Wessex diagnostic array design

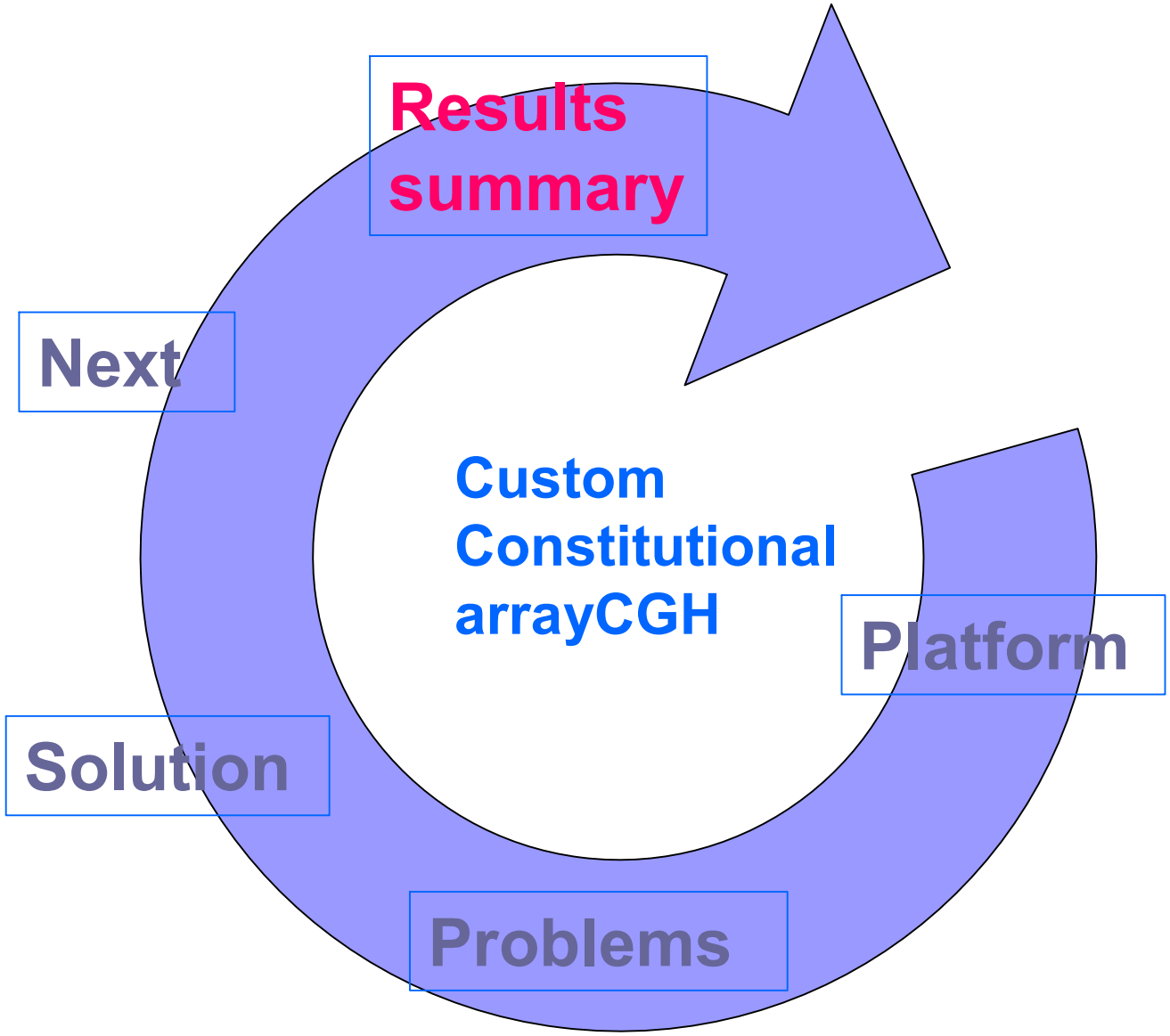
Shuwen Huang

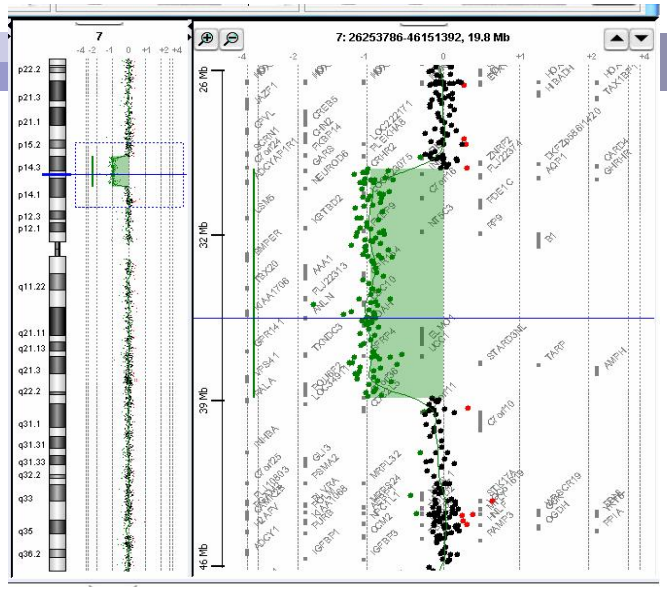
John Crolla

PART 1

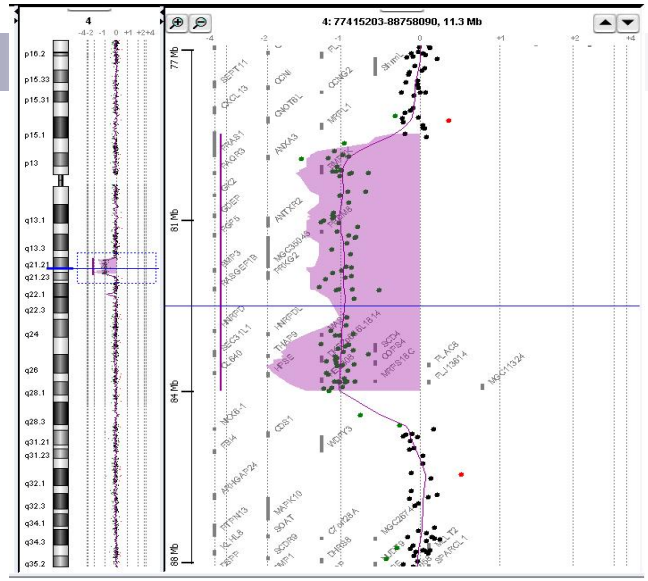
- DESIGN

Constitutional custom array-CGH design



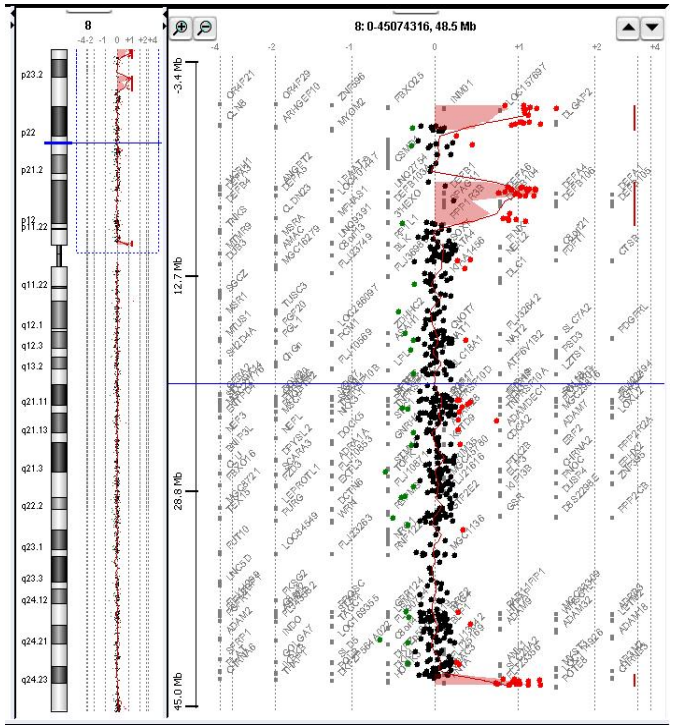


Chromosome 7p14.3-p14.1 9.11Mb deletion

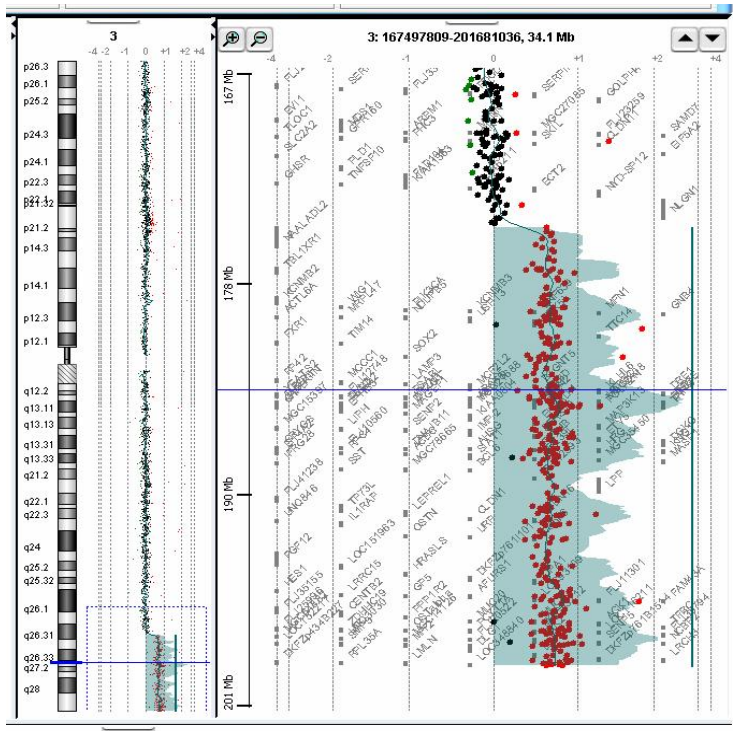


Chromosome 4 q21.21-21.23 5.91Mb deletion

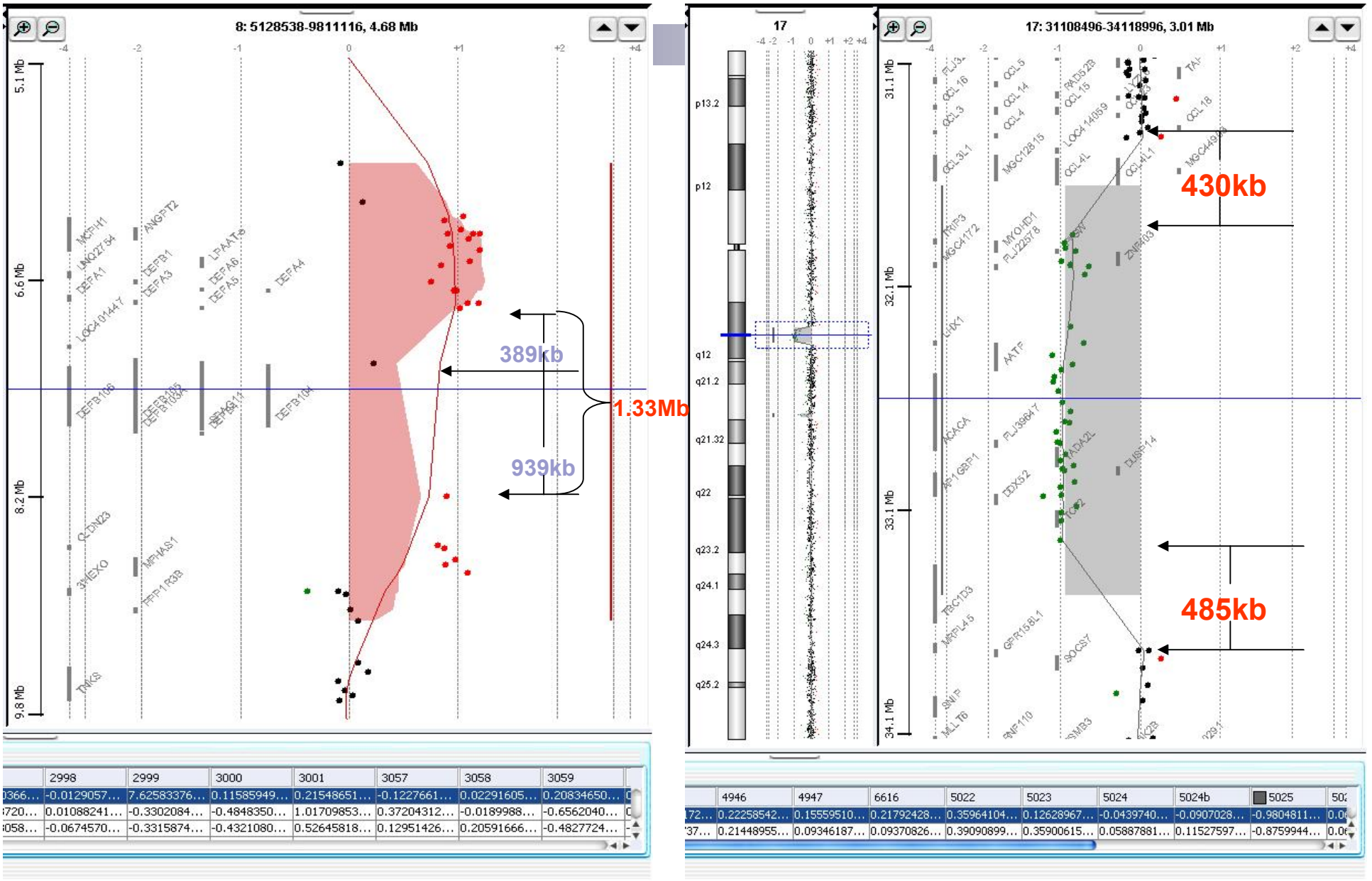
Agilent 44B



Chromosome 8 multiple amplifications



Chromosome 3qter duplication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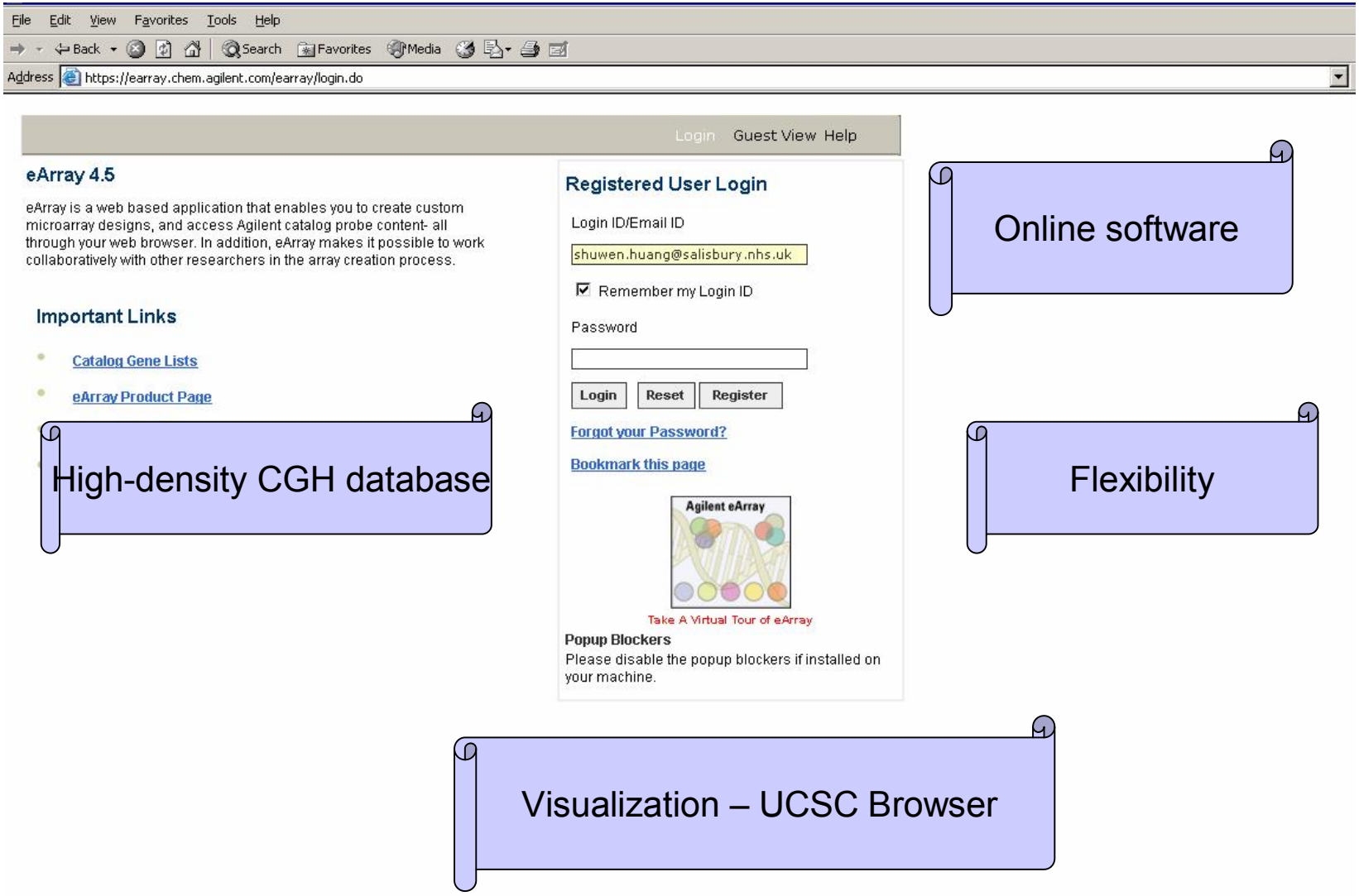
939kb
1.33Mb
430kb
485kb

Solution - custom constitutional array



Principles of our constitutional array design

1. Based on the 44B Agilent array contents;
2. Using 4 x 44k format (economical);
3. Probes more evenly distributed along the whole human genome;
4. More probes on micro-deletion/duplication regions.

Strategies of our constitutional array design

1. Make more features available for constitutional array;
2. Find all the big gaps in the current 44B array;
3. With the available features, number and size of the big gap, calculate the average spacing of the probes for big gaps;
4. Generate proper gap files for the eArray design, and design the array using eArray software version 4.5.
5. Check probe distributions along the whole genome.

eArray design tool

Agilent Technologies - Microsoft Internet Explorer

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Go Links

eArray - National Genetics Reference Laboratory (Wessex) Logout X

Home Collaborations Microarray Design Probe Group Probes Tools Administration Help

Create Search Browse Tra Search Upload Page Help

Simple GO Advanced Chromosomal Location HD-CGH HD-ChIP

Welcome Shuwen Huang Home

Getting Started

Common Probe Related Activites

- Use Simple Search to find probes
- Search CGH High Definition probe database
- Search ChIP High Definition probe database
- Upload new probes into your eArray account
- Design new probes for Gene Expression

Common Probe Group related Activities

- Create a new Probe Group.
- Search Probe Group.

Next >>

Common MicroArra

- Download Agile files.
- Create new custom MicroArray design.

Probes → Search → HD-CGH

Done Internet

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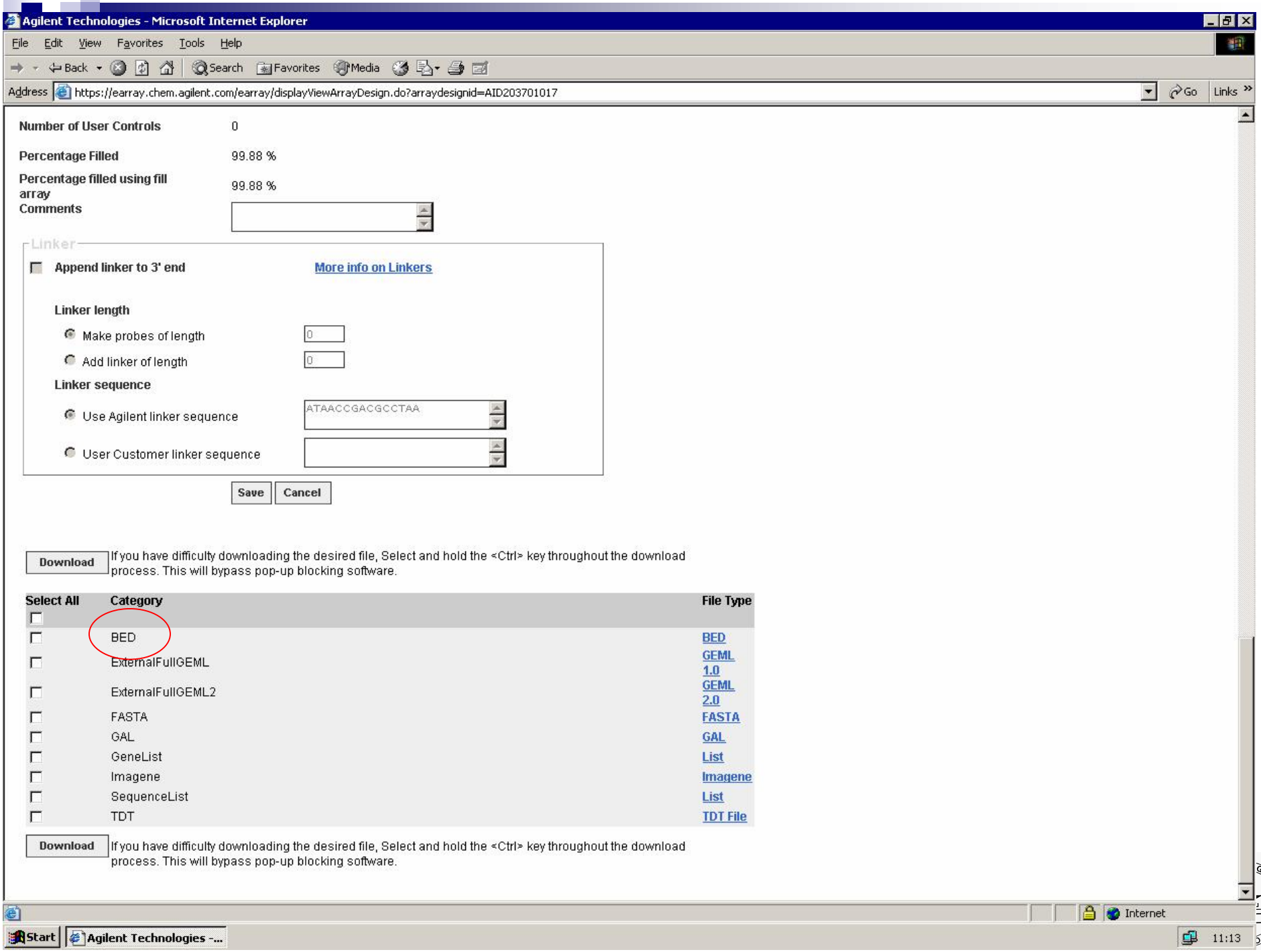
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Comments
Linker
Append linker to 3' end
More info on Linkers
Linker length
Make probes of length 0
Add linker of length 0
Linker sequence
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User Customer linker sequence
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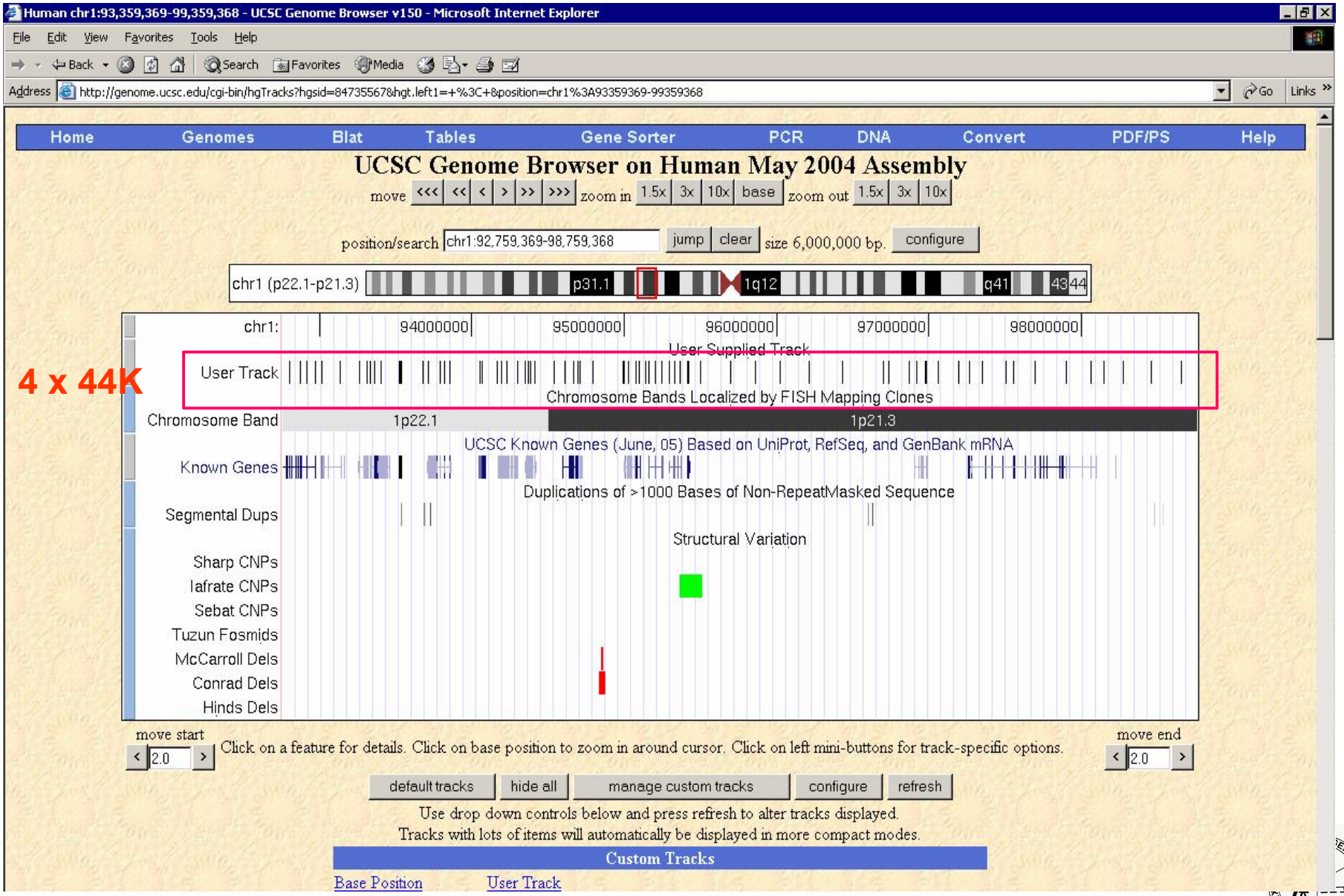
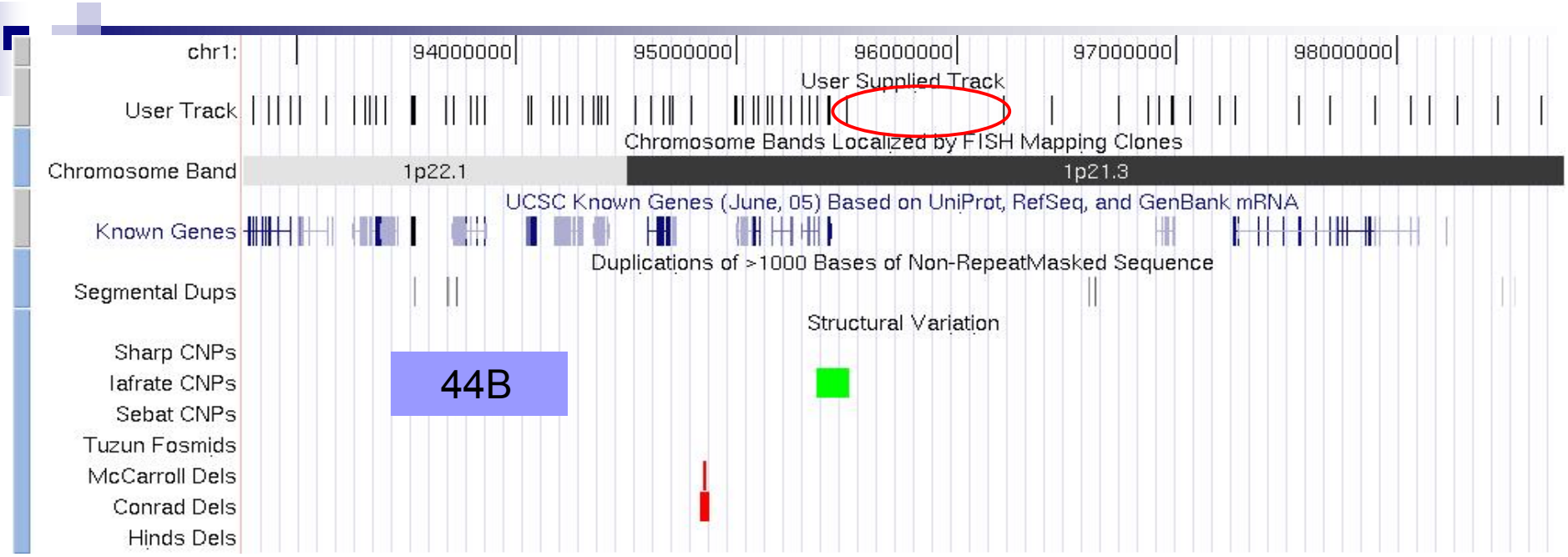
Check probe distributions using UCSC Genome Browser

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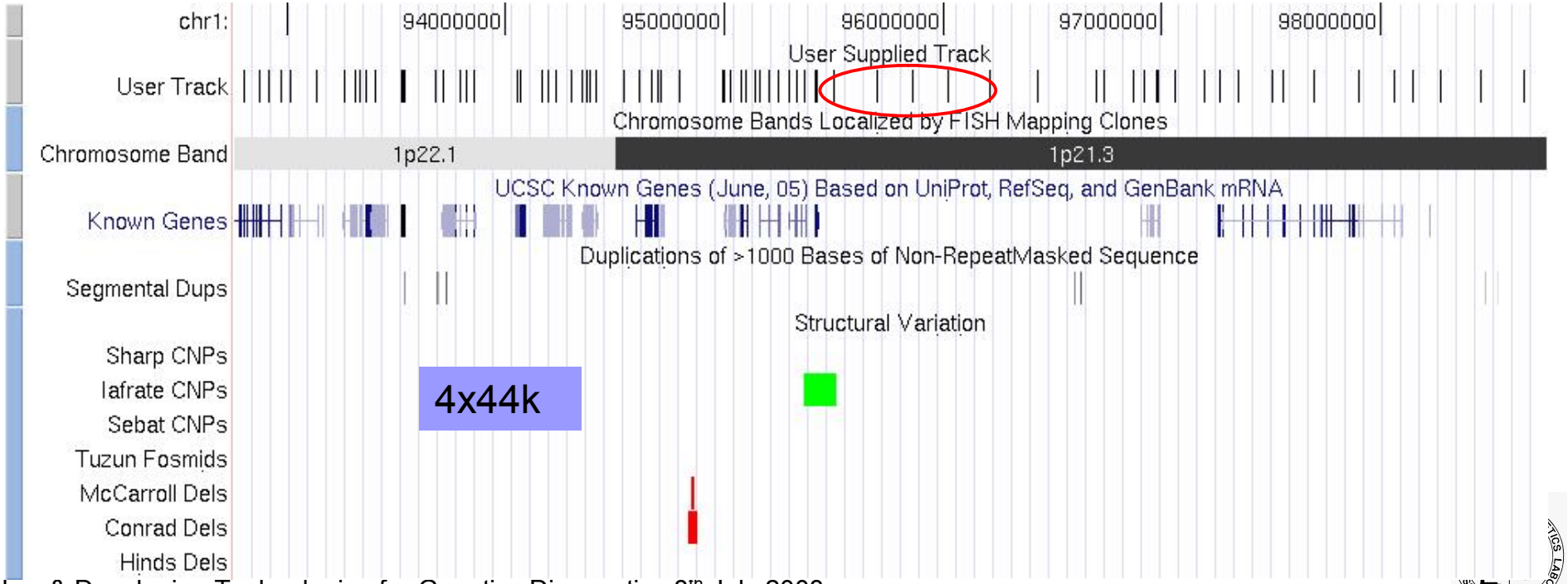
94000000
95000000
96000000
97000000
98000000
User Supplied Track
User Track
Chromosome Bands Localized by FISH Mapping Clones
Chromosome Band
1p22.1
1p21.3
UCSC Known Genes (June, 05) Based on UniProt, RefSeq, and GenBank mRNA
Known Genes
Duplications of >1000 Bases of Non-RepeatMasked Sequence
Segmental Dups
Structural Variation
Sharp CNPs
Iafrate CNPs
Sebat CNPs
Tuzun Fosmids
McCarroll Dels
Conrad Dels
Hinds Dels
44B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Tuzun Fosmids
McCarroll Dels
Conrad Dels
Hinds Dels
4x44k

Summary

- 4x44k custom array gives relatively even resolution without compromise of array quality
- Relatively inexpensive (slides, reagents)
- Efficient (3-day → 2-day)
- Collaboration

  16 collaborators from USA, UK, EU, Brazil, and Australia

- Contribution - Add novel findings to DECIPHER and other copy number variation websites

What next

- Refine the 4x44k custom array – 4x44k V2

  probes more evenly distributed
  update recently discovered interesting regions
  PAR1 region of sex chromosomes

- Next generation custom array – 4x180k

  higher resolution 35kb → ~8kb (HD database, 8.4m, 200bp)
  improved coverage of known microdeletion/microduplication regions
  improved coverage of telomere/peri-centromeric regions
  improved coverage of known haploinsufficiency genes
  improved coverage of targeted regions

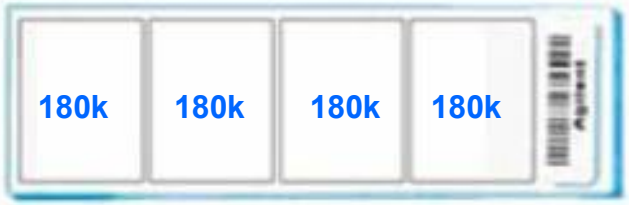
- SNP arrays

Next generation platforms

Highest Sensitivity

Single 244,000-probe arrays with highly refined replicate expression probes are our most sensitive arrays for gene expression experiments where you need to scan large regions in the finest detail.

Excellent Value



4-plex arrays provide four arrays on a single slide: each array comprises 44,000 probes. These are our most versatile arrays, the workhorse of the product line, optimized for both efficiency and coverage: an excellent value for whole-genome scanning.

Targeted Profiling

Our 8-plex arrays are our most extensible product. Eight targeted arrays with 15,000 probes each provide the perfect tool for the targeted profiling of a large number of samles at the best price.

PART 2

IMPLEMENTATION & RESULTS

Overview

- Brief outline of results
- Trends
- Copy number variation
- Some thoughts…….

ARRAY-CGH

- Not a new and developing technology
- First line test (has replaced karyotyping*) in several European and US laboratories
- UK widely funded for early implementation by the 2003 White Paper

Two main ascertainment groups

- Developmental delay, mental retardation, dysmorphism, congenital abnormality (DD/MR/CA) 350 Reported

- Further analysis of cytogenetic abnormalities 60 Reported
  - “Balanced” structural abnormalities (ABSCR/CCR)
  - Supernumerary marker chromosomes (SMC)

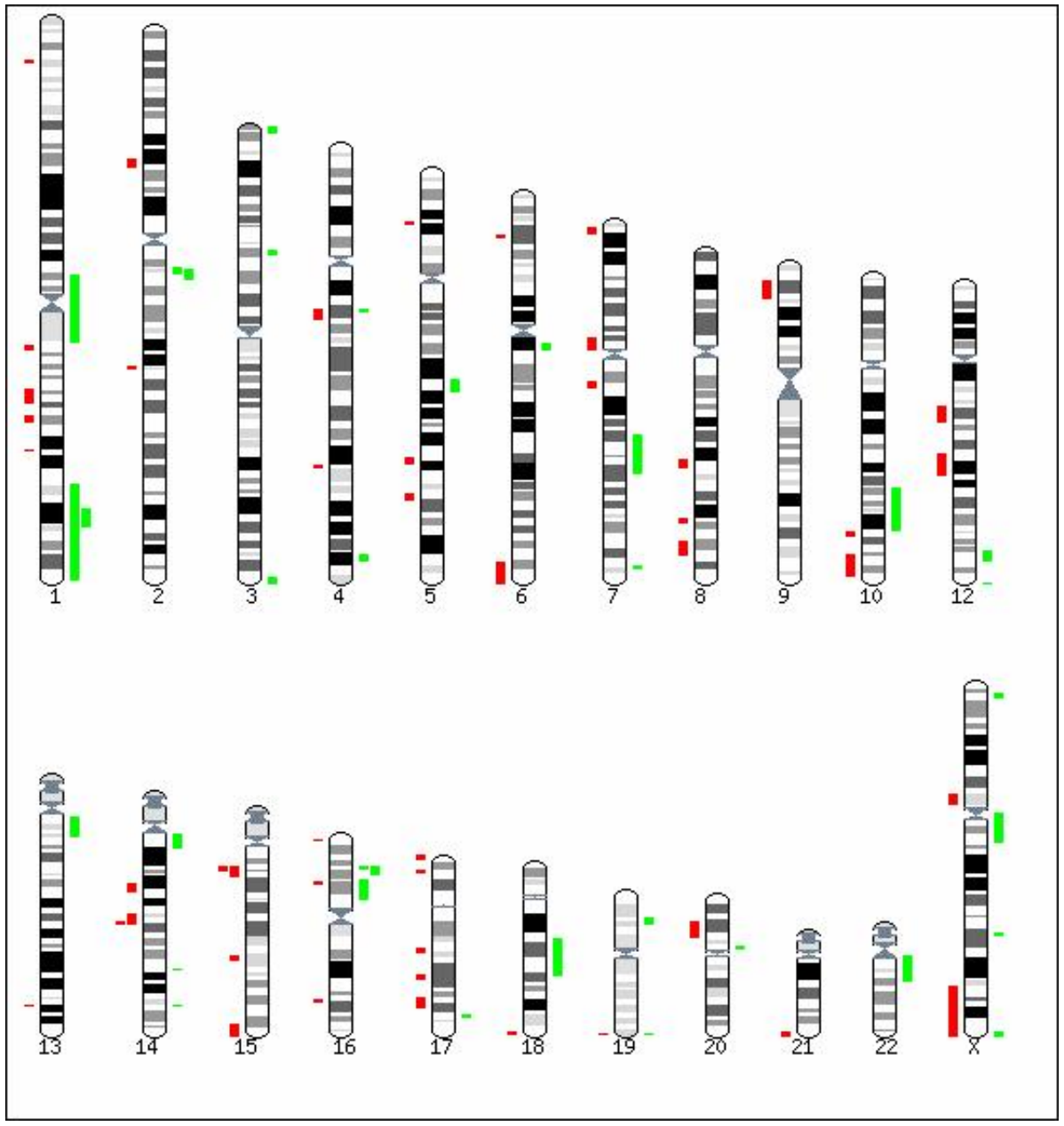
DD/MR/CA – Normal karyotypes, telomeres etc.

	de novo	Familial + phenotype	Follow up in progress	Novel CNV	Total CNV	Normal arrays	Total
dup	5	2	30	19	(56)	CNV	
del	13	3	20	9	(45)	CNV	
Total CNVs	18	5	50	28	(101)		
Number Cases	16* (4.6%)	4* (1.2%)	45* (12.8%)	26* (7.4%)	91 (26.0%)	249 (74.0%)	350 (100%)

* cases with two or more abnormalities

RESULTS SUMMARY (RETROSPECTIVE CASE ONLY)

- ~25% WITH COPY NUMBER CHANGES
- ~11-13% *DE NOVO*
- ~2% SEGREGATING WITH PHENOTYPE
- ~10- 12% NOVEL CNVs



deletions

duplications

1q21.1 Syndrome

del(1)(q21.1)pat	1.16 Mb	1q21 syndrome
del(1)(q21.1)nk	1.17 Mb	1q21 syndrome
del(1)(q21.1)mat	1.16 Mb	1q21 syndrome

del(15)(q13.1->13.3) syndrome

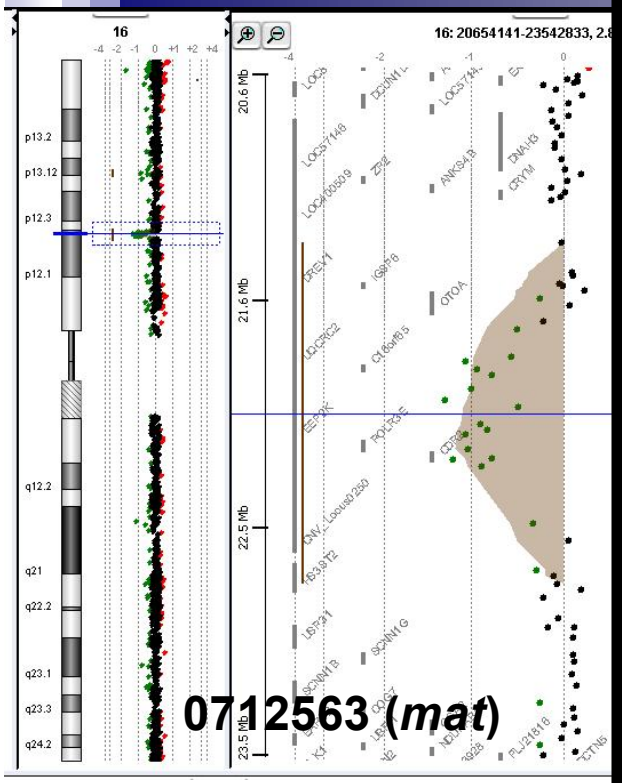
del(15)(q13.1q13.3)nk	3.56 Mb	Sharp et al 2008
del(15)(q13.1q13.2)pat	1.41 Mb	Sharp et al 2008
del(15)(q13.1q13.2)nk	1.41 Mb	Sharp et al 2008
dup(15)(q13.2q13.3)nk	1.58 Mb	

del(17)(q12) syndrome

del(17)(q12)de novo	1.32 Mb	*TCF2*
del(17)(q12)nk	1.77 Mb	*TCF2*

del(17(q21.31) syndrome

del(17)(q21.31)de novo	0.6 Mb	Sharpe et al 2007
del(17)(q21.31)nk	0.6 Mb	Sharpe et al 2007



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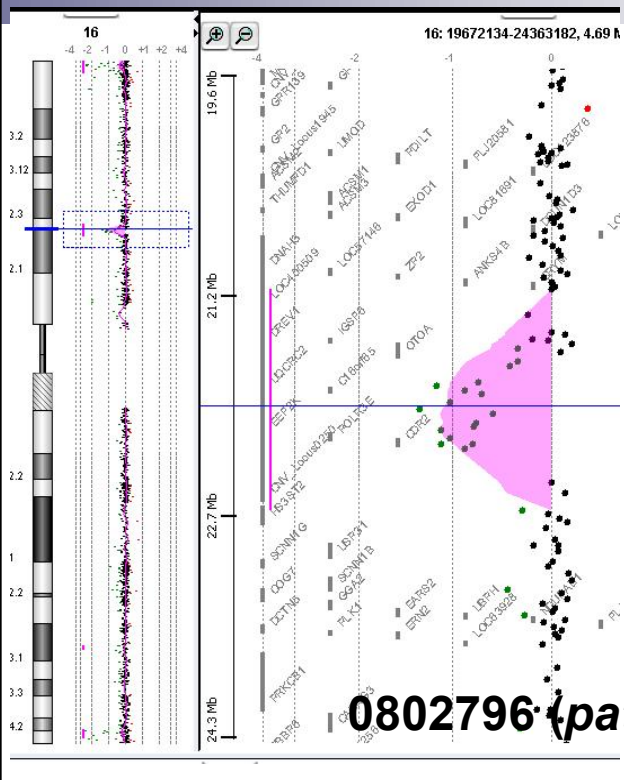

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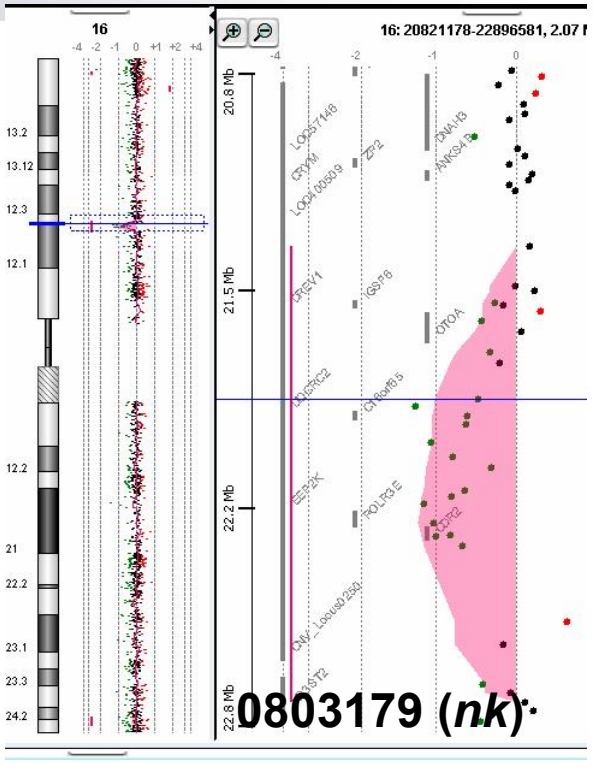

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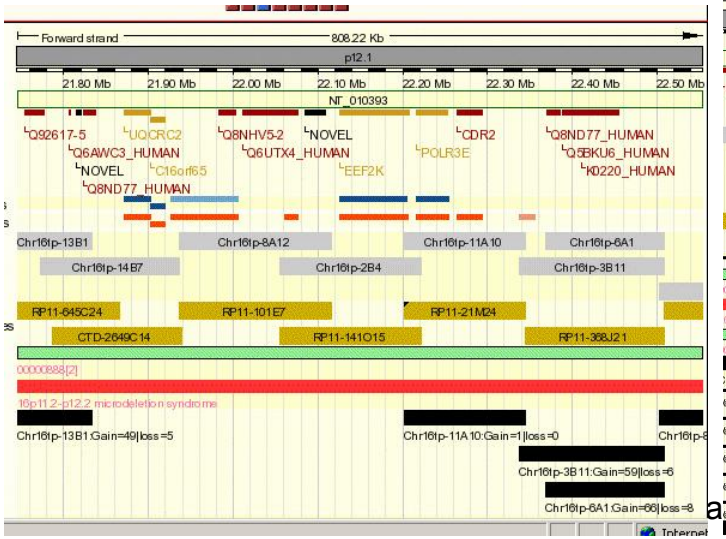
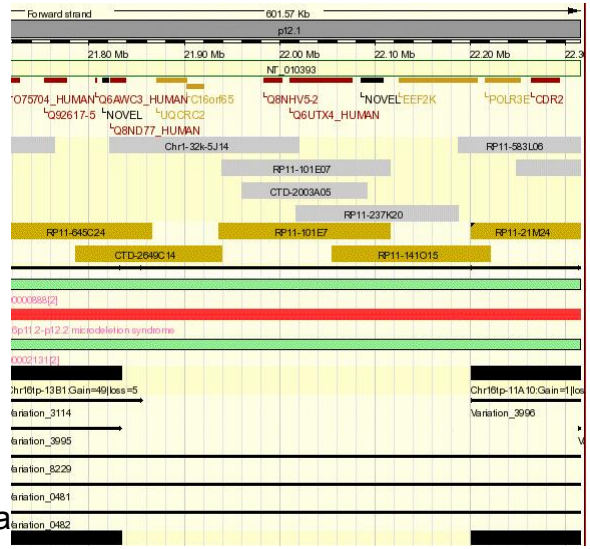
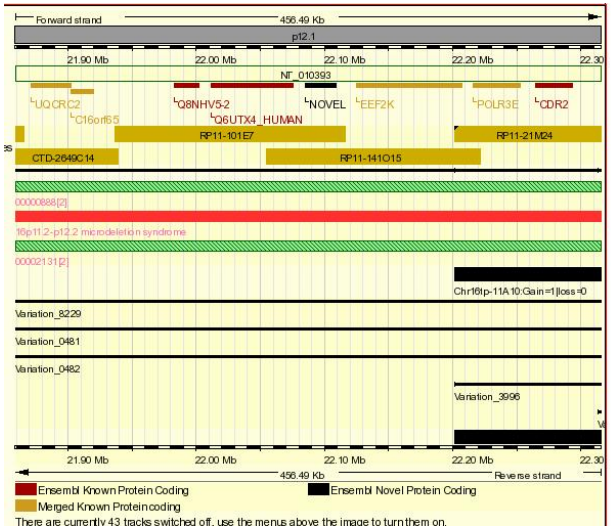






DGV June 2008

Most are gains but some losses

CYTOGENETIC CNV DATABASE

- **IS IT A GAIN OR A LOSS?**
- **PARENTAL ORIGIN**
- **SIZE**
- **FREQUENCY IN DIFFERENT POPULATIONS**

ATLANTA MEETING JUNE 08

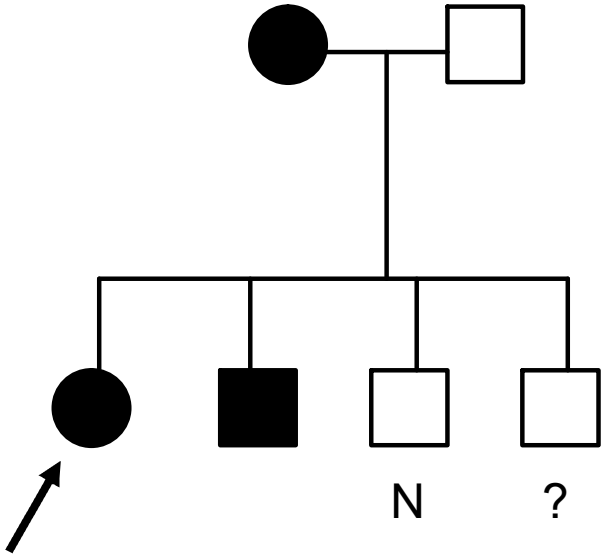
- Defined minimum resolution for a diagnostic array-cgh as 44k oligo

- Asked Decipher and/or other genomic browsers (UCSC/NCBI/DGV) to host "cytogenetic" DGV database for worldwide access – discussions in progress

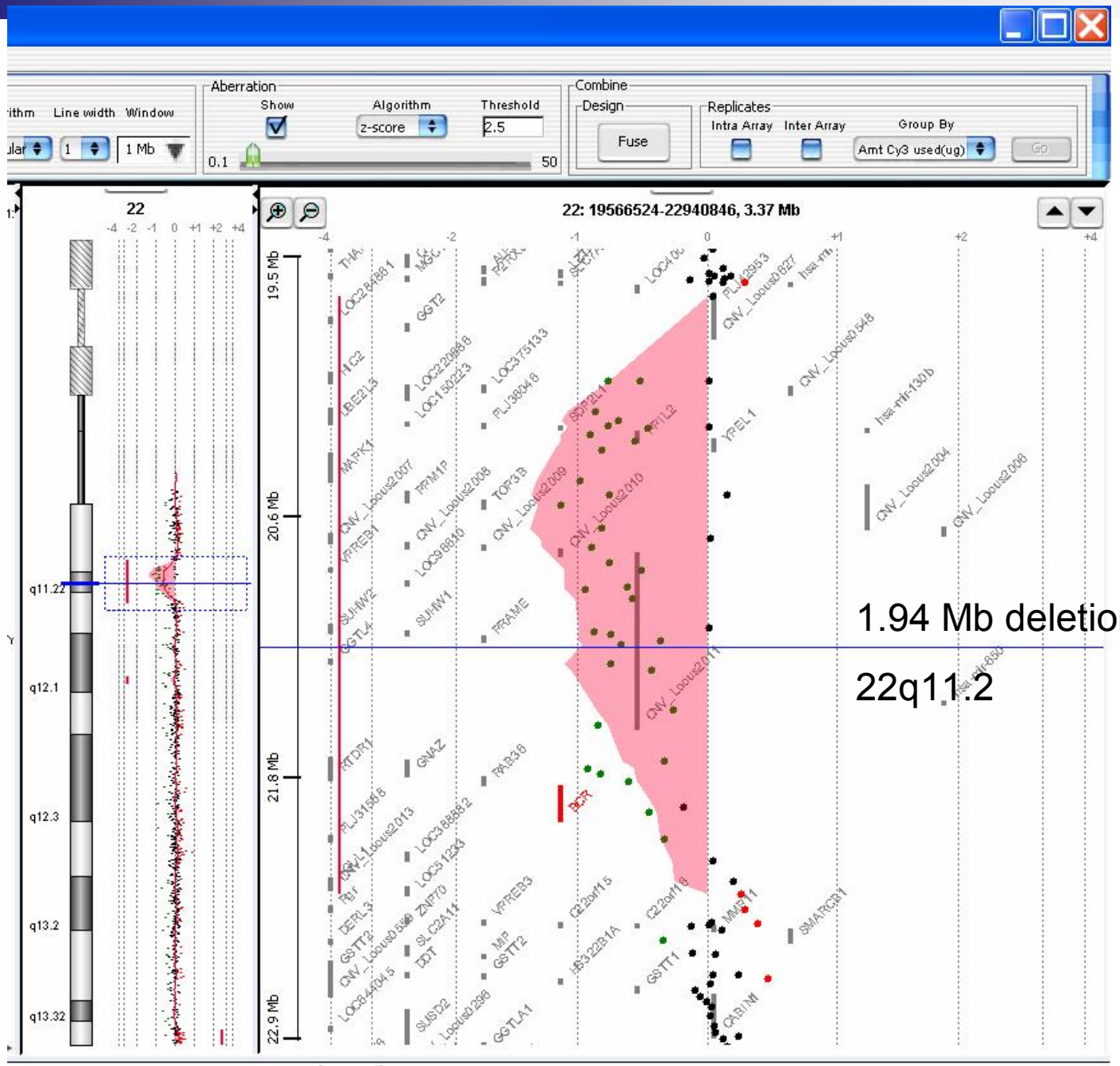
Karyotyping v array-cgh in the DD/MR/CA cohort

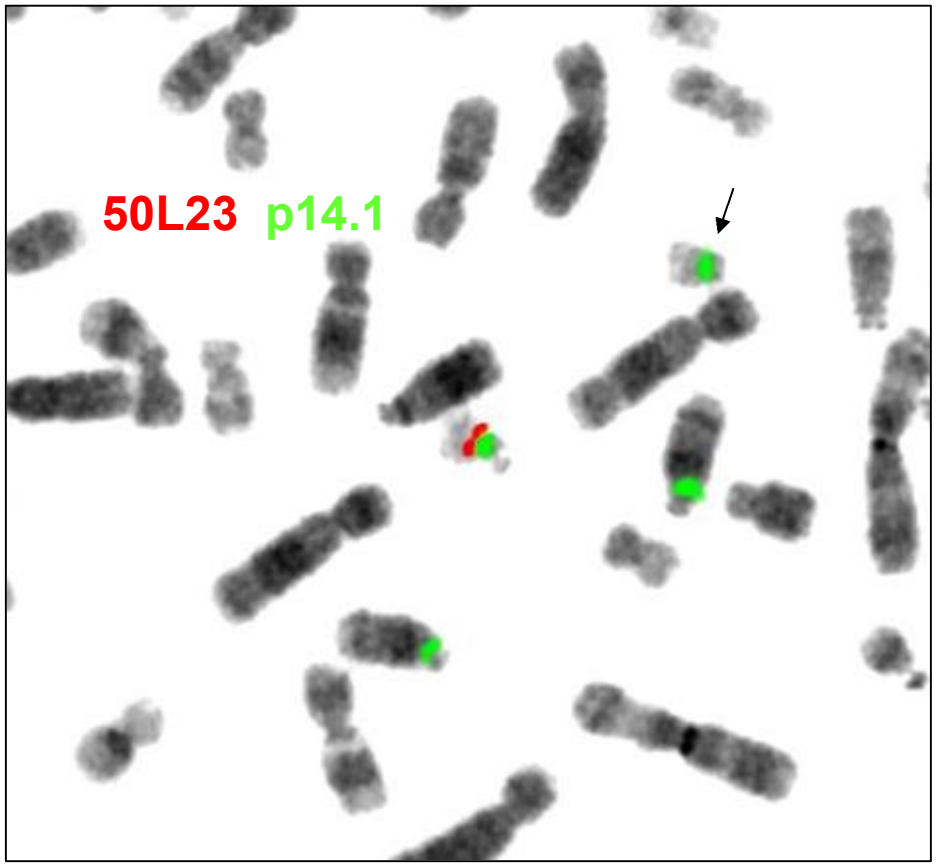
- A thought to leave you with…….

Pedigree



Karyotype January 2008: 46,XX (Q7)

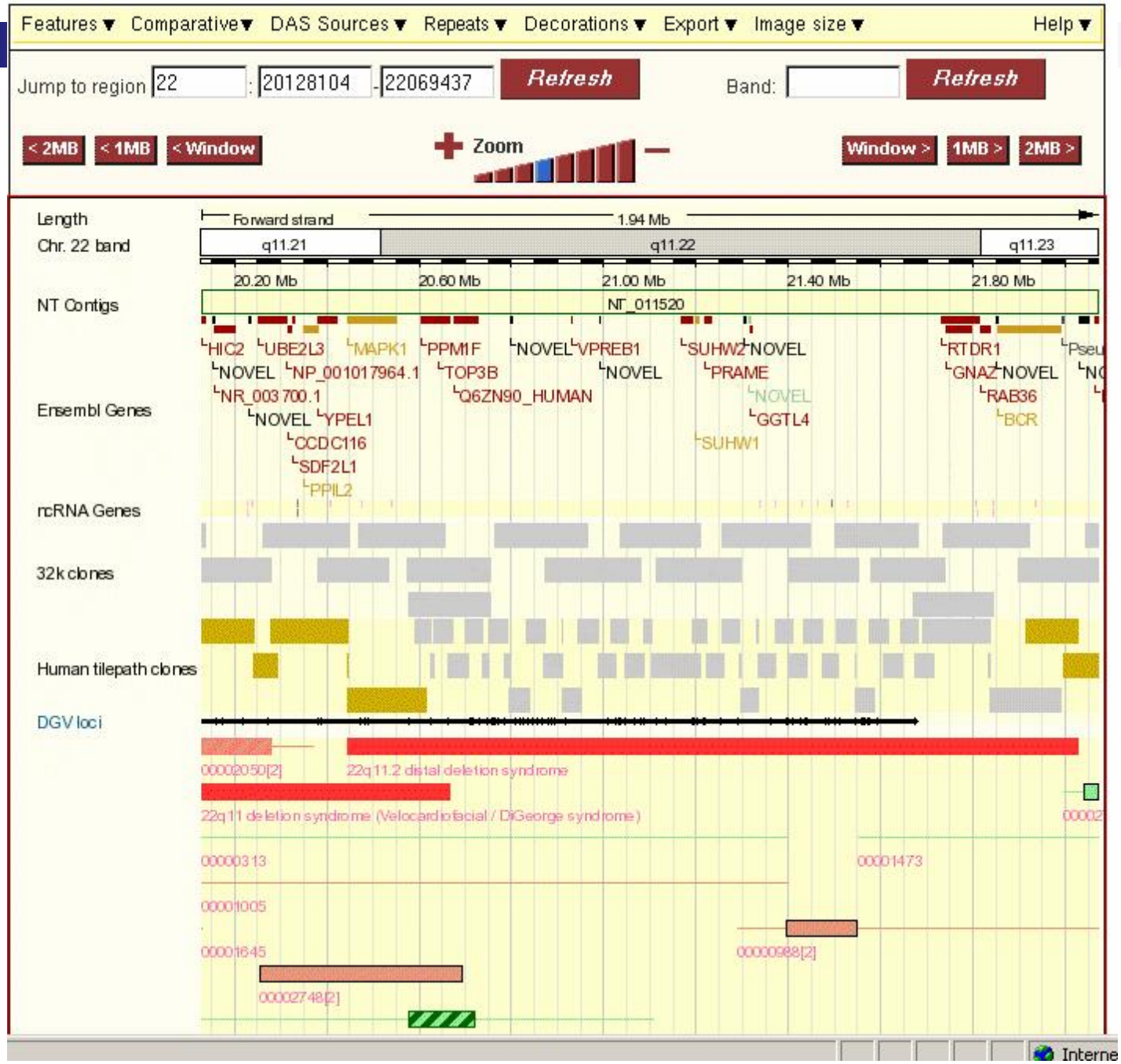

Aberration
Show
Algorithm
z-score
Threshold
2.5
Combine
Design
Fuse
Replicates
Intra Array
Inter Array
Group By
Amt Cy3 used(ug)
Line width
Window
1 Mb
22
22: 19566524-22940846, 3.37 Mb
19.5 Mb
20.6 Mb
21.8 Mb
22.9 Mb
q11.22
q12.1
q12.3
q13.2
q13.32
1.94 Mb deletion
22q11.2



22q11.2 Distal Deletion: A Recurrent Genomic Disorder Distinct from DiGeorge Syndrome and Velocardiofacial Syndrome

Shay Ben-Shachar et al

The American Journal of Human Genetics 82, 214–221, January 2008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q11.21
q11.22
q11.23
20.20 Mb
20.60 Mb
21.00 Mb
21.40 Mb
21.80 Mb
NT Contigs
NT_011520
Ensembl Genes
HIC2 UBE2L3 MAPK1 PPM1F NOVEL VPREB1 SUHW2 NOVEL RTDR1
NOVEL NP_001017964.1 TOP3B NOVEL PRAME GNAZ NOVEL
NR_003700.1 Q6ZN90_HUMAN NOVEL RAB36
NOVEL YPEL1 GGTL4 BCR
CCDC116 SUHW1
SDF2L1
PPIL2
rcRNA Genes
32k clones
Human tilepath clones
DGV loci
00002050[2]
22q11.2 distal deletion syndrome
22q11 deletion syndrome (Velocardiofacial / DiGeorge syndrome)
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00001473
00001005
00001645
00000988[2]
00002748[2]

Recurrence risk ~ 50%

- **Array-cgh currently detecting ~10-15% enrichment of *de novo* abnormalities not detectable by conventional karyotyping**

- **Will soon be actionable if an array had not been performed and a second affected child is born?**

And finally……

- The proven utility of array-cgh in the detection of copy number changes in “balanced” chromosome re-arrangements

De novo CCRs and translocations
Array detected abnormalities (+ abnormal phenotype)

	CCR		
Study	**Normal**	**Deletions**	**Total**
DeGregori	2	16	18
Present	1	6	7
Total	3	22 (88%)	25

	ABSCR		
Study	**Normal**	**Deletions**	**Total**
DeGregori	16	11	27
Baptista	12	4	16
Gribble	4	6	10
Total	32	21 (39%)	53

END OF PRESENTATION

- **THANK YOU FORYOUR ATTENTION AND SPECIAL ACKNOWLEDGEMENTS TO:**

- **SARAH BEAL**
- **VIV MALONEY**
- **ANNETTE COCKWELL**
- **NICK CROSS**
- **JOHN BARBER**
- **AGILENT INTERNATIONAL – ESPECIALLY UK**