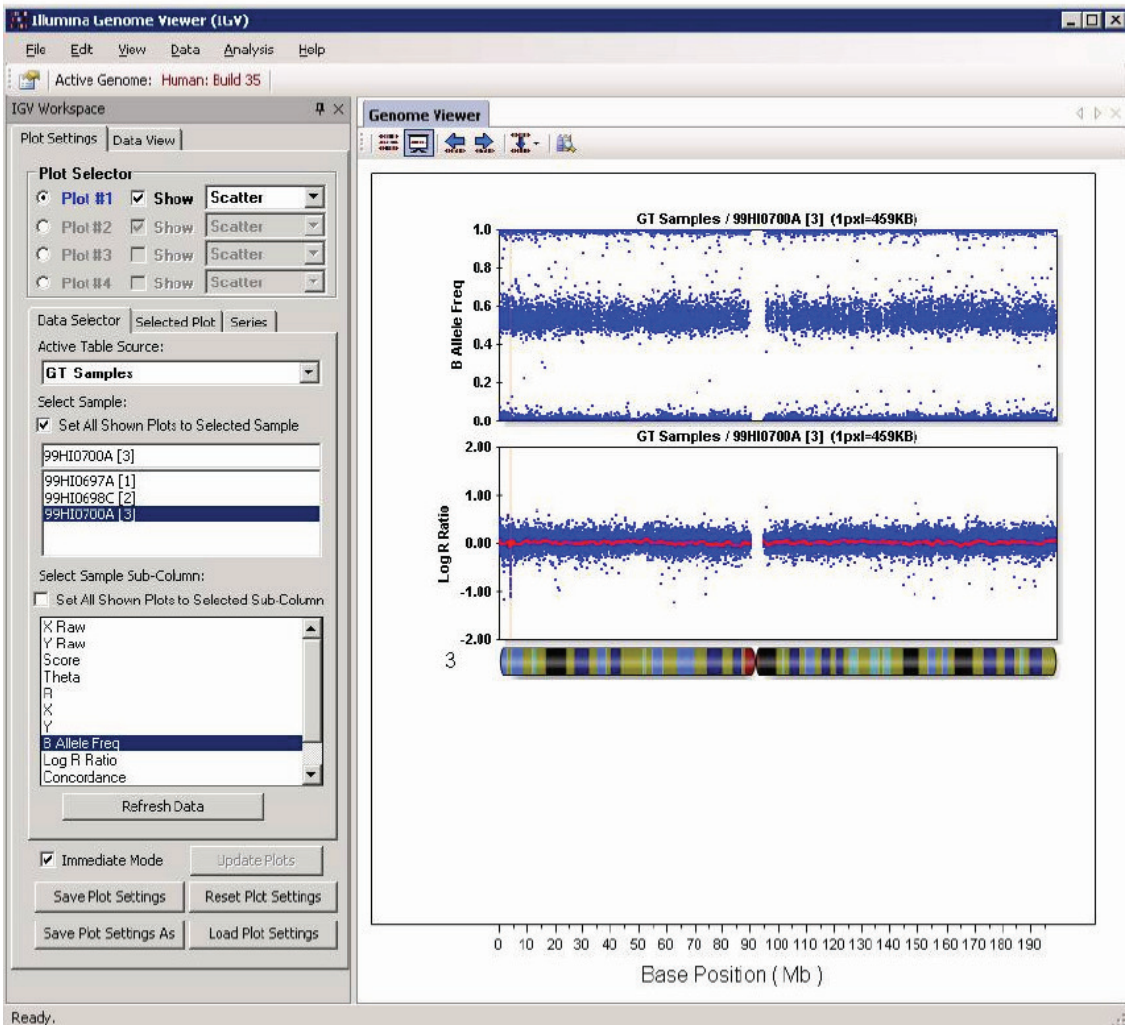

# Comparison with CBS






*Devin, Tumor 5, chr22 PennCNV results*

# Back to Genome Studio



# PennCNV quality assessment

- is done automatically
- identifies low-quality samples from a genotyping experiment
- several types of bad quality, see below ....

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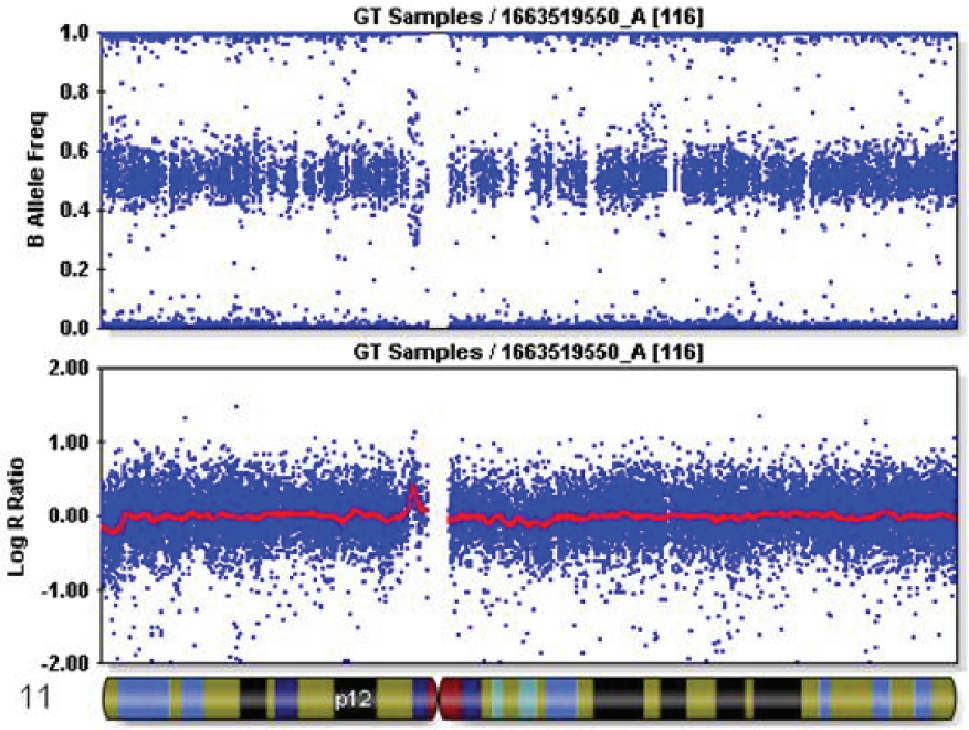
# Large variance of LRR values

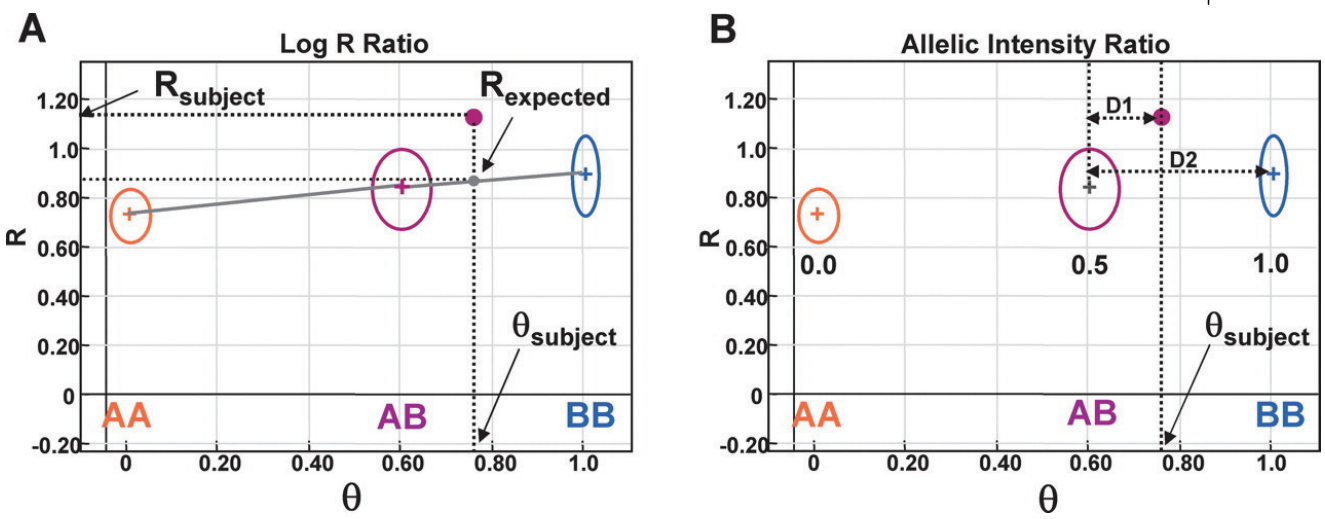



Logfile:
LRR_SD=0.2184

Threshold:
LRR_SD < 0.2

**possible cause:** use of non-optimal **canonical clustering** files

# Canonical clusters

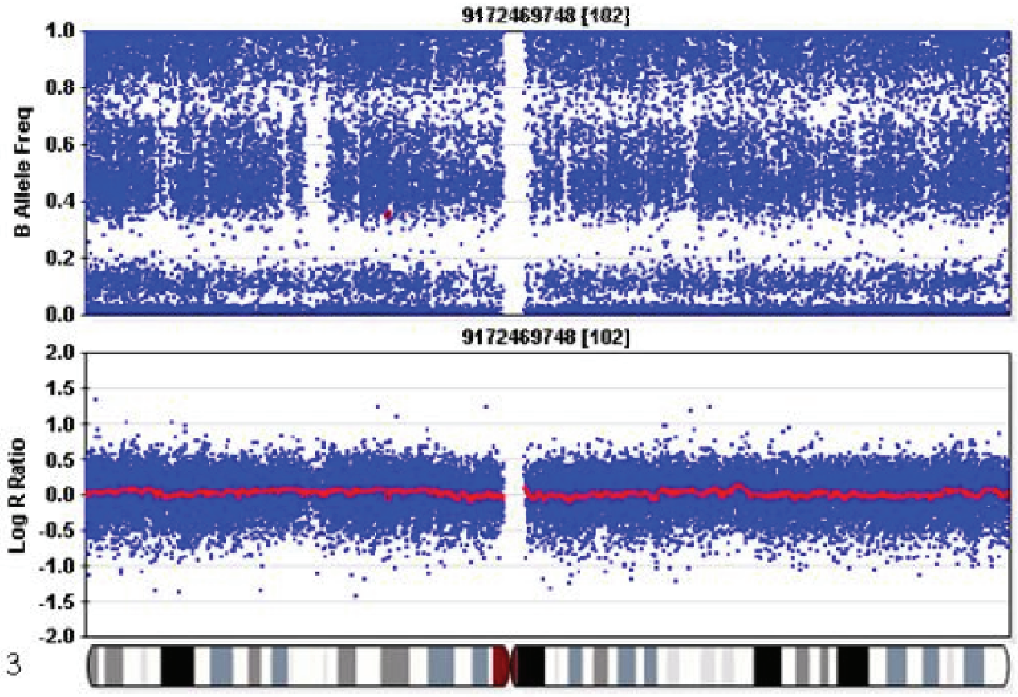



The canonical clusters are **not specific enough**

- clusters have to be defined for each machine
- or paired comparisons must be made

*Peiffer D. A. et.al. Genome Res. 2006;16:1136-1148*

# Large variance of BAF-values

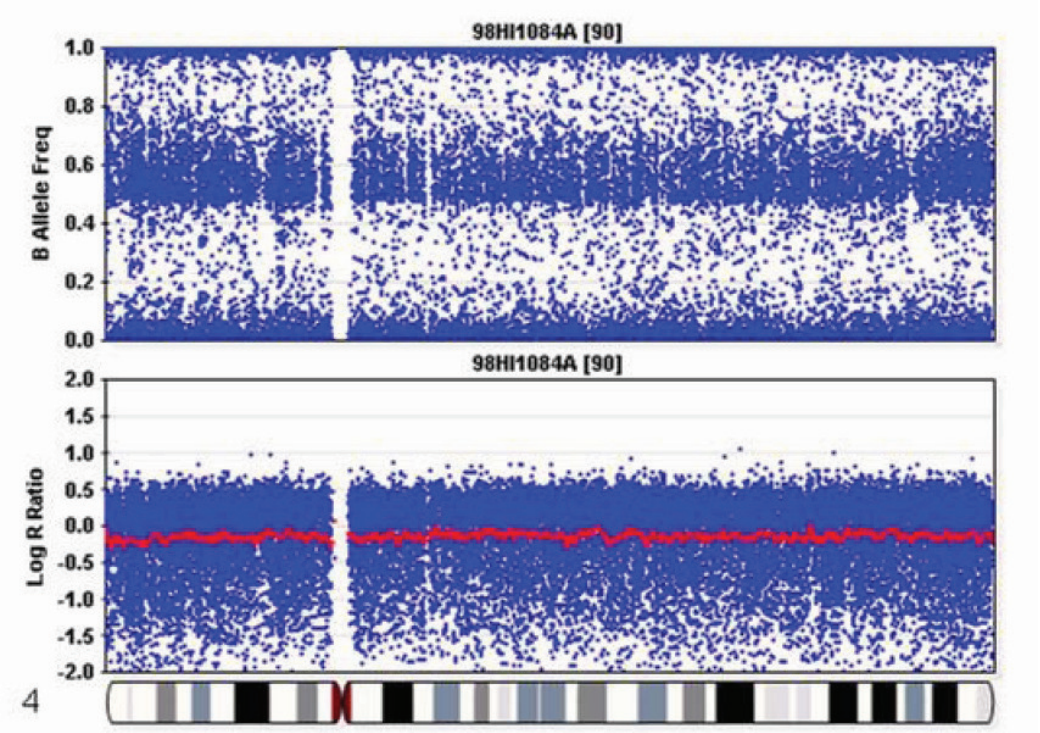



**BAF_SD**: stdev. of all autosome BAF values between 0.25 and 0.75

**possible cause:** mixing two different genomes (?)

*PennCNV_quality.pdf*

# Failure on one allele

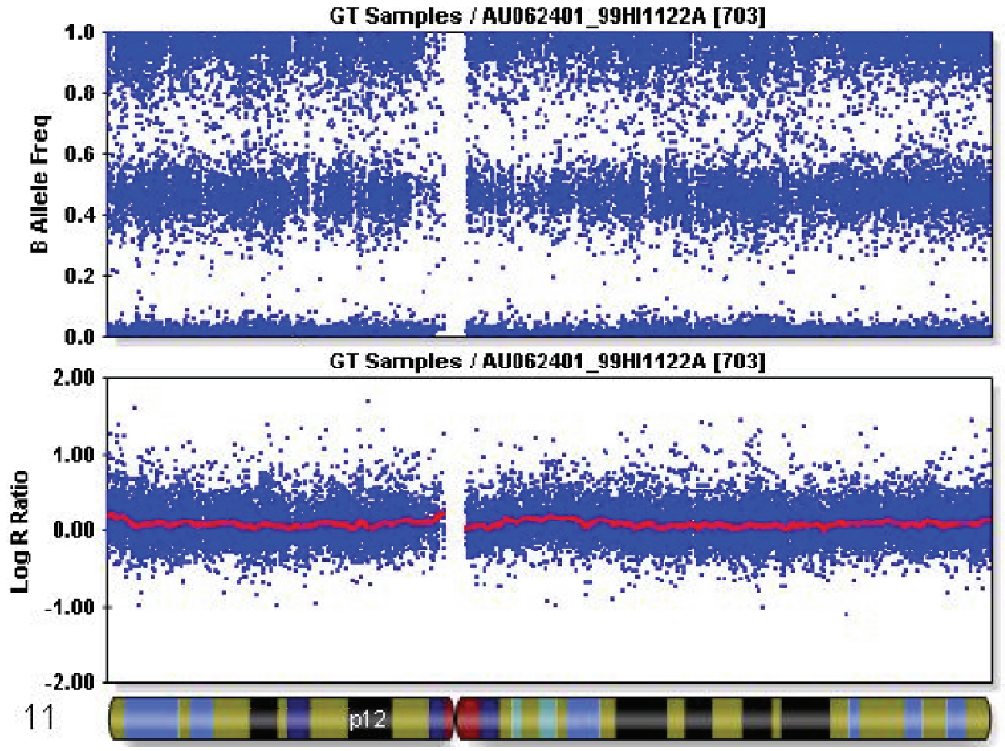



Allele A generally not measured well

**cause:** unknown

*PennCNV_quality.pdf*

# Upshift or downshift of BAF values

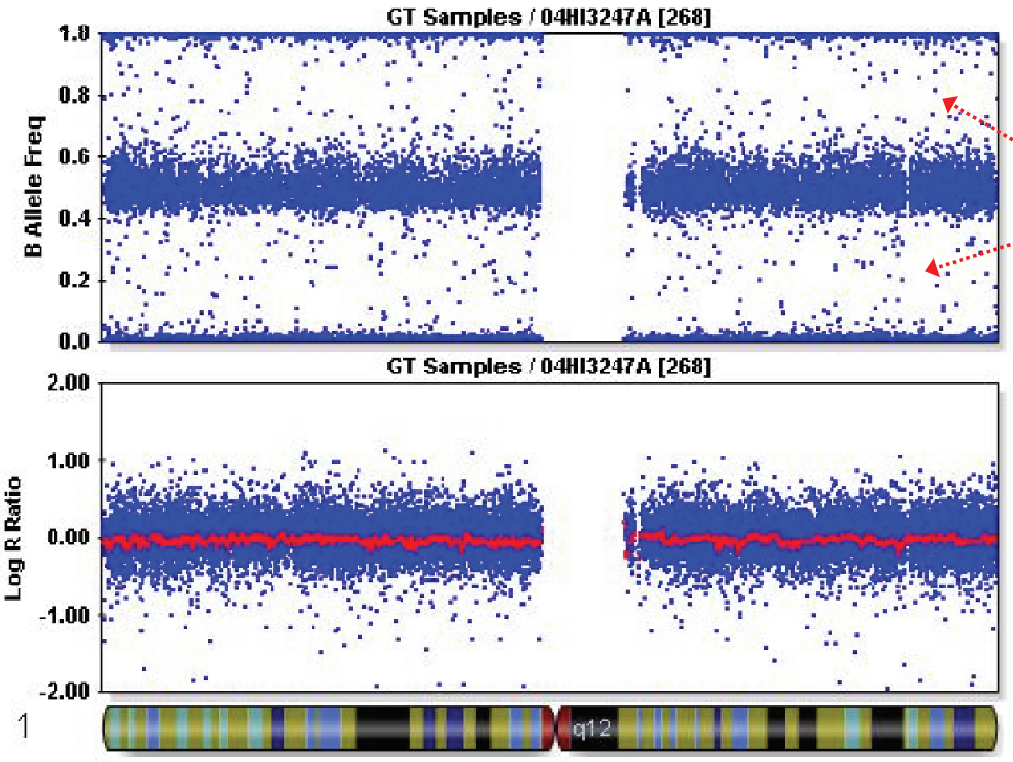



downshift

causes false positive CNV duplication calls

**cause:** unknown

# Random failures

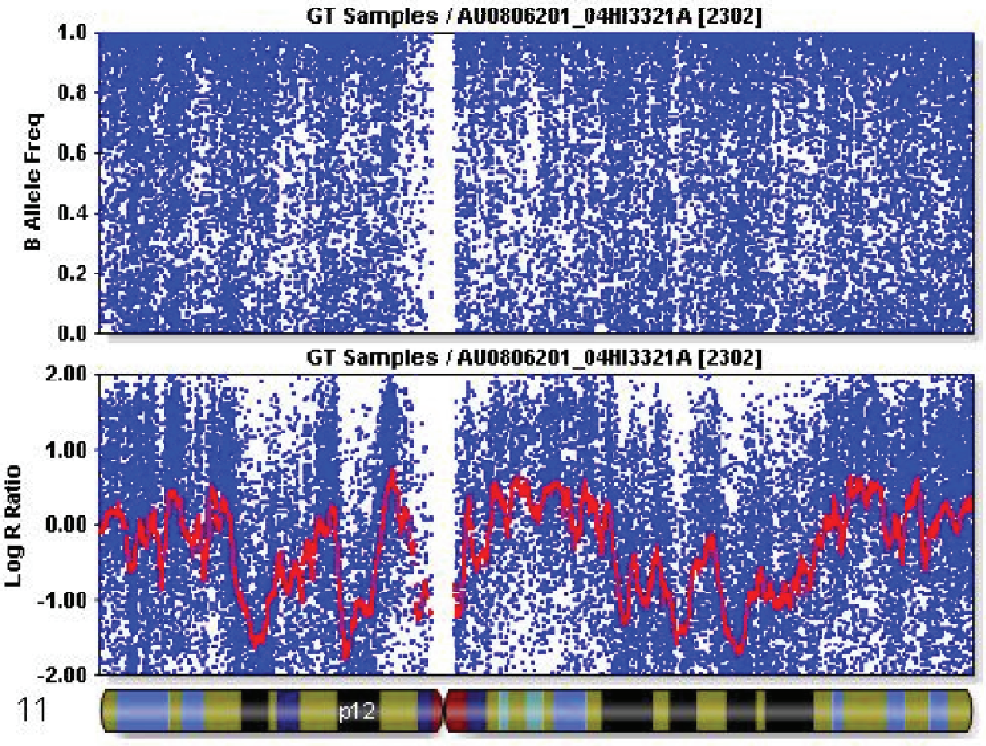



here should be nothing!

→ false positive CNV calls on homozygous deletions

BAF_DRIFT

# Waviness




**cause:** too much or too little DNA (or something else)

# Quality ETJ1

| | |
|---|---|
| Sample1 | large SD for LRR 0.241 |
| Sample2 | large SD for LRR 0.225 |
| Sample3 | large SD for LRR 0.327 |
| Sample4 | large SD for LRR 0.349 |
| Sample4 | drifting BAF values drift=0.0020 |
| Sample5 | large SD for LRR 0.2296 |
| Sample6 | large SD for LRR 0.2320 |
| Sample7 | large SD for LRR 0.4999 |
| Sample7 | drifting BAF values drift=0.002147 |
| Sample8 | large SD for LRR 0.3747 |
| Sample8 | drifting BAF values drift=0.004070 |
| Sample9 | large SD for LRR 0.216 |
| Sample9 | waviness factor values wf=-0.0822 |
| Sample10 | large SD for LRR 0.2011 |
| Sample11 | large SD for LRR 0.3709 |
| Sample12 | large SD for LRR 0.467 |
| Sample12 | drifting BAF values drift=0.00236 |
| Sample13 | large SD for LRR 0.2119 |
| Sample14 | large SD for LRR 0.2155 |
| Sample15 | large SD for LRR 0.2576 |
| Sample16 | large SD for LRR 0.4398 |

# PennCNV parameters

Optional arguments:

| | |
|---|---|
| -v, --verbose | use verbose output |
| -h, --help | print help message |
| -m, --man | print complete documentation |
| --train | train optimized HMM model (not recommended) |
| --test | test HMM model to identify CNV |
| --trio | posterior CNV calls for father-mother-offspring trio |
| --quartet | posterior CNV calls for quartet |
| --joint | joint CNV calls for trio (available soon) |
| --summary | generate descriptive summary for signal quality |
| --listfile <file> | a list file containing path to files to be processed |
| --output <file> | specify output root filename |
| --exclude_heterosomic | empirically exclude CNVs in heterosomic chromosomes |
| --hmmfile <file> | HMM model file |
| --pfbfile <file> | population frequency for B allelel file |
| --cnvfile <file> | specify CNV call file for use in family-based CNV calling |
| --wavemodelfile <file> | a file containing regression model for wave adjustment |
| --sample_index <int> | index of sample in input file (default=1) (obselete argument) |
| --minsnp <int> | minimum number of SNPs within CNV (default=3) |
| --minlength <int> | minimum length of bp within CNV |
| --minconf <float> | minimum confidence score of CNV (experimental feature) |
| --loh | display copy-neutral LOH information (obselete option) |
| --chrx | use chrX-specific treatment |
| --chry | use chrY-specific treatment (not implemented yet!) |
| --fmprior <numbers> | prior belief on CN state for regions with CNV calls |
| --denovo_rate <float> | prior belief on genome-wide de novo event rate |
| --logfile <file> | write notification/warningn messages to this file |
| --confidence | calculate confidence for each CNV (experimental feature) |
| --tabout | use tab-delimited output |
| --coordinate_from_input | get marker coorindate information from signal file (rather than PFB file) |

Function: generate CNV calls from high-density SNP genotyping data that contains Log R Ratio and B Allele Frequency for each SNP

# Other Programs: QuantiSNP

- similar to PennCNV
- several advantages of PennCNV:
  - state-specific and distance-dependent transition probabilities
  - better adapted to Illumina BAF calculation procedure
  - population frequency of the B allele considered
  - family information can be included (CNV-NDPs)

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# Other Programs: Birdsuite

The Birdsuite is a fully open-source set of tools to detect and report SNP genotypes, common Copy-Number Polymorphisms (CNPs), and novel, rare, or de novo CNVs in samples processed with the Affymetrix platform. While most of the components of the suite can be run individually (for instance, to only do SNP genotyping), the Birdsuite is especially intended for integrated analysis of SNPs and CNVs. Support for chips and platforms other than the Affymetrix SNP 6.0 is currently limited, but we are currently working on creating the supporting files for other common genotyping platforms.

# Other Programs: SNPRank (Nexus)

Dear Uwe,

The algorithm is new and we have developed it ourselves. It is called SNPRank. Are you working with Hanna Göransson at Uppsala?

-Soheil

# dChip

Windows (Java), free

DANA-FARBER CANCER INSTITUTE

Harvard School of Public Health


Research

People

Publications

Software

Links

**dChip Software: Analysis and visualization of gene expression and SNP microarrays**

| | | | |
|---|---|---|---|
| Introduction | Manual | References | Tutorials |
| Download | User Group | Updates | User Help |
| Workshops | | | |

New: dChip course in Boston, Jan 26 – 28, 2009, offered by bioinformatics.org.

**Search manual, site & user group**

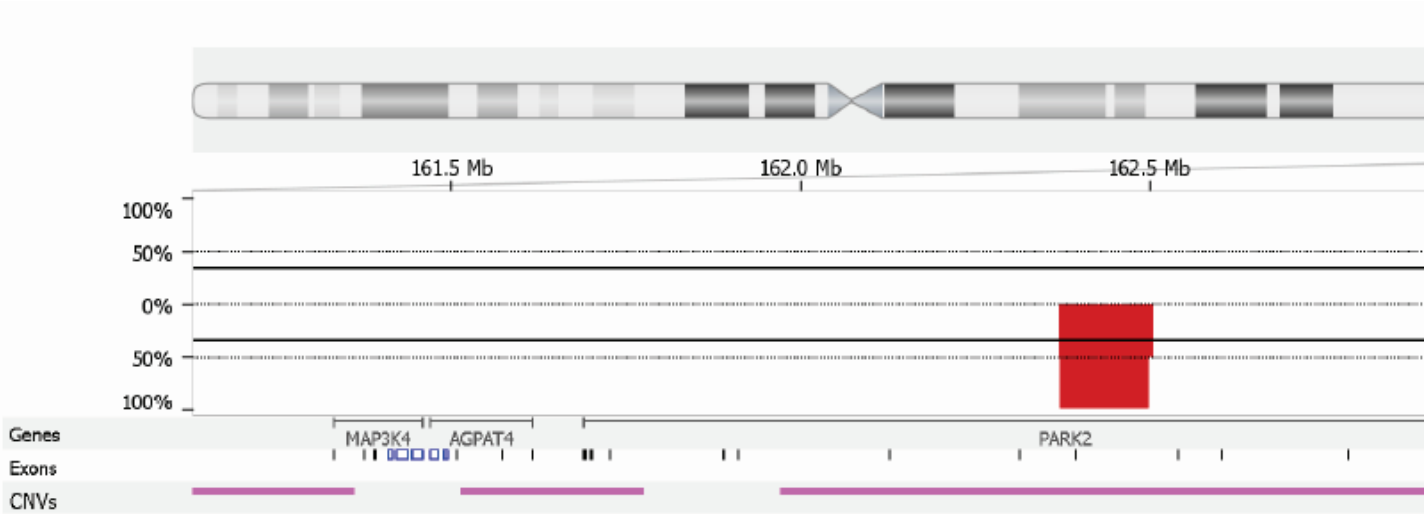
Google™ Custom Search Search

**HMM** to infer copy number:

Zhao et al. "*An Integrated View of Copy Number and Allelic Alterations in the Cancer Genome Using Single Nucleotide Polymorphism Arrays*" [Cancer Research 64, 3060-3071, May 1, 2004]

# Comparison of samples

- Frequency plots (Nexus):




# Comparison of samples

- STAC:

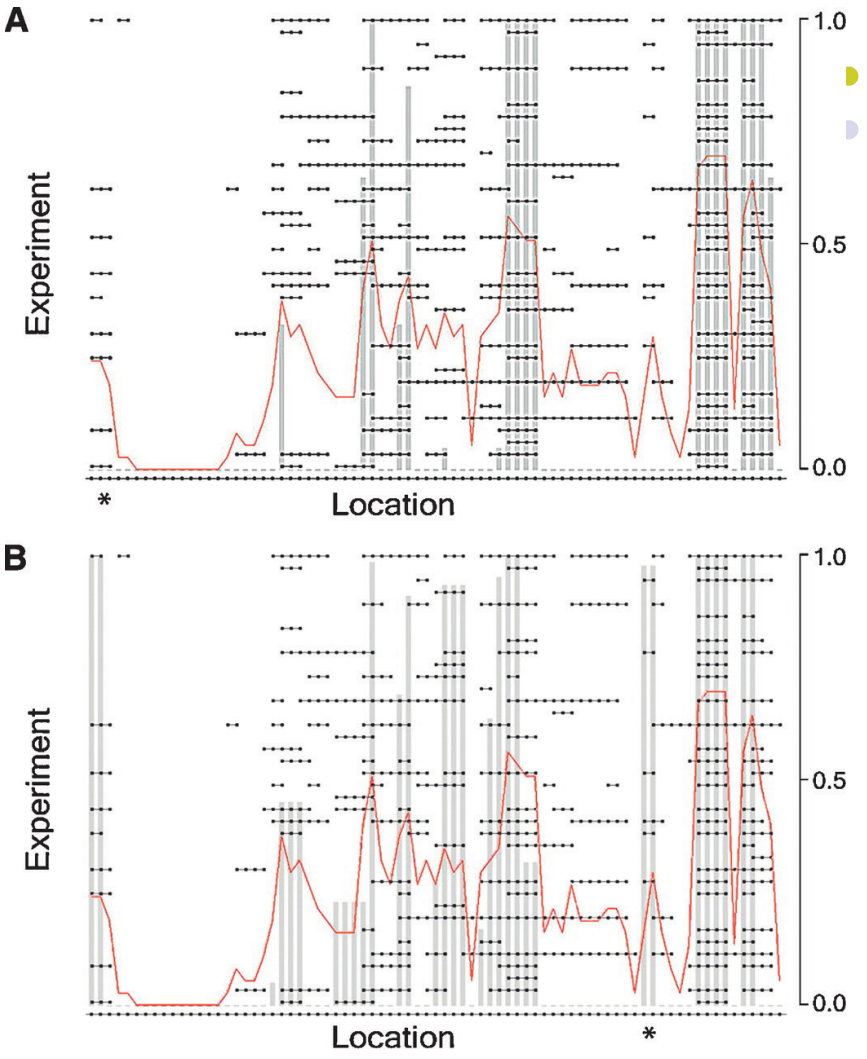
CSH PRESS GENOME RESEARCH

**STAC: A method for testing the significance of DNA copy number aberrations across multiple array-CGH experiments**

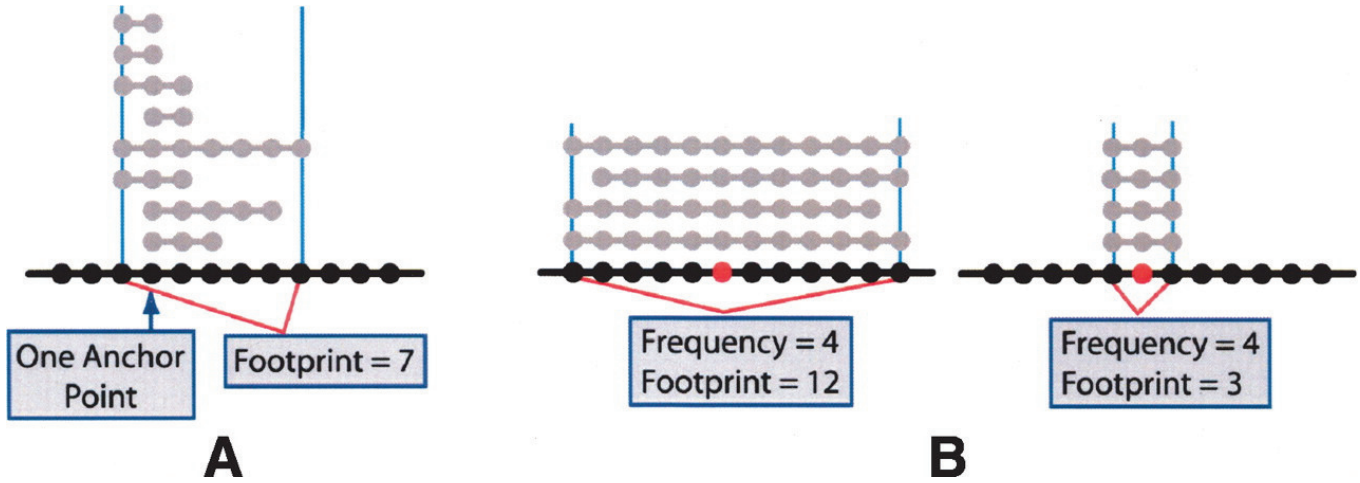
Sharon J. Diskin, Thomas Eck, Joel Greshock, et al.

*Genome Res.* 2006 16: 1149-1158
Access the most recent version at doi:10.1101/gr.5076506

# STAC-results




# STAC-results

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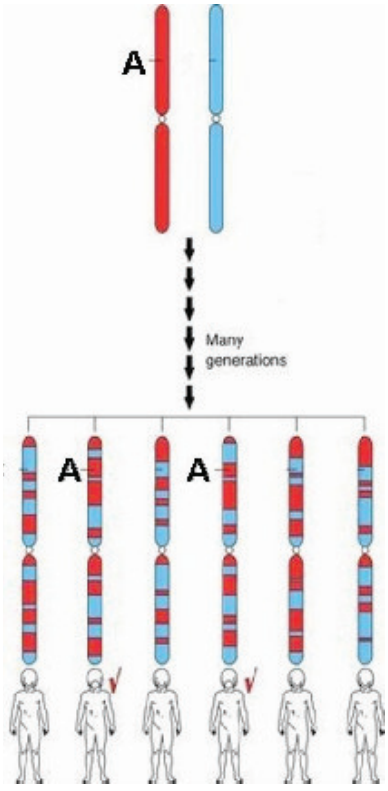
*Thanks!*

*http://www.hapmap.org/whatishapmap.html.en*

# Haplotypes and tag SNPs




Over the course of many generations, segments of the ancestral chromosomes in an interbreeding population are shuffled through repeated recombination events. Some of the segments of the ancestral chromosomes occur as regions of DNA sequences that are shared by multiple individuals (Figure 1). These segments are regions of chromosomes that have not been broken up by recombination, and they are separated by places where recombination has occurred. These segments are the haplotypes that enable geneticists to search for genes involved in diseases and other medically important traits.

A given haplotype can occur at different frequencies in different populations.

# Haplotypes and tag SNPs

- In many parts of our chromosomes, just a handful of haplotypes are found in humans.
- In a given population, 55 % of people may have one version of a haplotype, 30 % may have another, 8 % may have a third, and the rest may have a variety of less common haplotypes.
- The HapMap Project is identifying these common haplotypes in four populations from different parts of the world.
- It also is identifying **"tag" SNPs** that uniquely identify these haplotypes:
  - testing an individual's tag SNPs (“ genotyping”) → identification of the collection of haplotypes in that person's DNA
  - The number of tag SNPs that contain most of the information about the patterns of genetic variation is estimated to be about 300,000 to 600,000, which is far fewer than the 10 million common SNPs

*http://www.hapmap.org/index.html*