

Multiplex paralogue ratio tests (PRT) for high-throughput CNV typing

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Copy number variation measurement
for association studies

Measuring copy number variation
with PRT

Examples: beta-defensins

Multiplex PRT: *CCL3L1*

Measuring copy number accurately for association studies

Typical challenges:

0-4 copies (*CCL3L1*, UK)

2-7 copies (beta-defensins)

4-10 copies (*CCL3L1*, Africa)

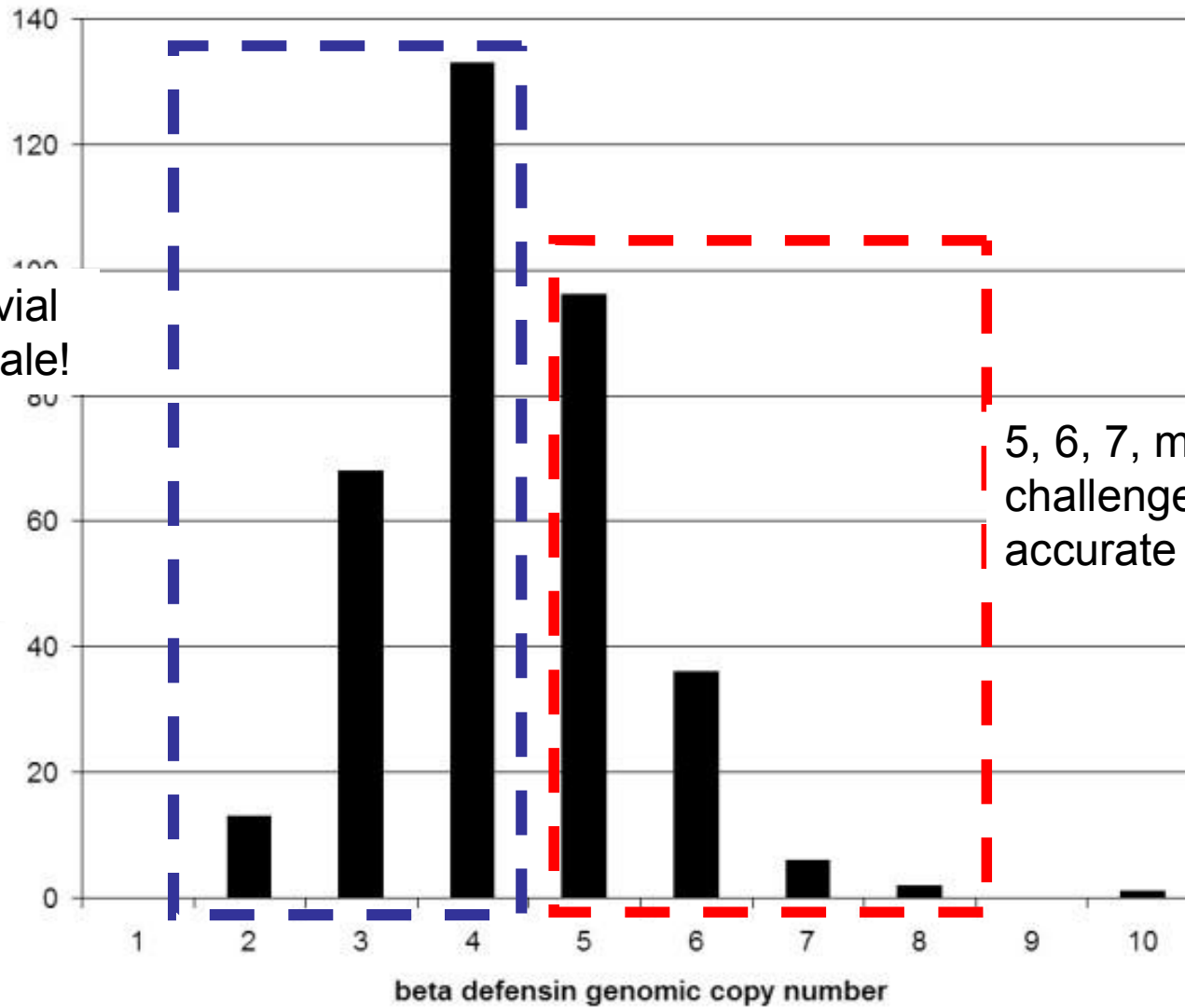
4-11 copies (alpha-defensins)

Accuracy not easily combined with high throughput!

Ideally, want accuracy comparable to SNPs

UK population: beta-defensin variation

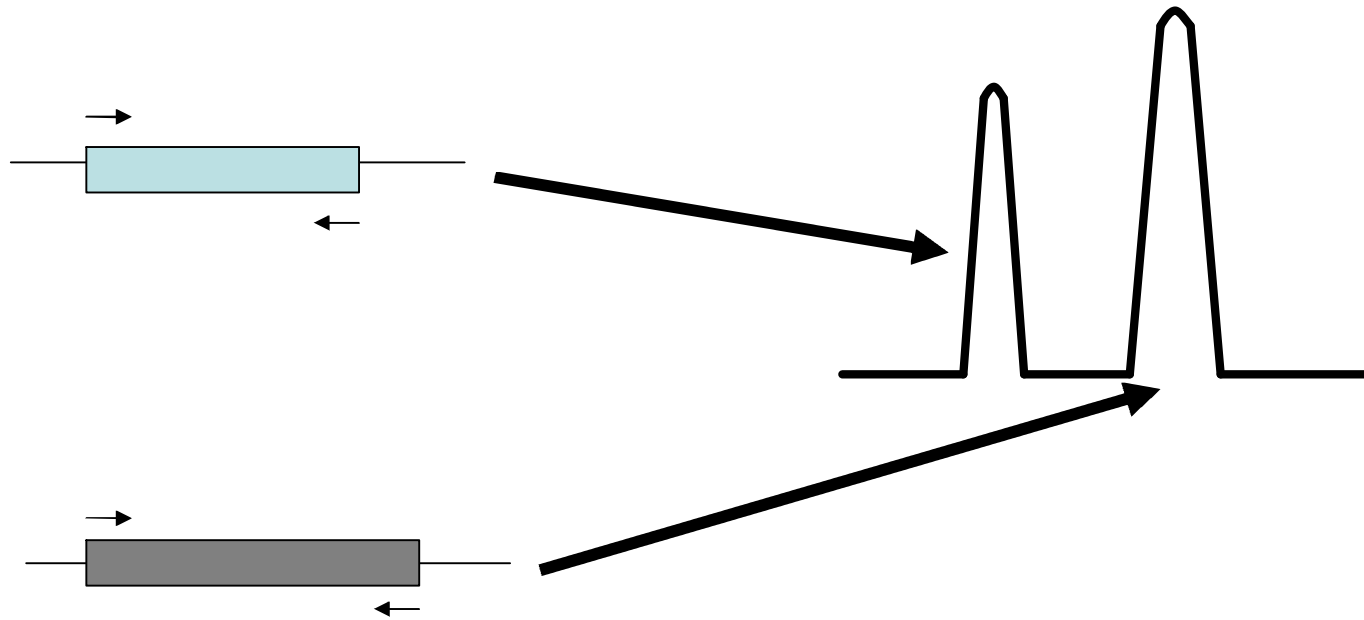
2, 3, 4 not trivial on a large scale!



5, 6, 7, more copies: challenge for accurate typing

A simple approach: comparative multiplex PCR

(“quantitative” PCR: includes QF-PCR, QMPSF, real-time, etc.)



Why doesn't it work?

(at least: why doesn't it work very well?)

Solution: use the same primers for the test and reference PCRs

“Paralogous sequence quantification” of
Deutsch et al.

(applied to aneuploidies)

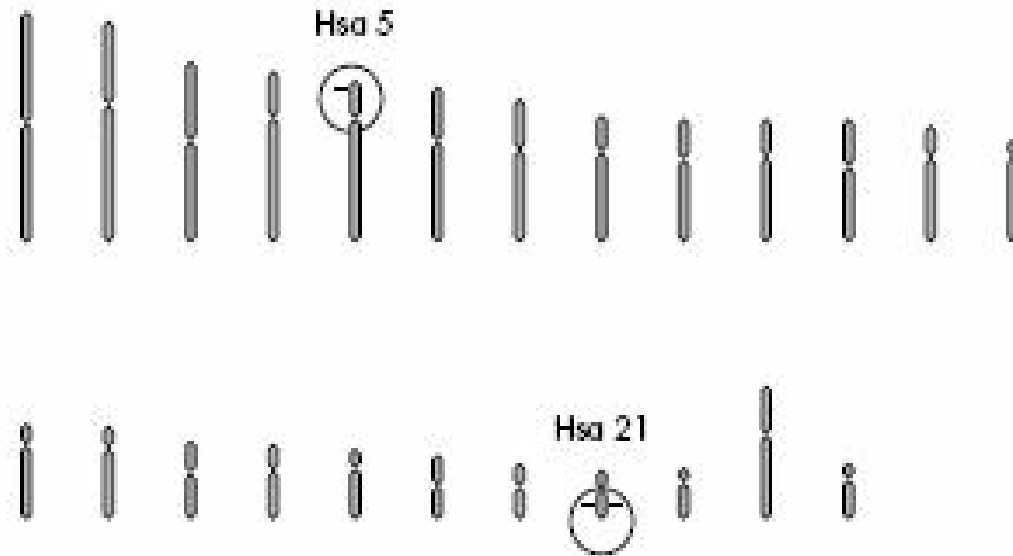


Detection of aneuploidies by paralogous sequence quantification

S Deutsch, U Choudhury, G Merla, C Howald, A Sylvan and S E Antonarakis

J. Med. Genet. 2004;41:908-915
doi:10.1136/jmg.2004.023184

A



B

Hsa 21
 Hsa 5

Hsa 21
 Hsa 5

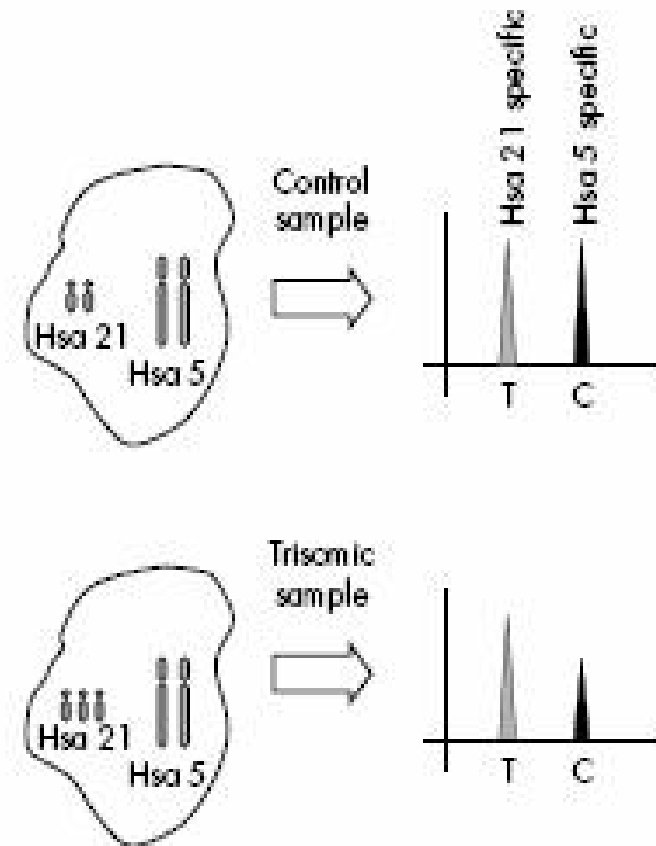
Hsa 21
 Hsa 5

Hsa 21
 Hsa 5

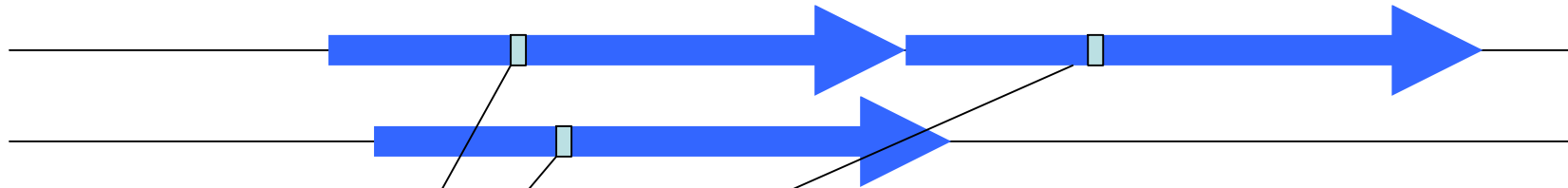
Hsa 21
 Hsa 5

PSM

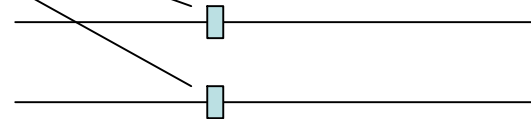
C



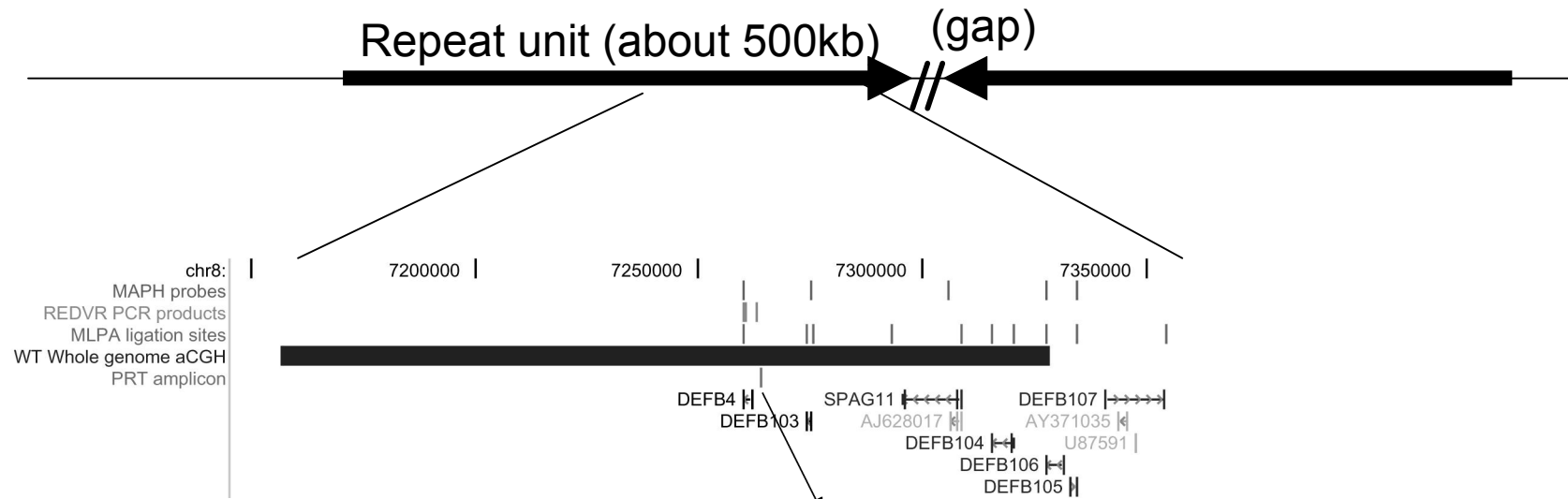
Variable *DEFB4* repeat unit
Chr 8 (about 500kb)



Pseudogene *HSPDP3*
also present on chr 5 (constant 2 copies)

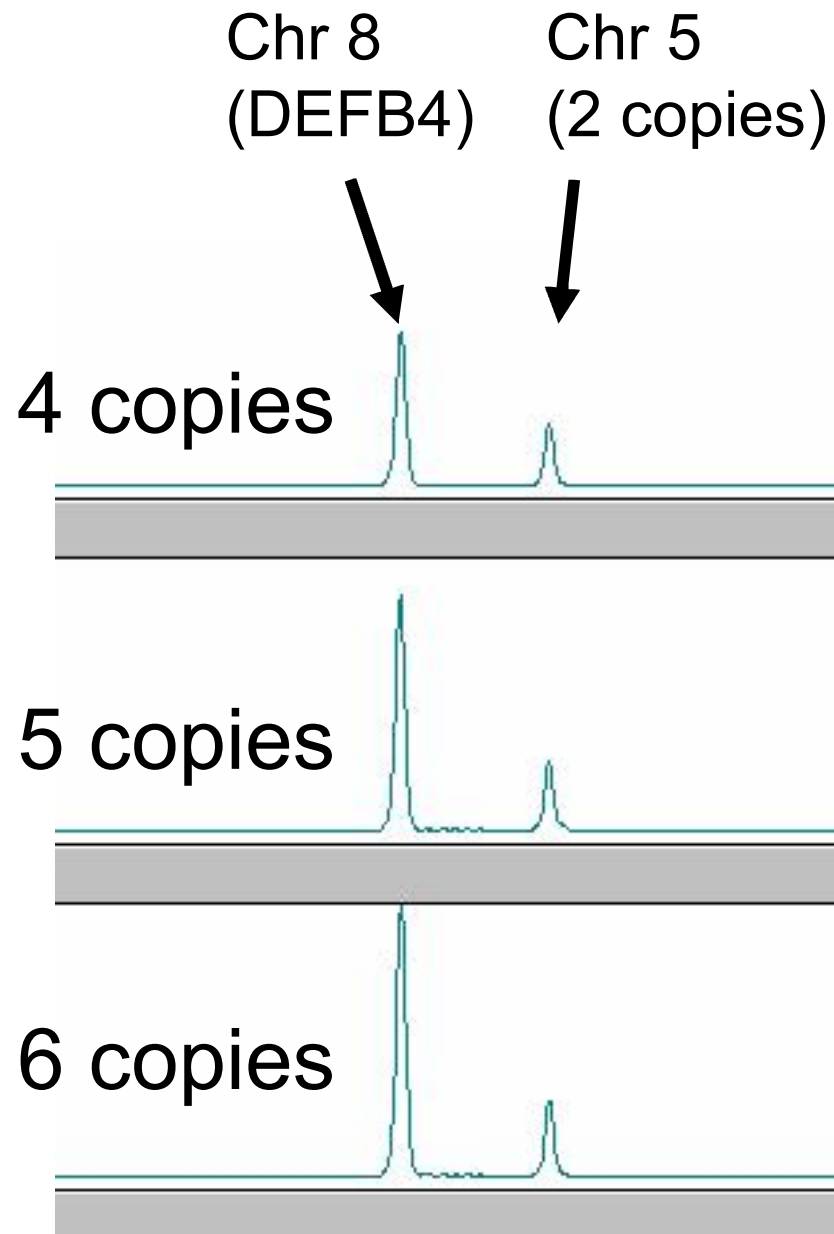
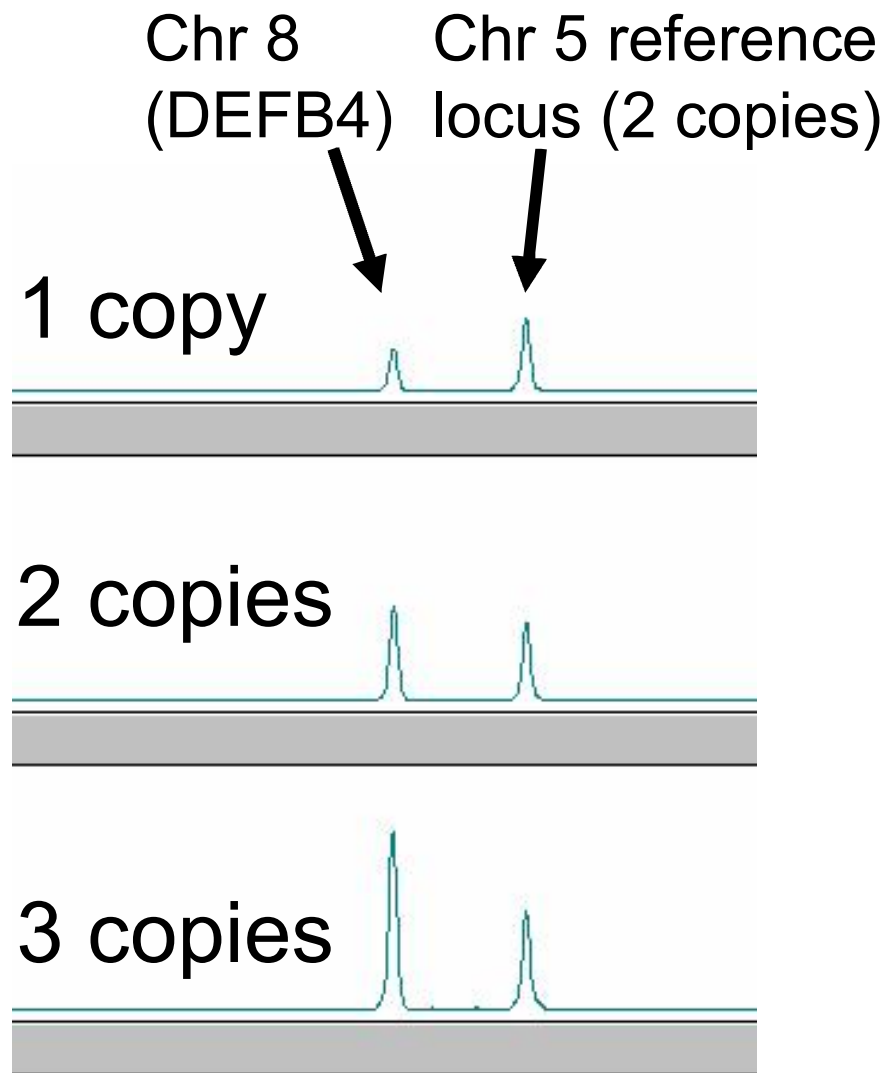


PCR with single primer pair
for *HSPDP3*
Measure chr8:chr 5 ratios



Pseudogene *HSPDP3*
(approx. 2kb)

chr8 (DEFB4)	CCAGATGAGACCAGTGTCC..	443bp	..GCTGTAATTGCTGAACTTAAAA
chr5	CCAGATGAGACCAGTGTCC..	447bp	..GCTGTAATTGCTGAACTTAAAA
hspdcDNA	gcagatgagaccggtgtcc. [2 introns]		.gctgtaattgctgaacttaaaa
chr12A	CCAGATGAGACT TGGTGTCC..		..GCTGTAATTGCTGAACTTAAAA
chr3	--AGATA AAGACCATTGTCC..		..GCTGTAATAGCTGAACCTAAAA
chr4	CCAGATA AAGACCAGTGTCC..		..GCTATAATCGCTGAACCAAAAA
chr13A	C T AGATGAGAA ACTGTGTCC..		..GCTGTAATTGCTGAACTTGAAA
chr21	T CAGATGAC CACCAGTGTCTG..		..GCTGTAATTGCTGAACTTAAAA
chr6	CCAGATGAGACC GGTGTCC..		..GTTATAATTGCTGAACTTAAGA
chr13B	CCA AATAAGACTGGTGTCC..		..ACTGTAATTGCTGAACTTAGGA



Accuracy and error: evidence?

Repeat testing

Clustering around integers

Alternative methods

MLPA/CGH/MAPH (RT-PCR)

microsatellites/MSV ratios

Segregation

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Repeat testing

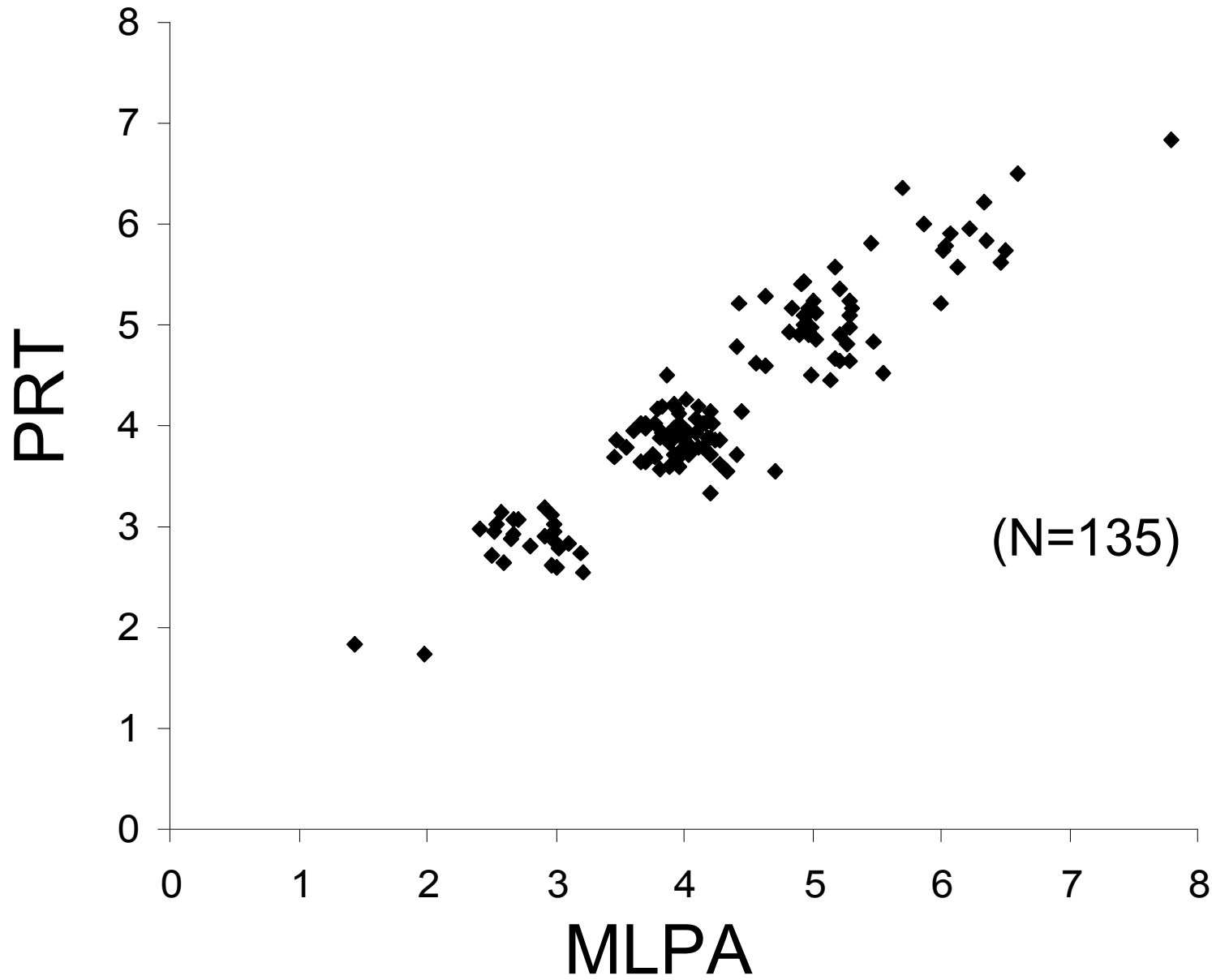
Clustering around integers

Alternative methods

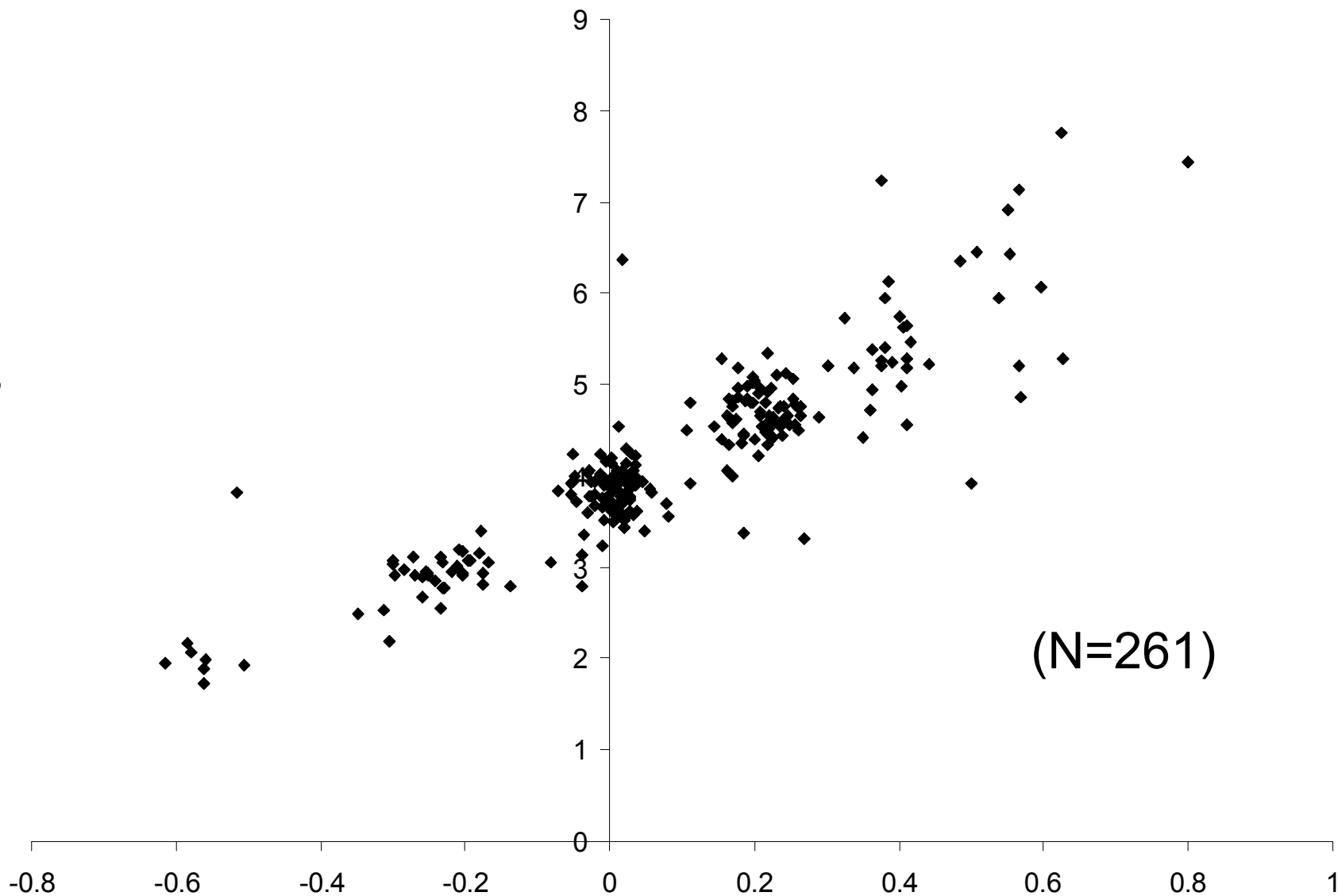
MLPA/CGH/MAPH (RT-PCR)

microsatellites/MSV ratios

Segregation



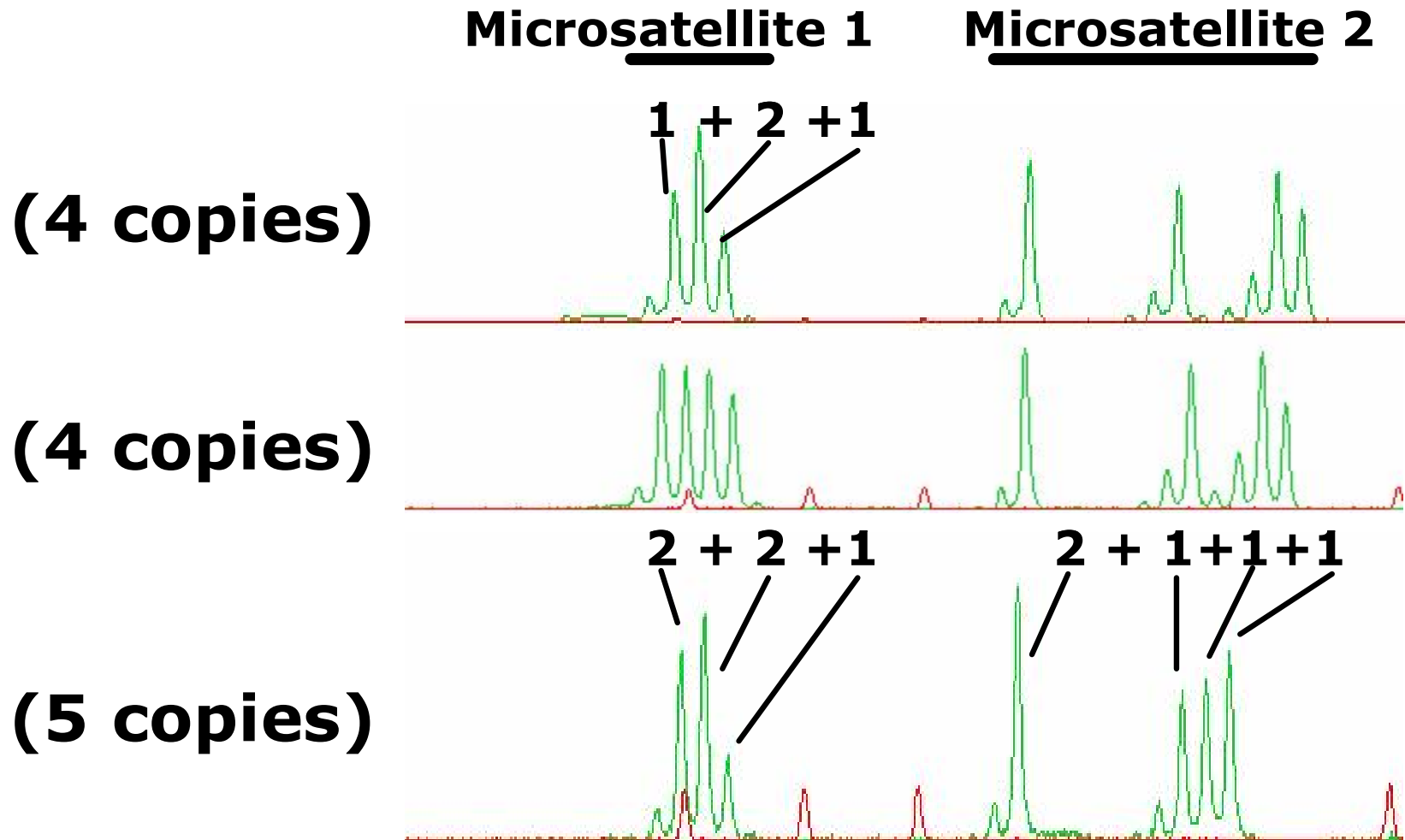
PRT copy number



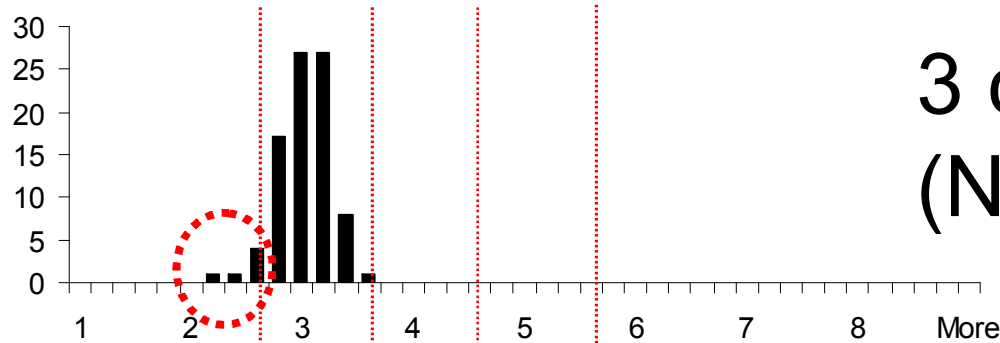
(N=261)

\log_2 array-CGH signal (clone 10C3)

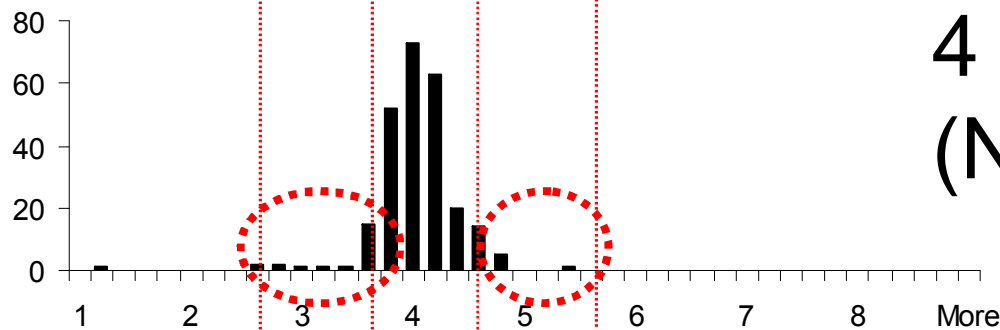
Microsatellite confirmation



Copy numbers established from combined methods – results of single PRT:

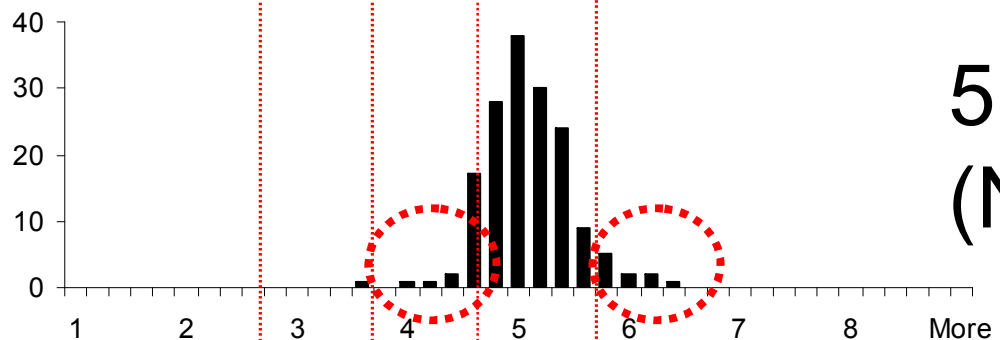


3 copies
(N=86)



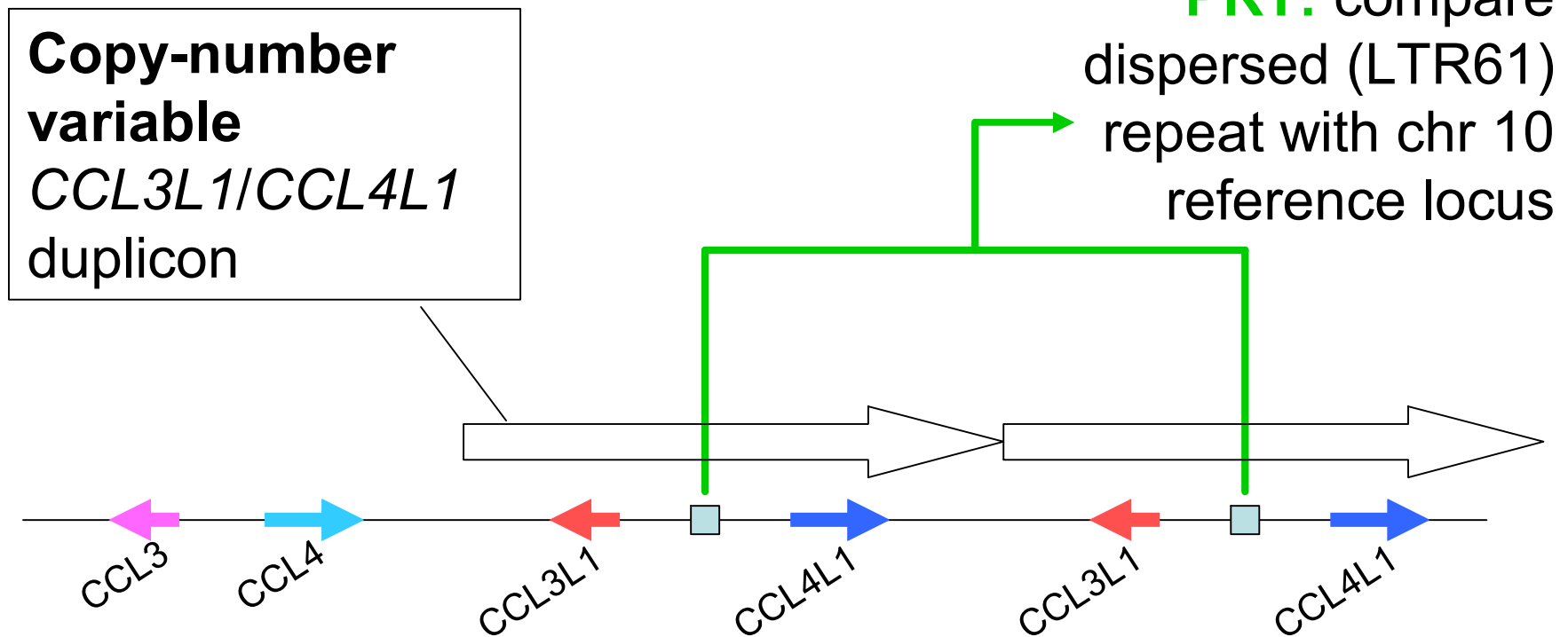
4 copies
(N=251)

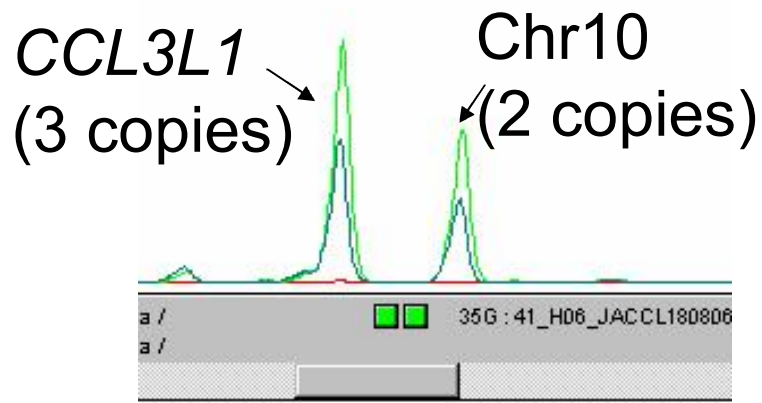
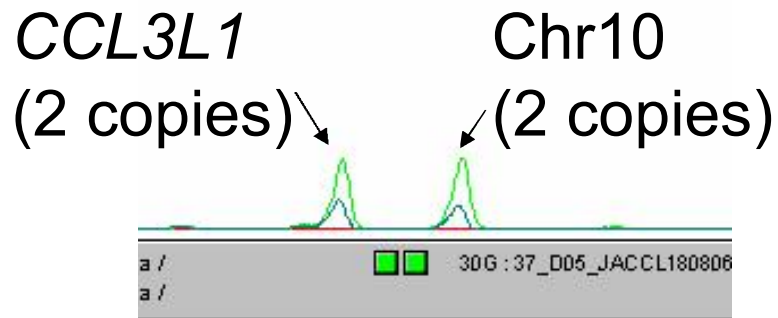
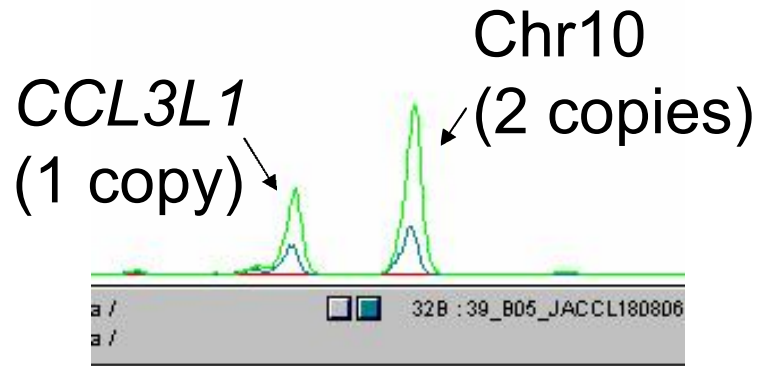
Next: *multiplex*
PRTs



5 copies
(N=161)

PRT using LTR61 at *CCL3L1*





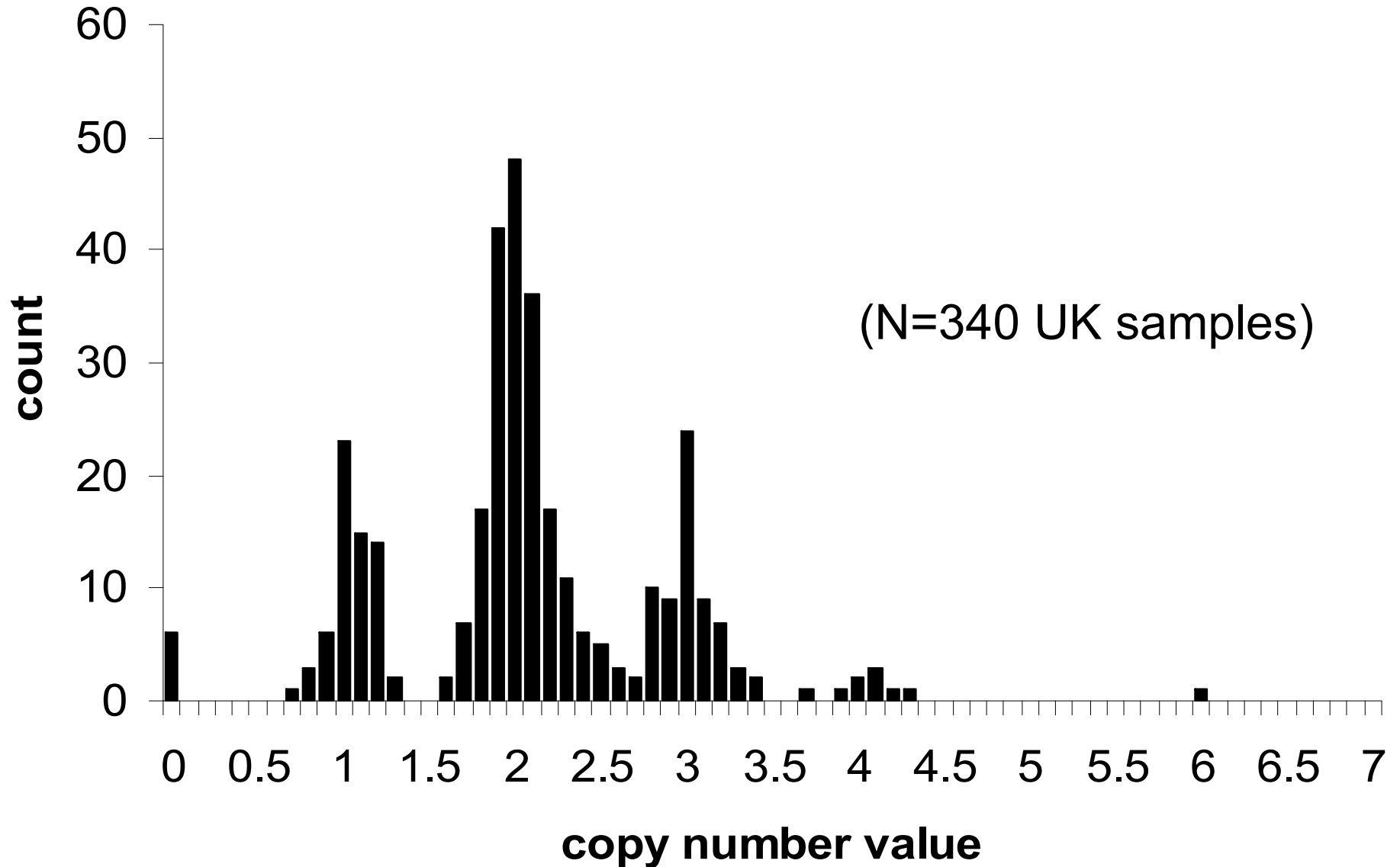
PRT using LTR61

Measure signal from LTR61 copy near CCL3L1

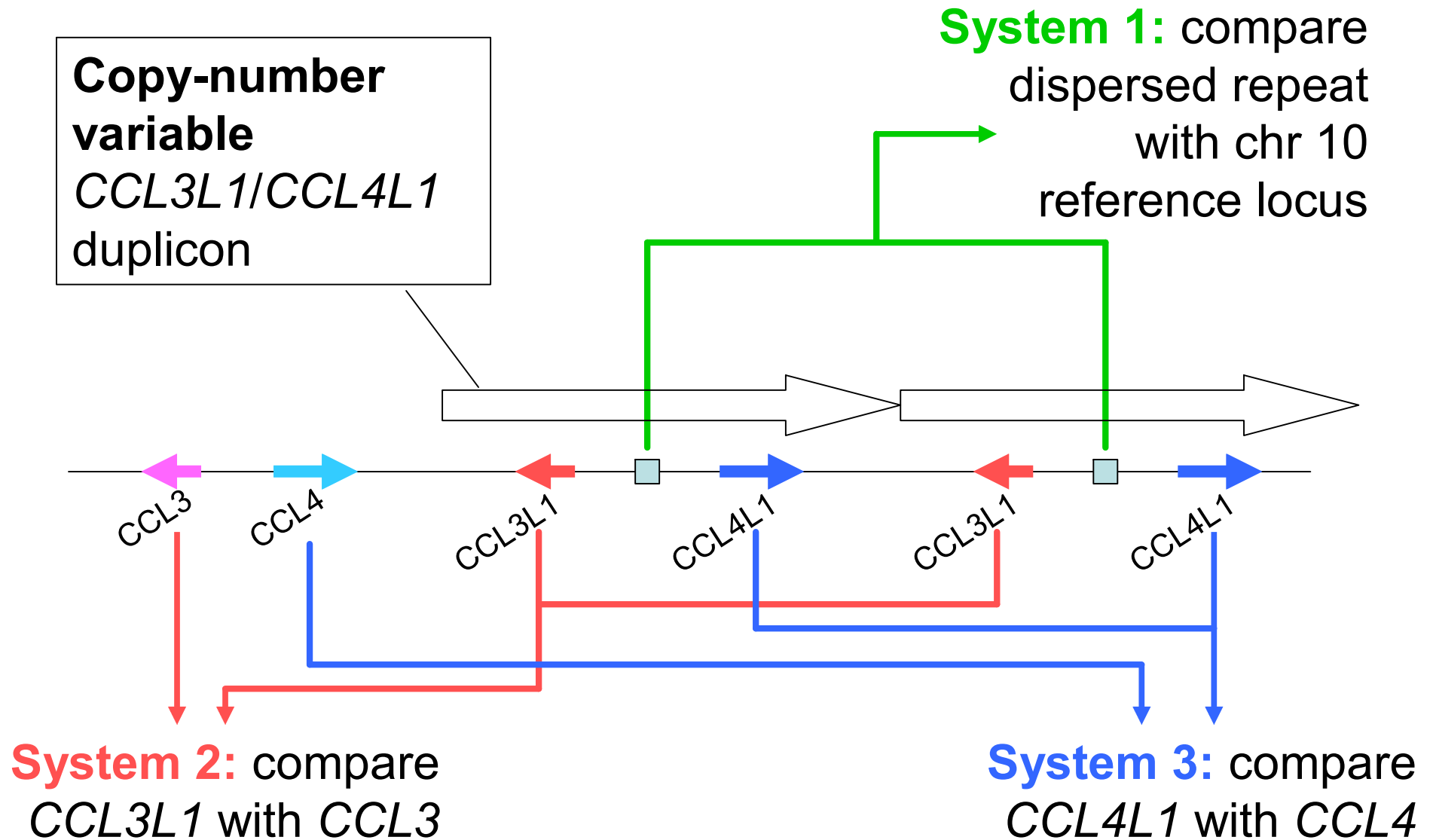
(chromosome 10 reference locus)

Two PCRs (HEX and FAM) per sample – mix and run together

PRT using LTR61 at *CCL3L1*

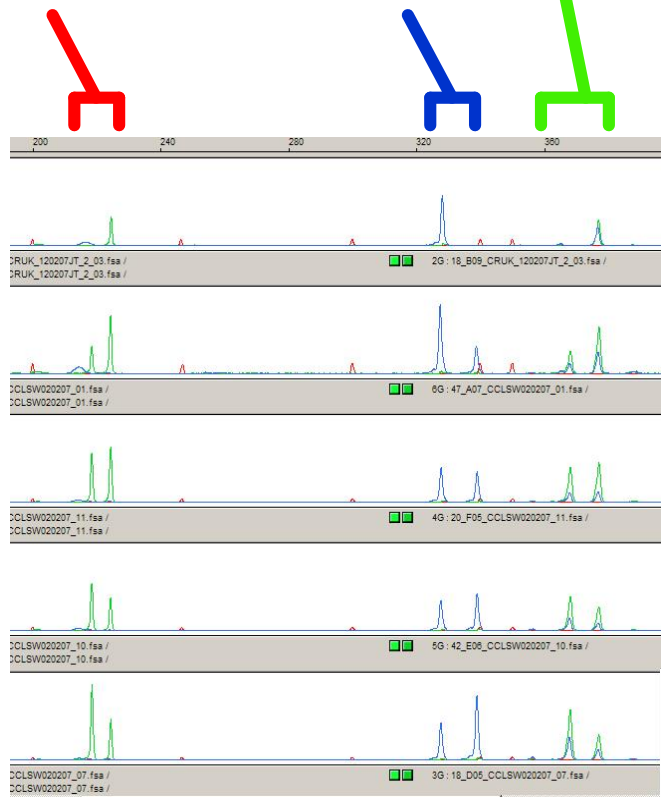


Combined PRT systems at *CCL3L1*



Combined PRT systems at *CCL3L1*

System 2 (v *CCL3*) **System 3** (v *CCL4*) **System 1** (unlinked)



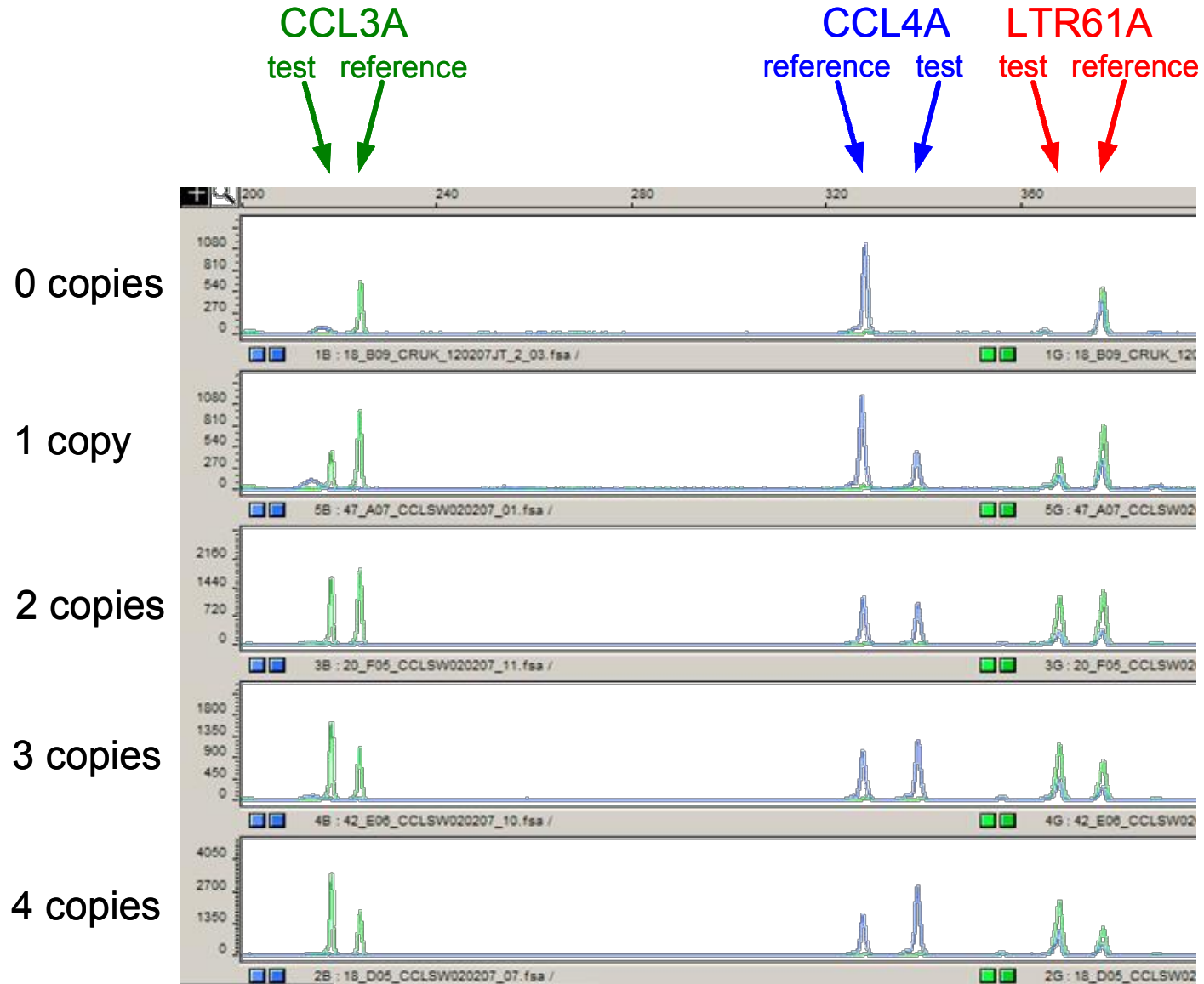
0 copies
1 copy
2 copies
3 copies
4 copies

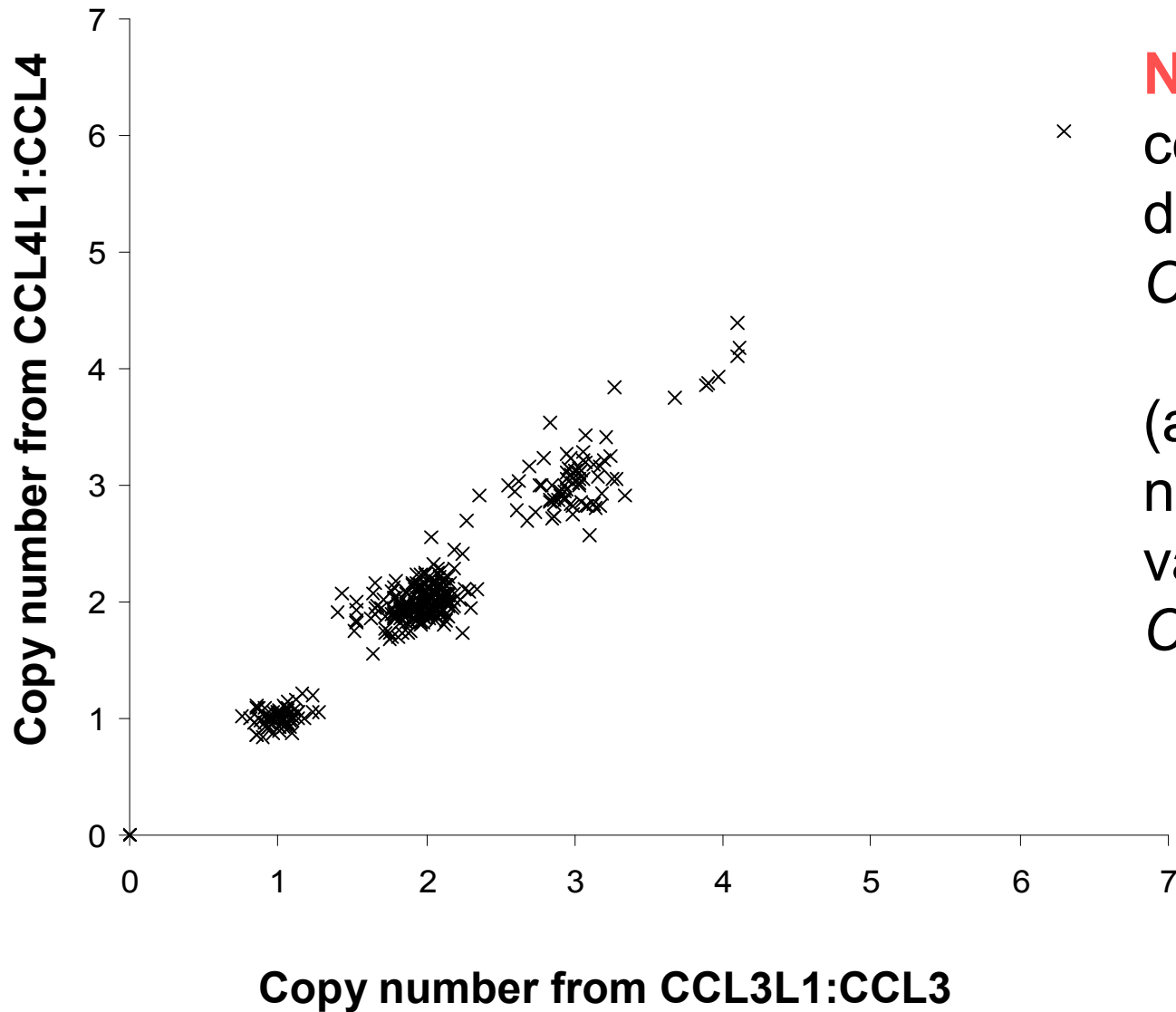
Two PCRs, single capillary

Advantages:
combined accuracy very high (“What I tell you three times is true”)

Combine products on single capillary run, so adds little to the cost per test

Multiplex PRT at CCL3L1/CCL4L1



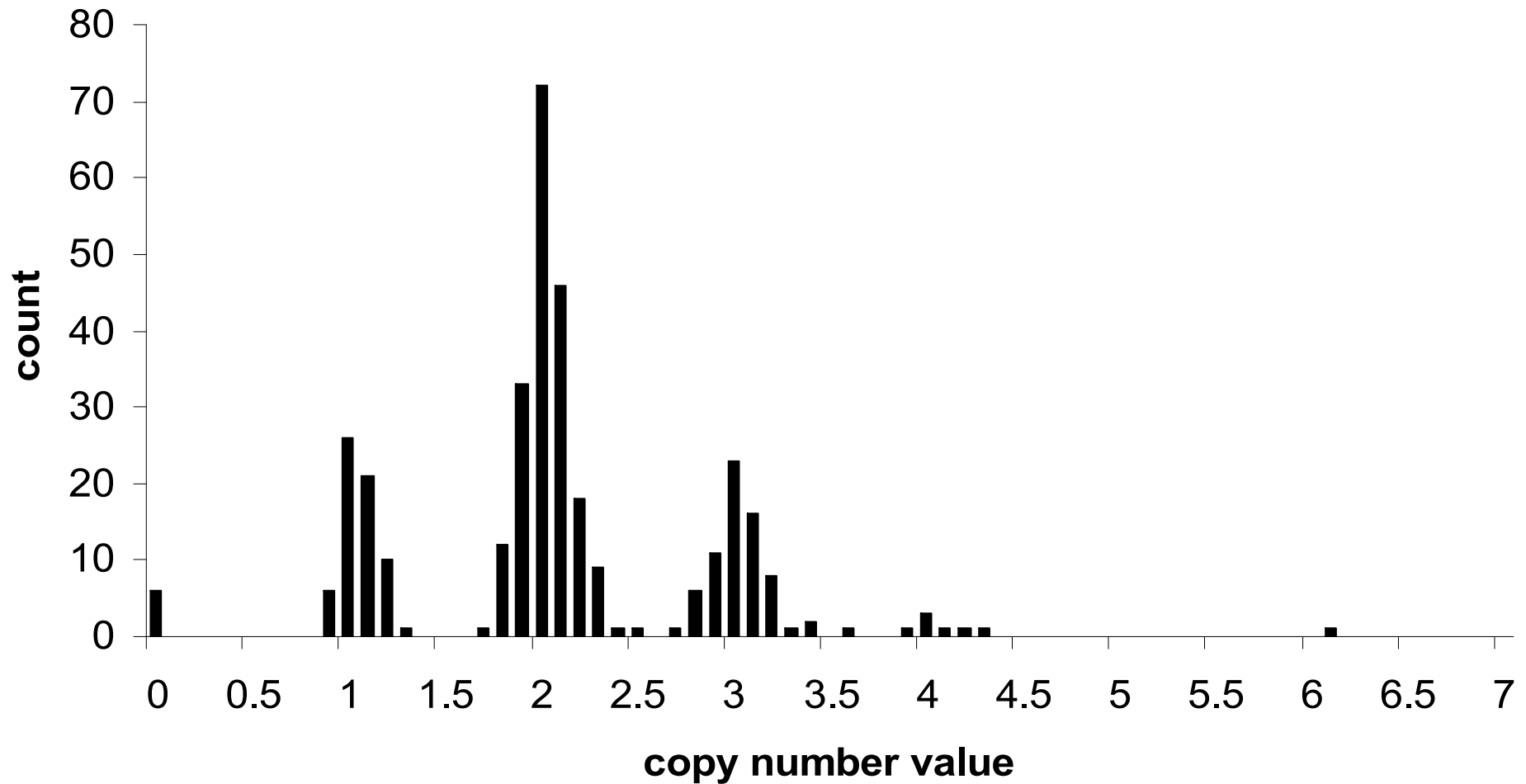


N=340 (UK)

No evidence for
consistently
discordant
CCL3L1/CCL4L1

(and LTR61 agrees:
no evidence for
variation in *CCL3* or
CCL4)

Combining data from all three PRT systems (340 UK samples):



Current formats

CCL3L1/CCL4L1:

Either 2 PCRs/ 1 capillary [4 ratios]

Or single tube, 1 capillary [3 ratios]

Beta-defensins: 2 PRTs, 1 indel

(4 PCRs? 1 capillary [6 ratios])

To come: alpha-defensins (4-11!)