Chromosomal Variation in Lymphoblastoid Cell Lines

Matt Shirley
Pevsner Lab
December 10

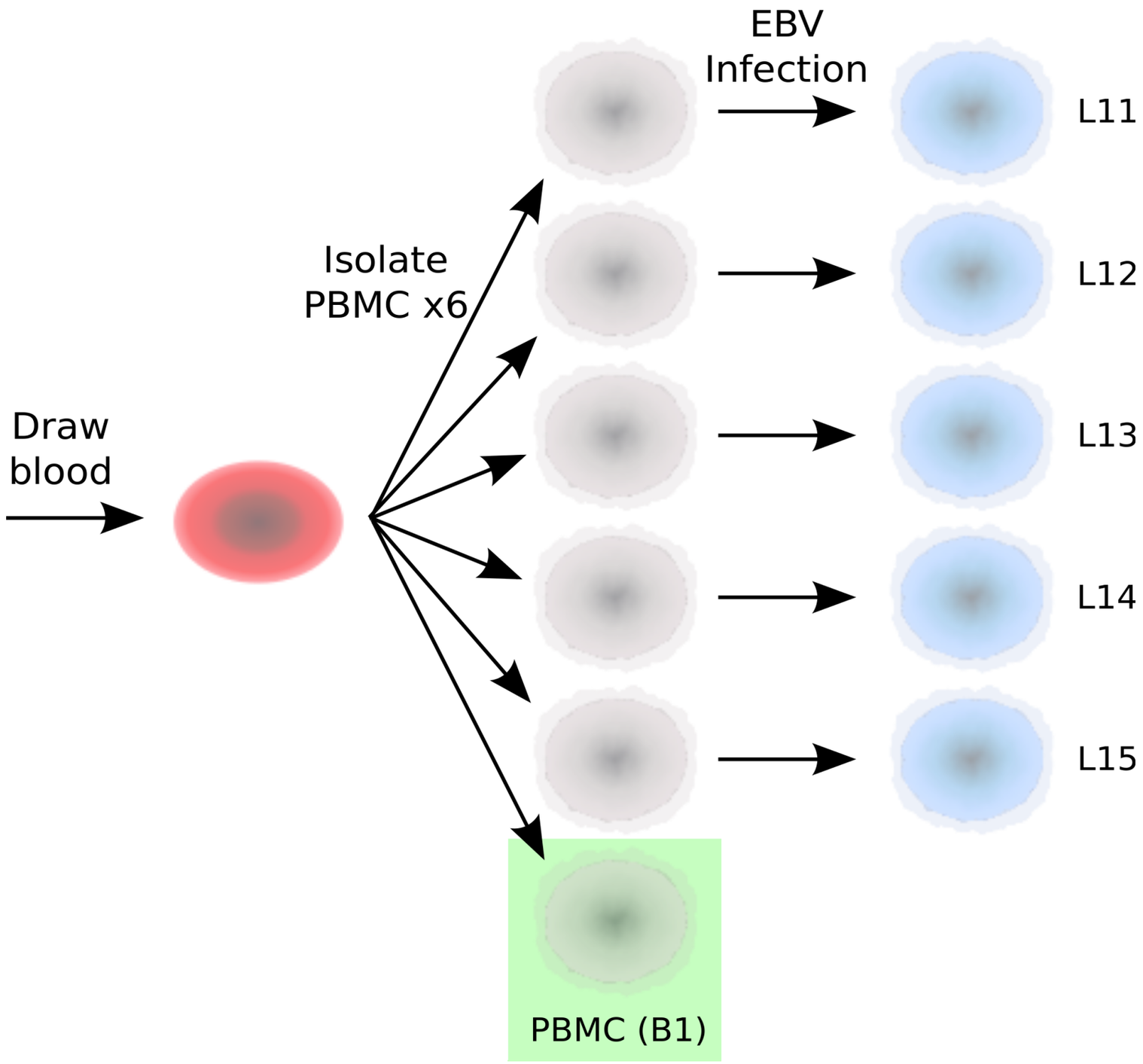
1. What are lymphoblastoid cell lines?
2. Methods
    1. Establishment of cell lines
    2. DNA microarray technology
3. Results
    1. QC and genotype concordance
    2. DNA copy number concordance
    3. Associated regions of variable copy number

A ready source of DNA

- Peripheral blood mononuclear cells (PBMCs) are infected with Epstein-Barr virus (EBV), resulting in immortalization and establishment of lymphoblastoid cell lines (LCLs).
- Large genomic studies want easy future access to source DNA material
- Cell lines may also be used for experiments

1. What are lymphoblastoid cell lines?
2. Methods
    1. Establishing cell lines
    2. DNA microarray technology
3. Results
    1. QC and genotype concordance
    2. DNA copy number concordance
    3. Associated regions of variable copy number

Establishing cell lines



1. What are lymphoblastoid cell lines?
2. Methods
    1. Establishing cell lines
    2. DNA microarray technology
3. Results
    1. QC and genotype concordance
    2. DNA copy number concordance
    3. Associated regions of variable copy number

DNA microarrays

- Comparative hybridization between DNA from sample and reference genome (usually a mixture of unrelated genomes)
- Differential hybridization signals are used to determine sample genotype and DNA copy number at polymorphic sites (“probes”)
- Affymetrix Human SNP 6.0 array contains roughly 1.8 million probes

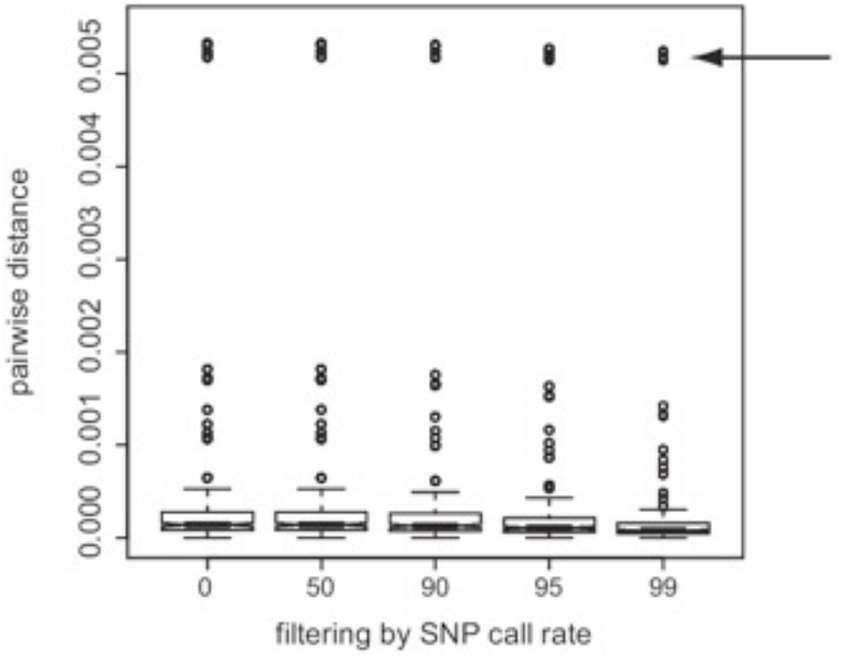
DNA microarrays

- Genotyping is performed after signal normalization by comparing results from multiple arrays and clustering signal from each probe
- Hybridization signal (logR) is also a surrogate for DNA copy number (logR ratio)
- Segmentation of logR ratios allows analysis of DNA copy gain or loss (CNV)

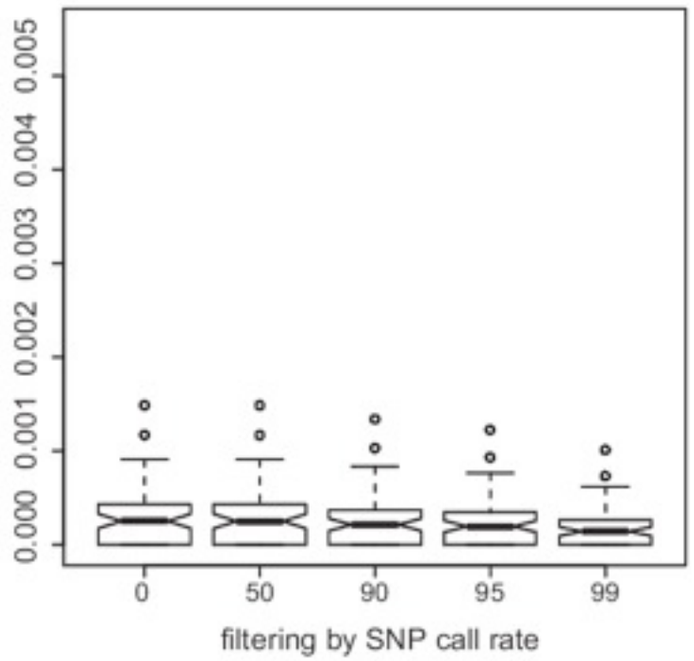
1. What are lymphoblastoid cell lines?
2. Methods
    1. Establishing cell lines
    2. DNA microarray technology
3. Results
    1. QC and genotype concordance
    2. DNA copy number concordance
    3. Associated regions of variable copy number

Genotype concordance

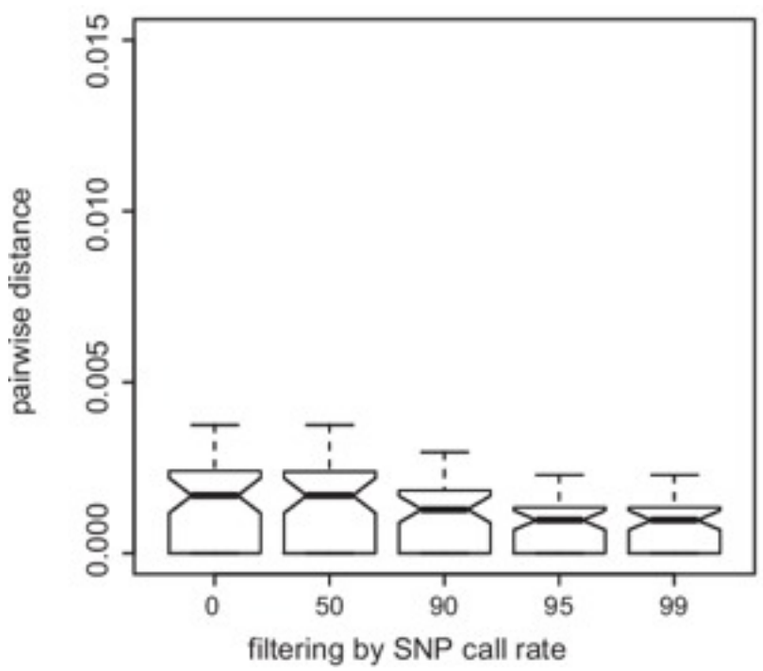
A



B

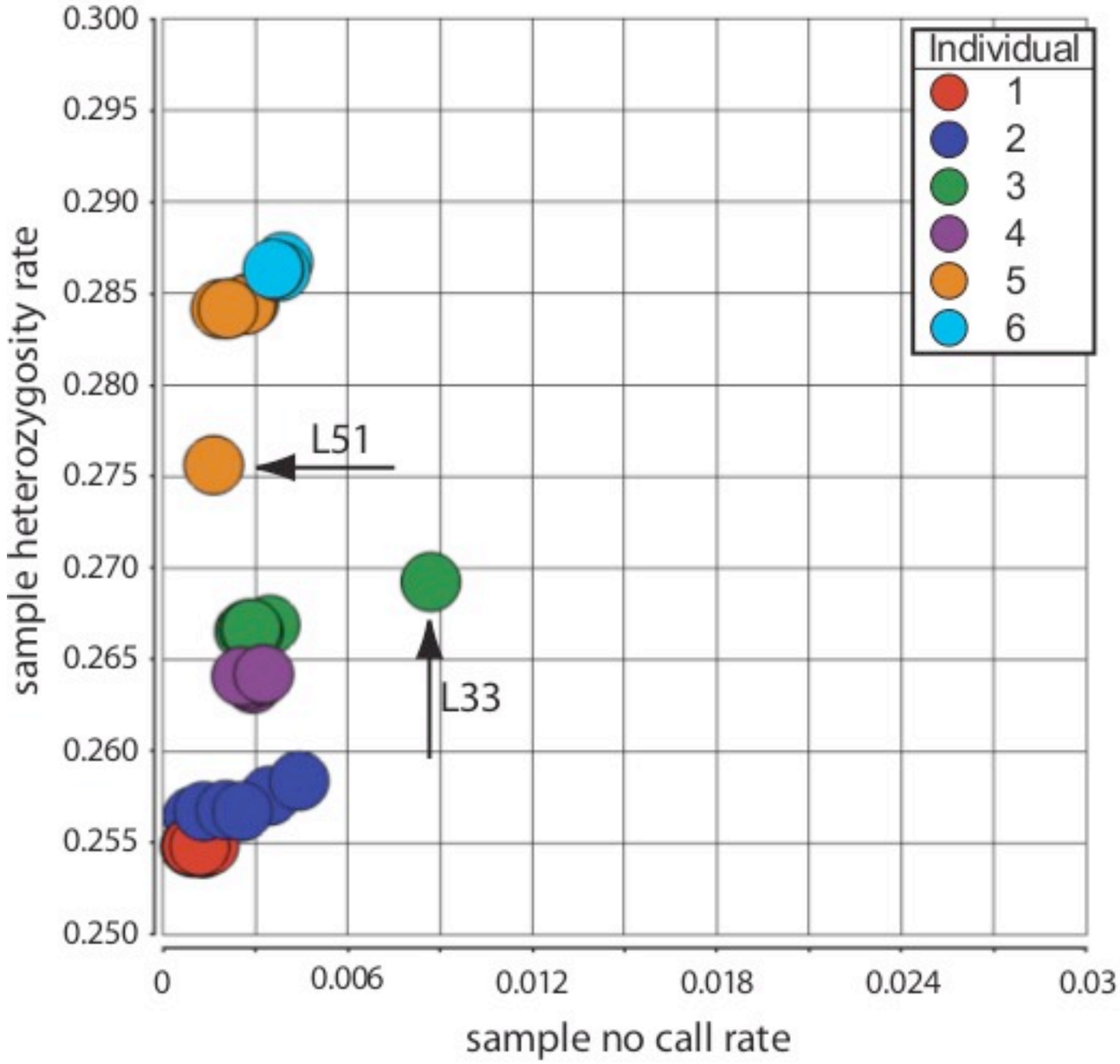


C

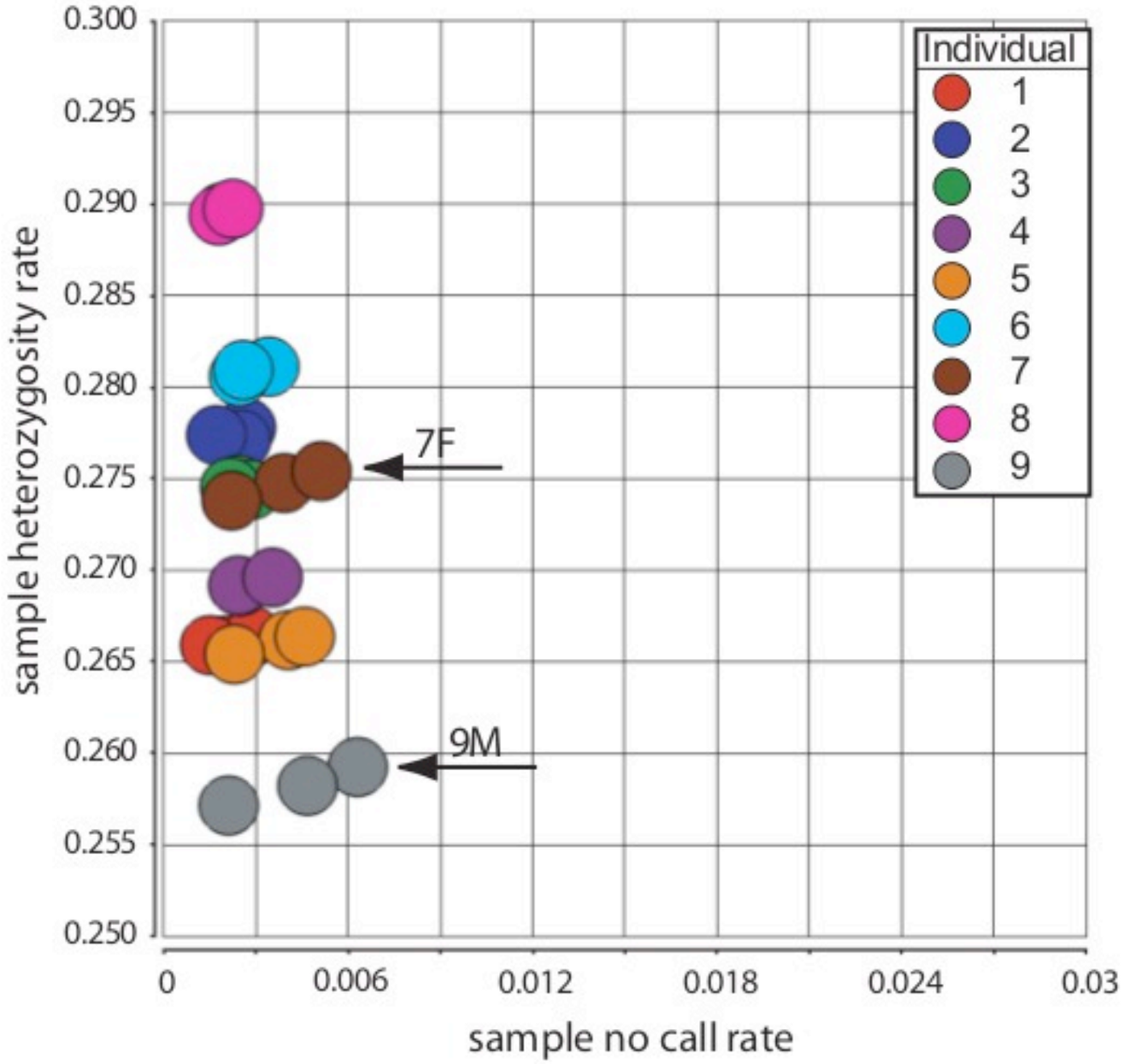


A: Lymphoblastoid cell lines
B: Differentiated cell lines
C: Technical replicates

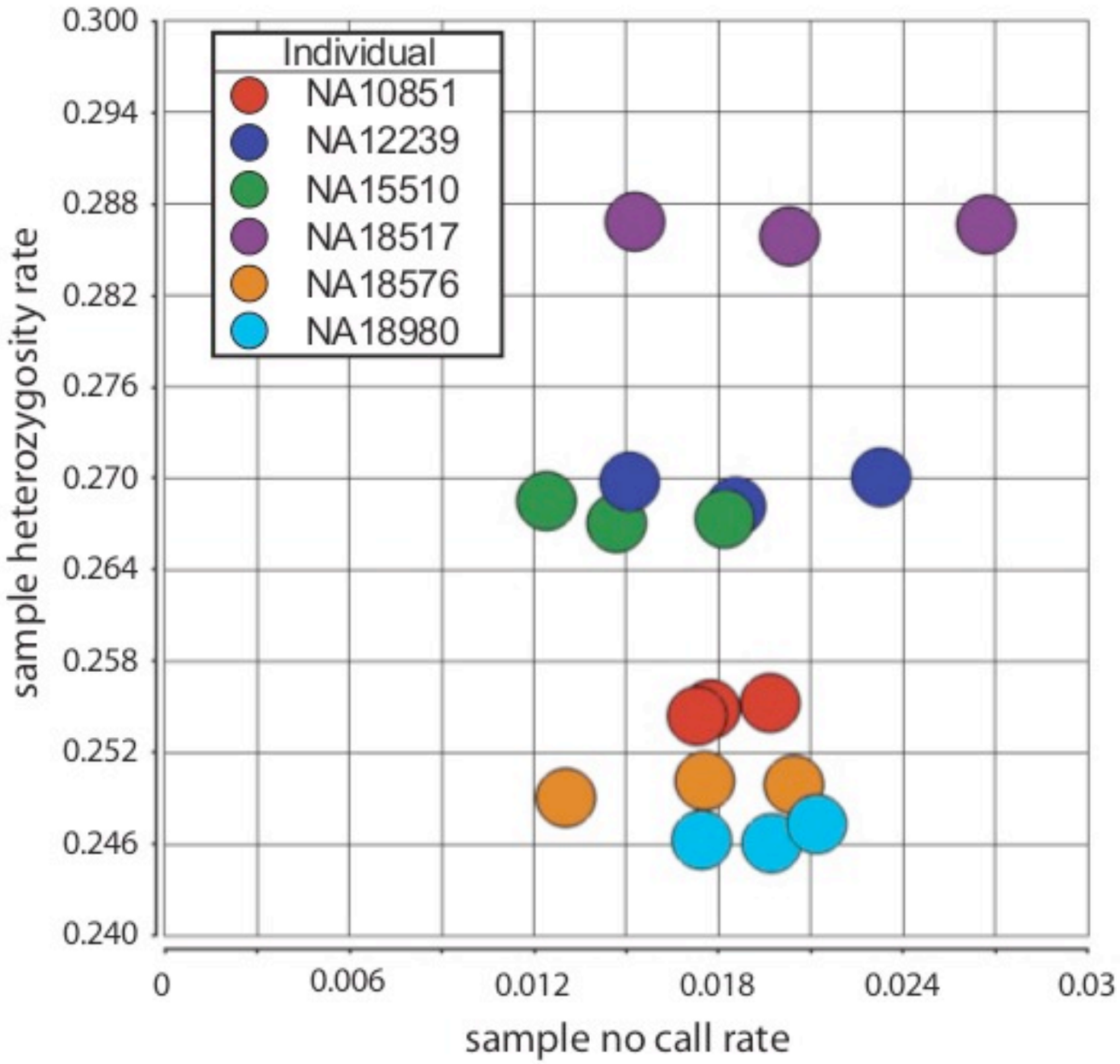
LCL heterozygosity rate vs. no call rate



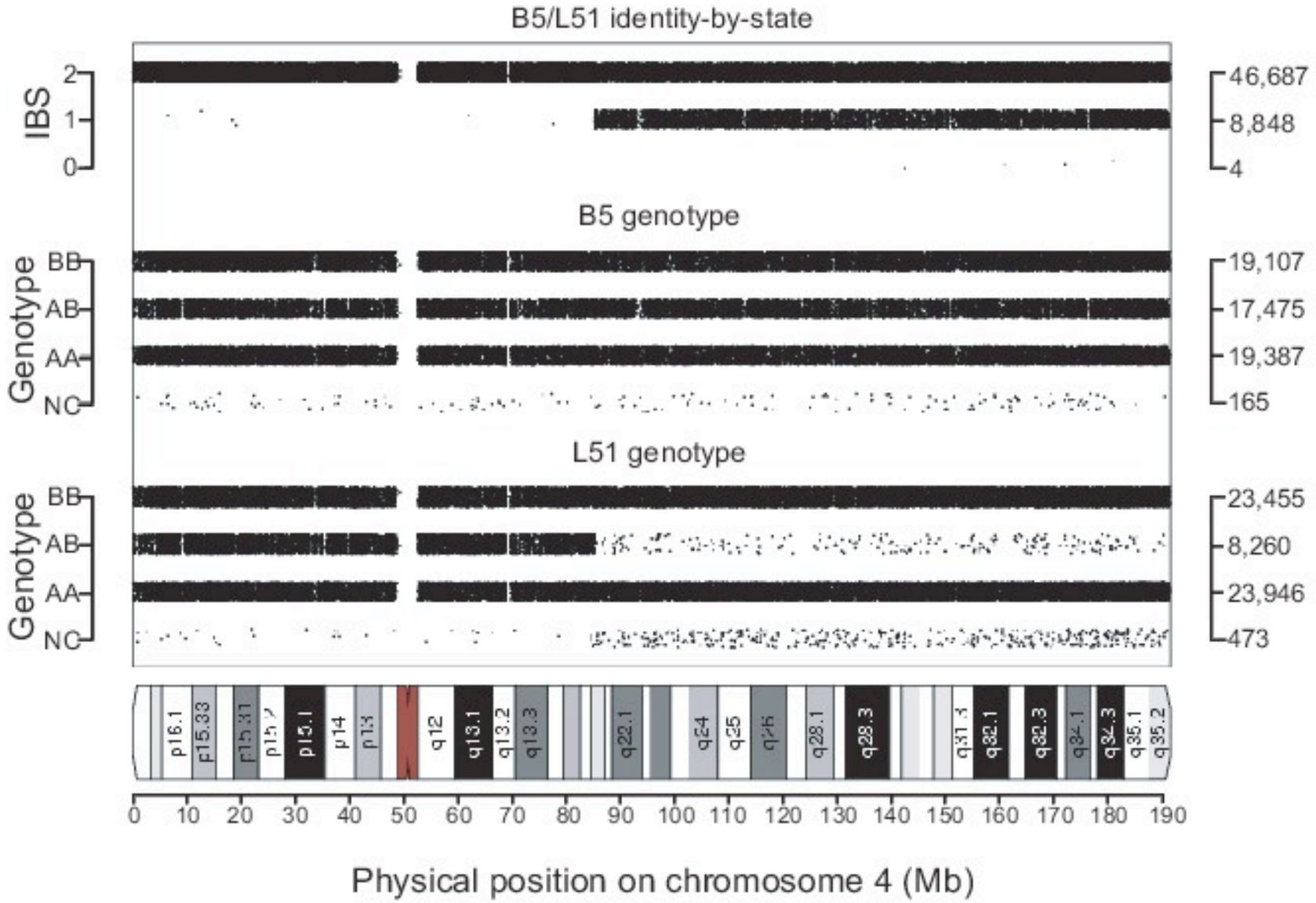
DCL heterozygosity rate vs. no call rate



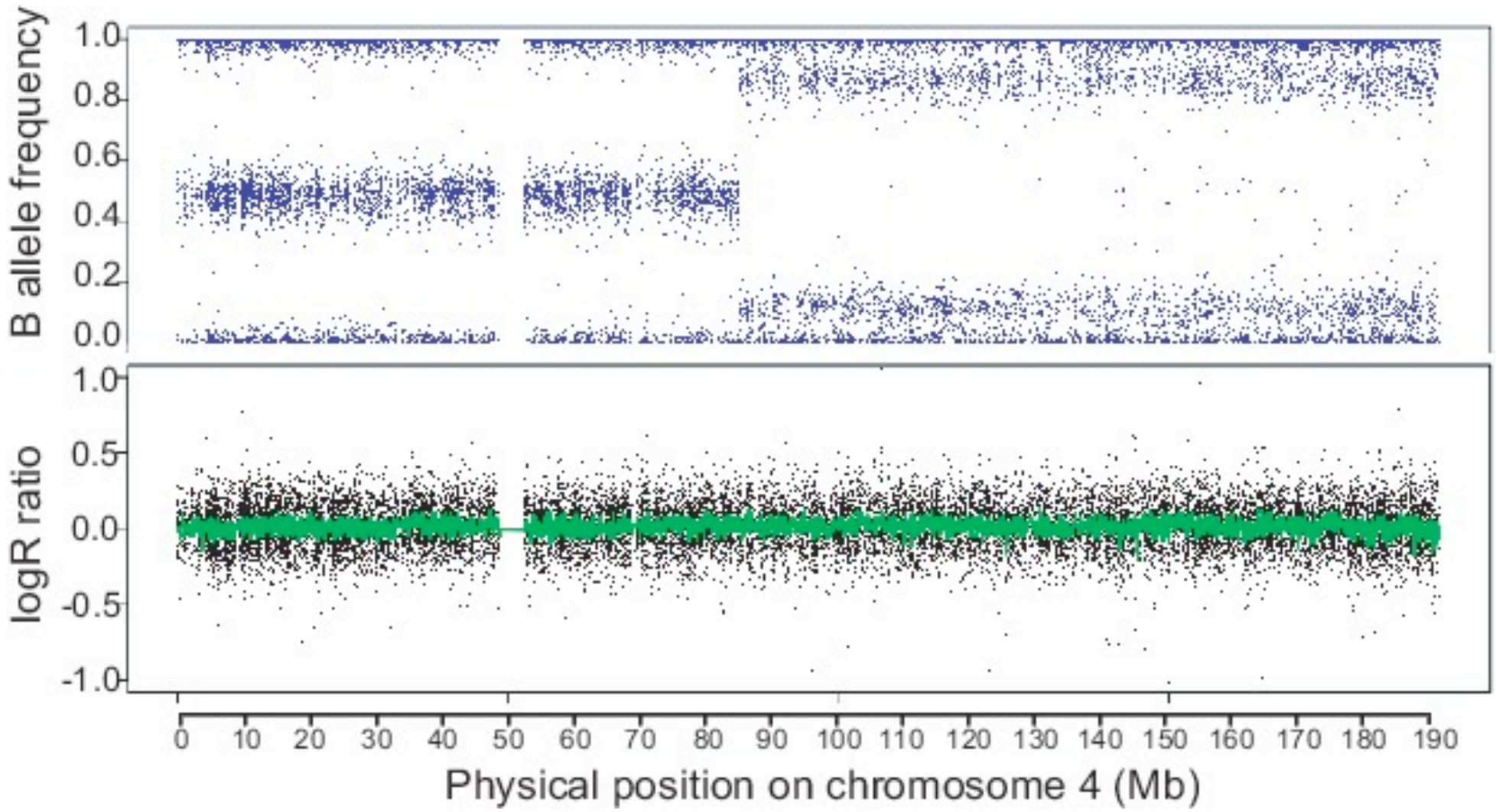
Replicate heterozygosity rate vs. no call rate



Cell line #1 from individual #5 mosaic uni-parental isodisomy



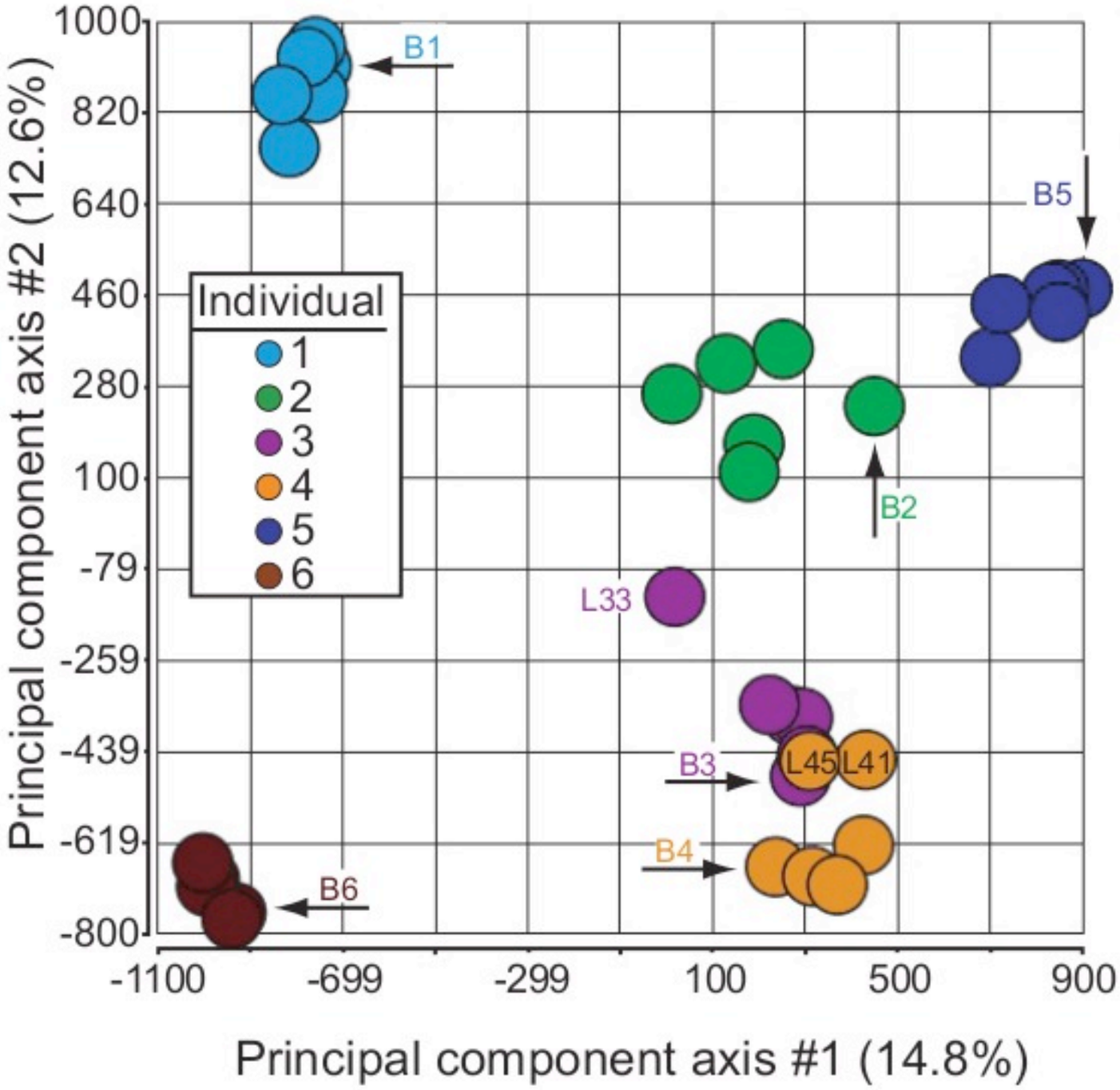
Cell line #1 from individual #5 mosaic uni-parental isodisomy



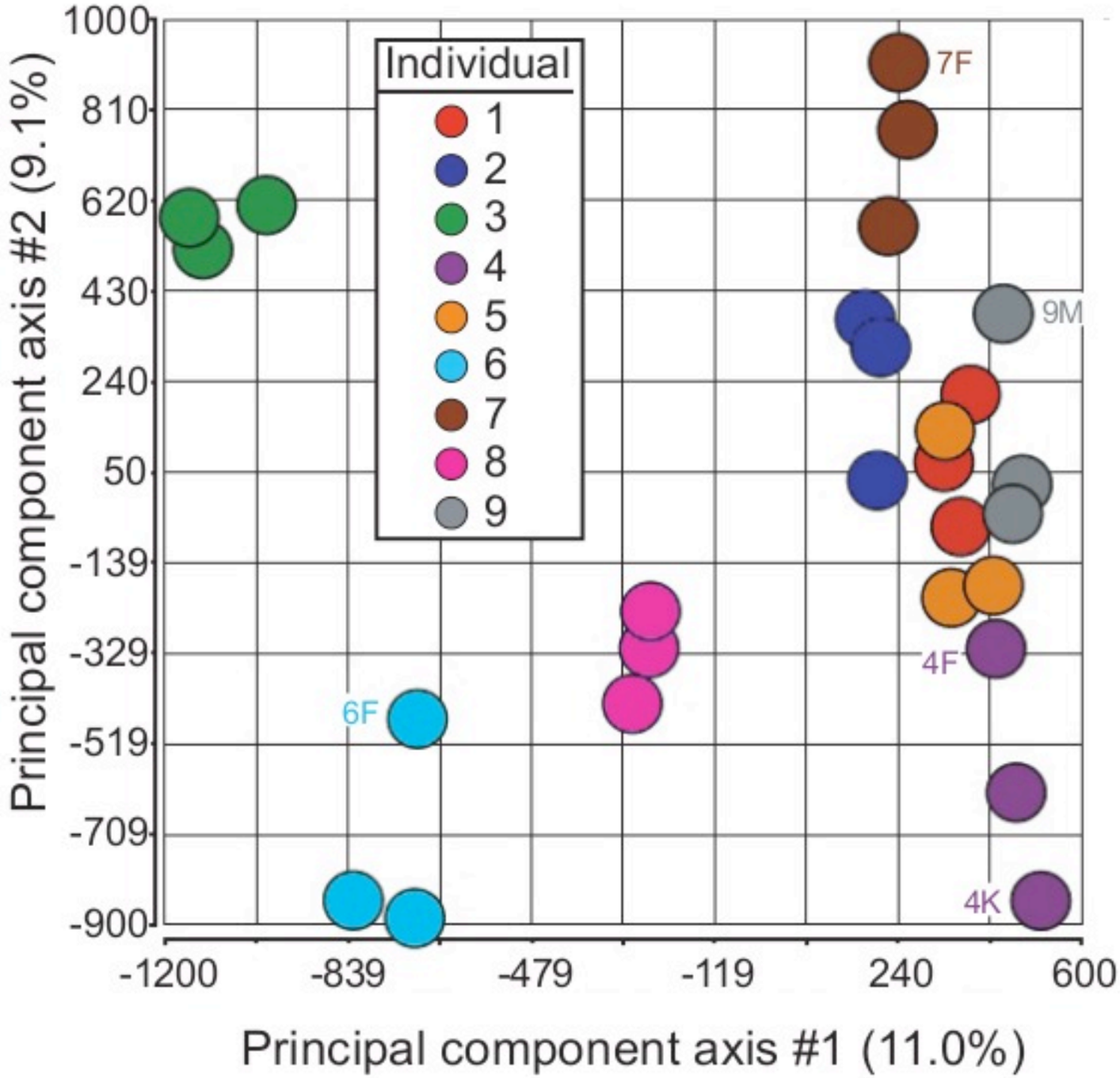
1. What are lymphoblastoid cell lines?
2. Methods
    1. Establishing cell lines
    2. DNA microarray technology
3. Results

1. QC and genotype concordance
2. DNA copy number concordance
3. Associated regions of variable copy number

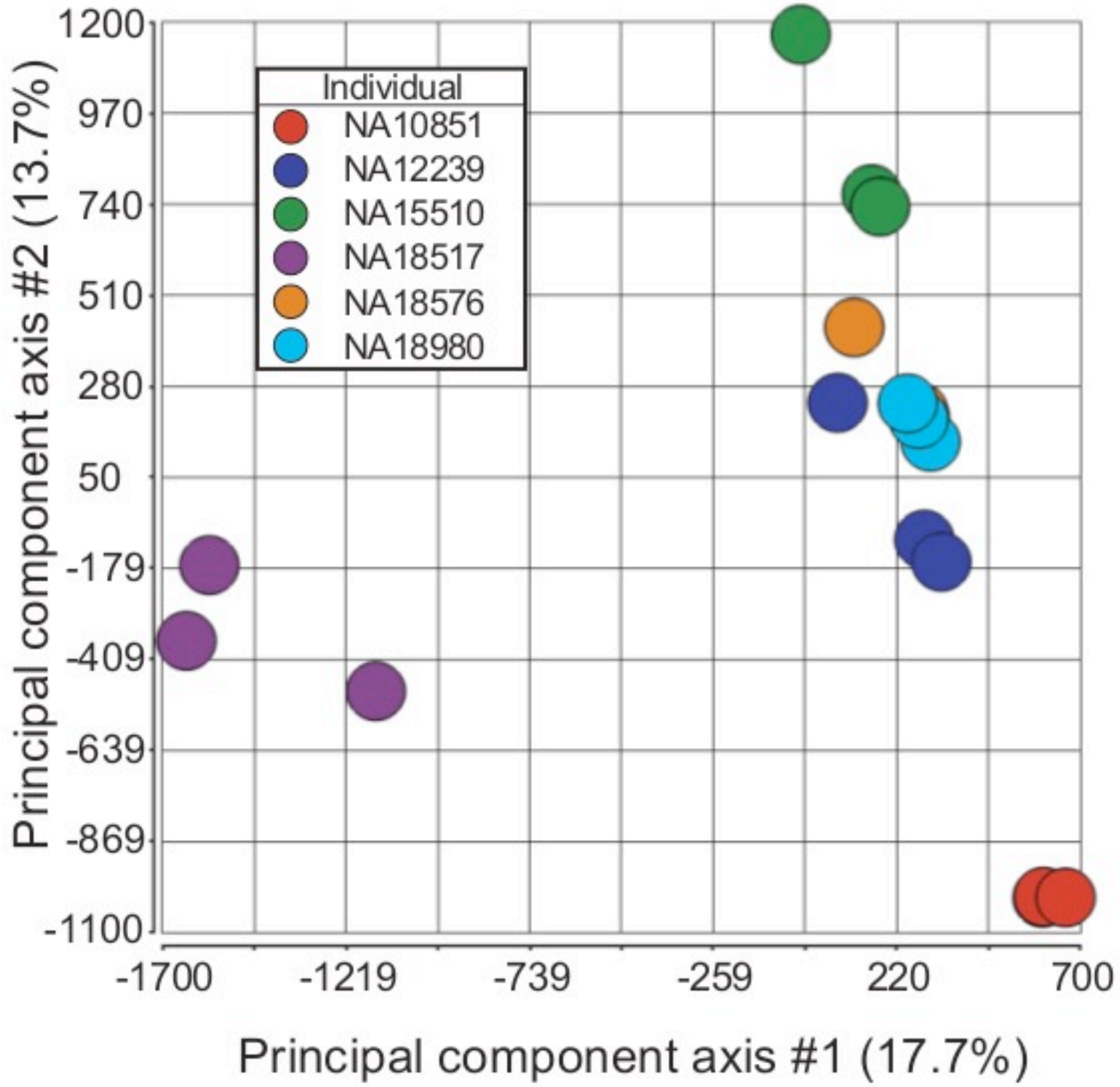
LCL principal components analysis of logR ratio



DCL principal components analysis of logR ratio

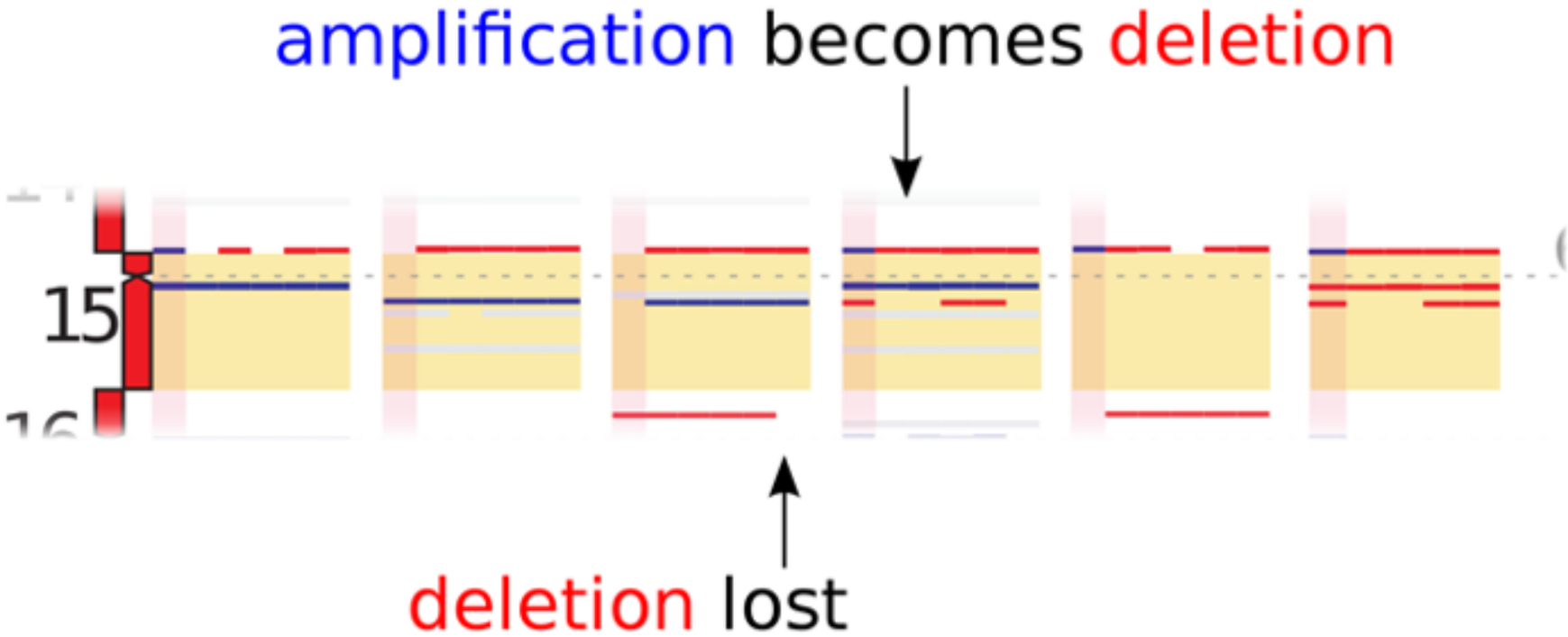


Replicate principal components analysis of logR ratio




PBMCs and corresponding LCLs
Differentiated cell lines (F, K, M)
HapMap replicates
Jaccard mean
±S D
Individual
Gender
Table 2 index
Chromosome
Legend
Deletion
Amplification
Homozygosity

Copy number variants are mostly conserved across samples



Jaccard correlation coefficients reveal decreased CNV fidelity of LCLs

	PBMCs and corresponding LCLs					
Jaccard mean	0.65	0.57	0.55	0.54	0.54	0.58
±S D	0.03	0.08	0.15	0.09	0.02	0.03

	Differentiated cell lines (F, K, M)								
Jaccard mean	0.64	0.60	0.70	0.70	0.78	0.74	0.66	0.77	0.58
±S D	0.04	0.06	0.02	0.04	0.02	0.09	0.03	0.06	0.05

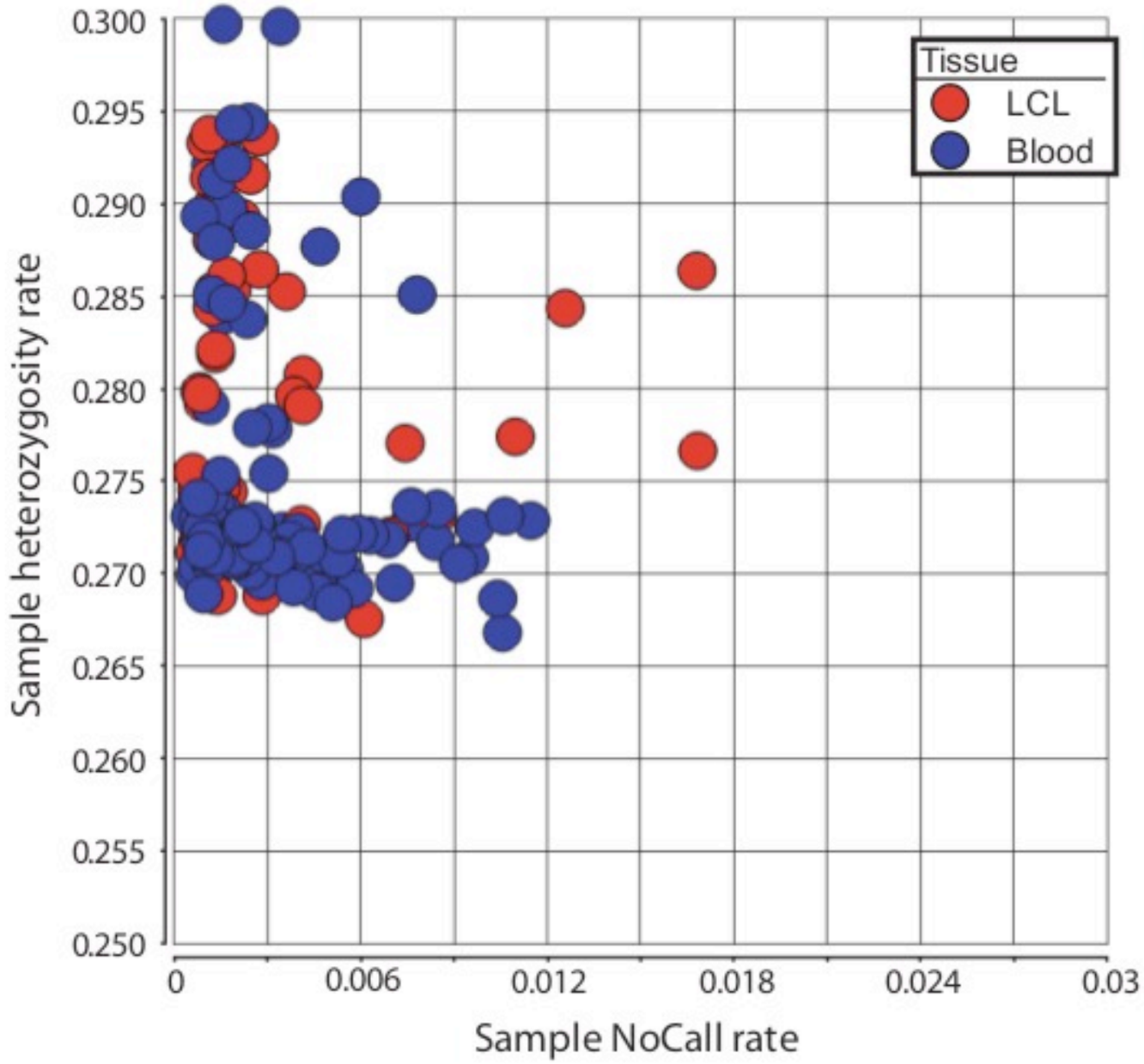
	HapMap replicates					
Jaccard mean	0.73	0.77	0.71	0.69	0.69	0.60
±S D	0.02	0.05	0.04	0.04	0.08	0.09

1. What are lymphoblastoid cell lines?
2. Methods
    1. Establishing cell lines
    2. DNA microarray technology
3. Results
    1. QC and genotype concordance
    2. DNA copy number concordance
    3. Associated regions of variable copy number

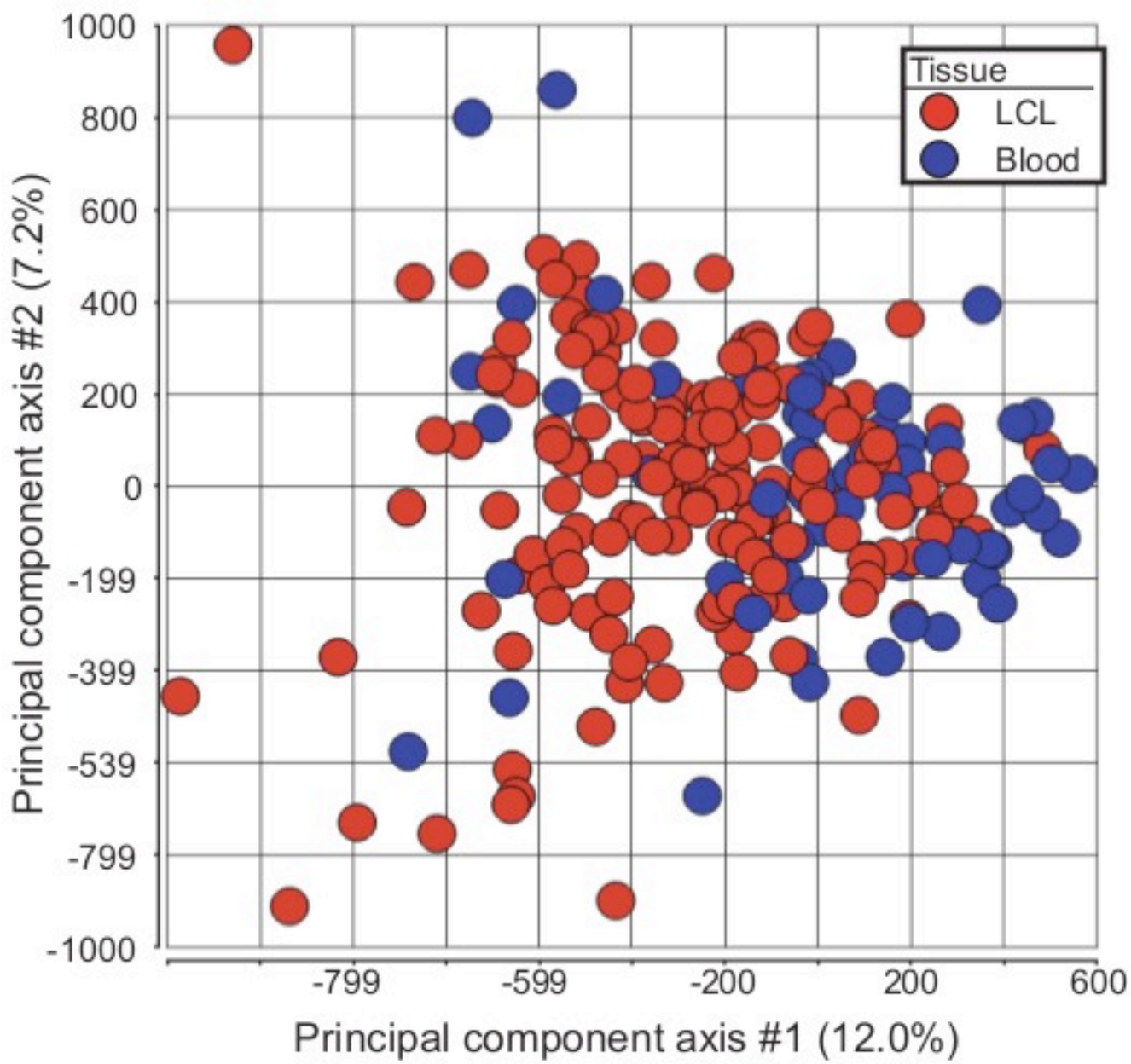
Extending the hypothesis to a large GWAS (GENEVA SAGE)

- Our sample size of 35 is large enough to localize variable regions specific to LCLs
- Therefore, we apply our analyses to a larger number of samples

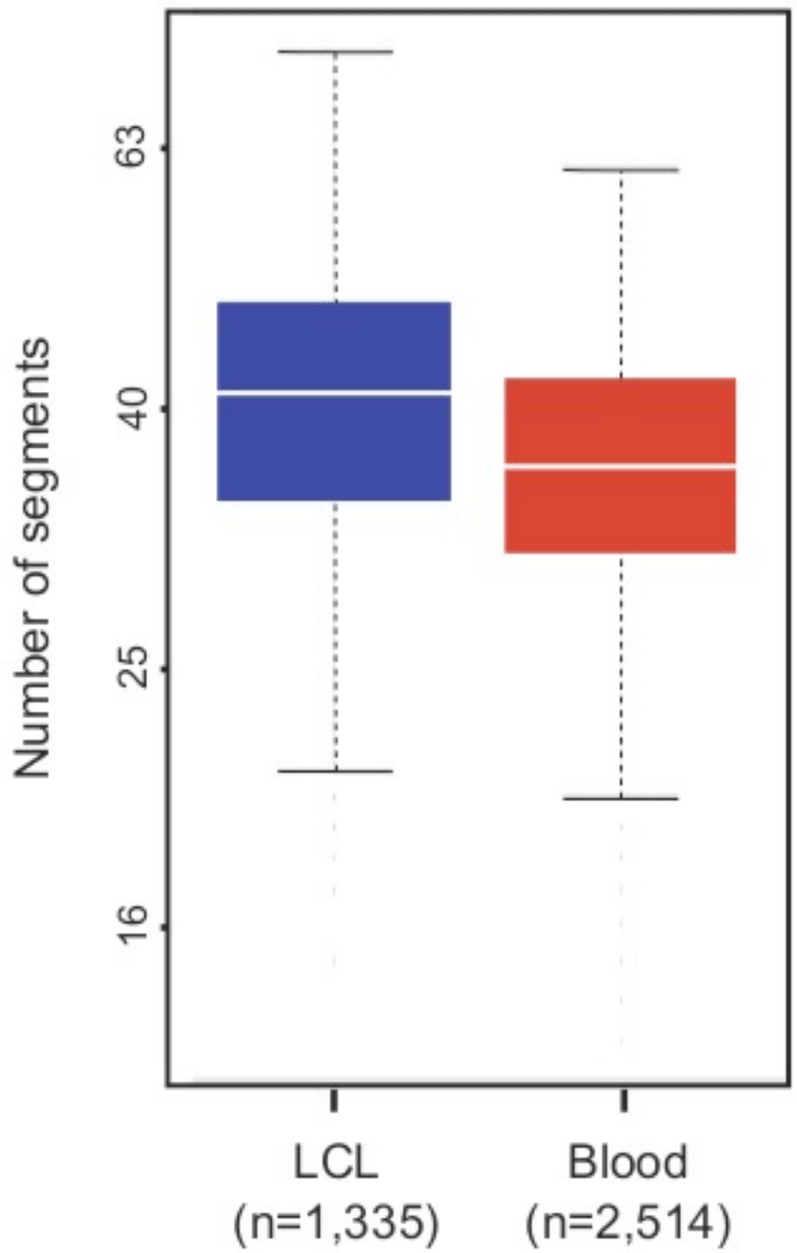
GWAS heterozygosity rate vs. no call rate: no separation based on DNA source



GWAS PCA of logR ratio: no separation based on DNA source

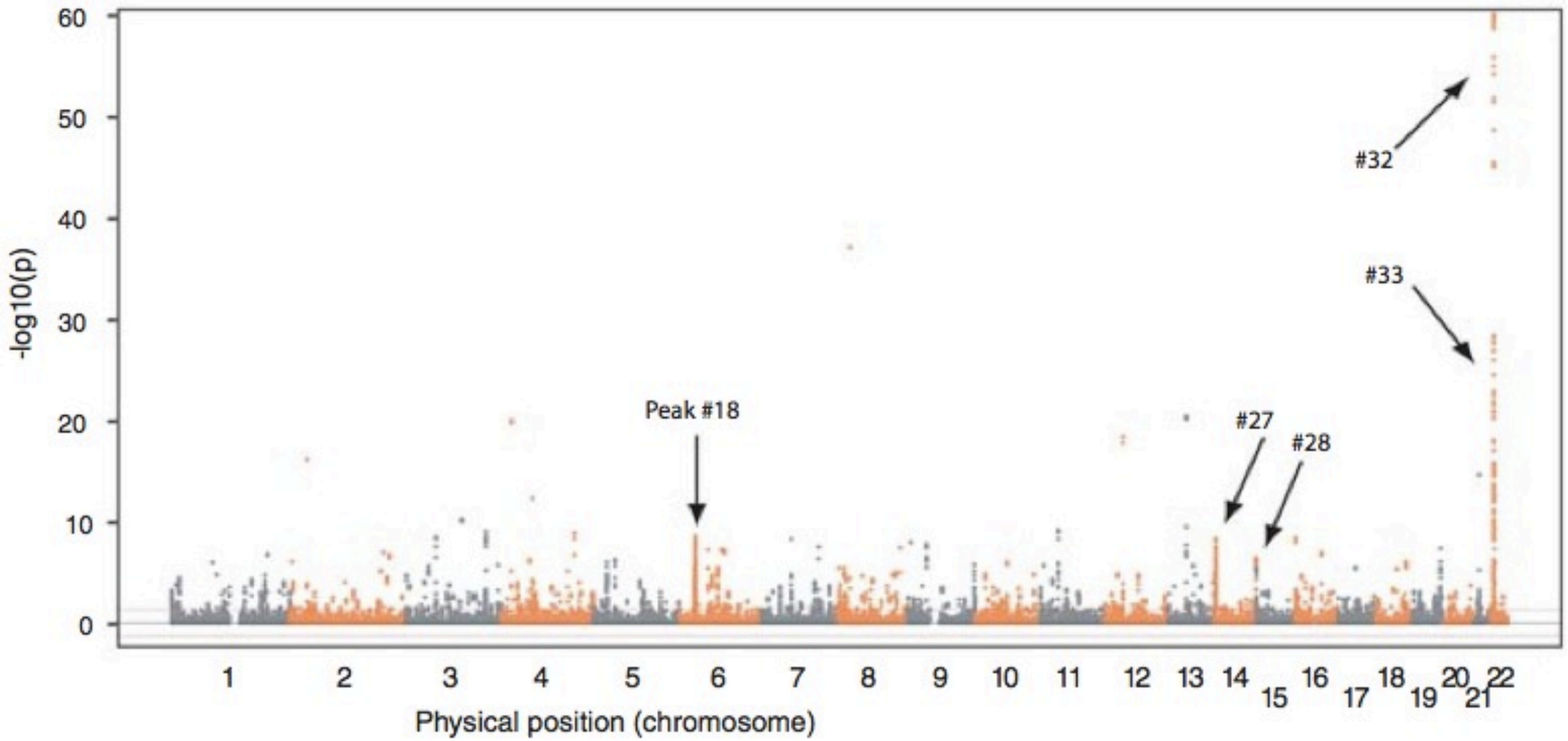


LCL derived samples have a greater number of CNVs per sample than blood

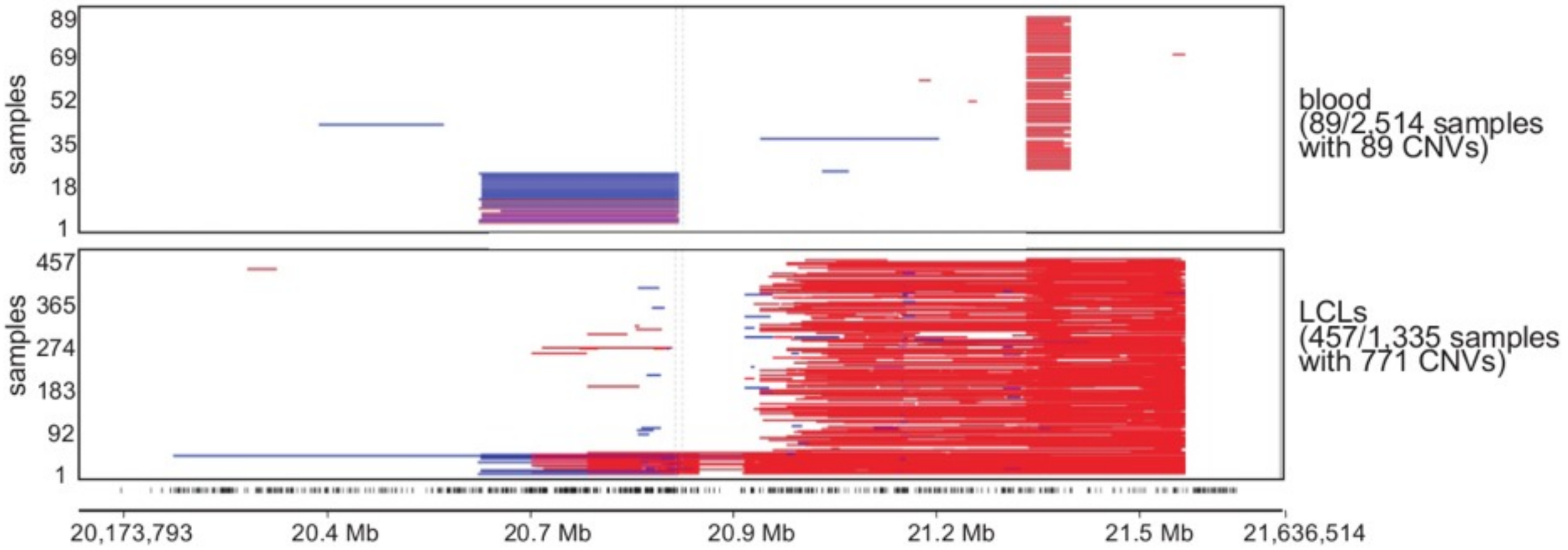


Association test with CNVs in blood or LCL samples reveals hot spots for LCL variability

Peaks correspond to chromosomes 6, 14, and 22



Chromosome 22 immunoglobulin lambda region is highly variable in LCLs



Acknowledgements

Pevsner Lab:

Jonathan Pevsner

Joe Baugher

Eric Stevens

Corriel Cell Repository:

Dorit Berlin

Zhenya Tang

Norman Gerry

Christine Beiswanger

Shirley MD, Baugher JD, Stevens EL, Tang Z, Gerry N, Beiswanger CM, Berlin DS, Pevsner J. *Chromosomal variation in lymphoblastoid cell lines*. Human Mutation. 2012.