Array based targeting for massively parallel sequencing

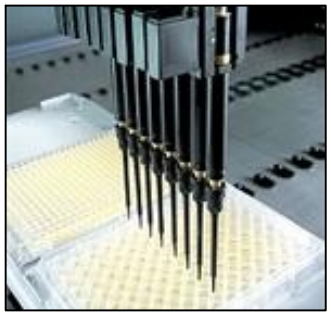
Patrick Tarpey
Team 78

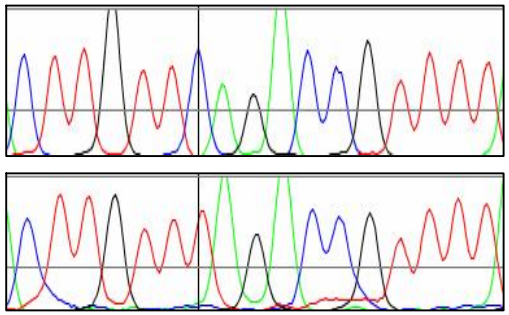
High Throughput Sequencing in the CGP

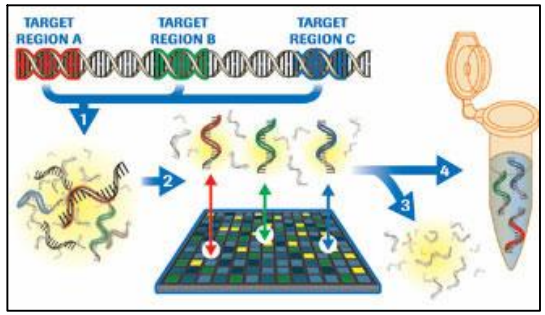
sample preparation

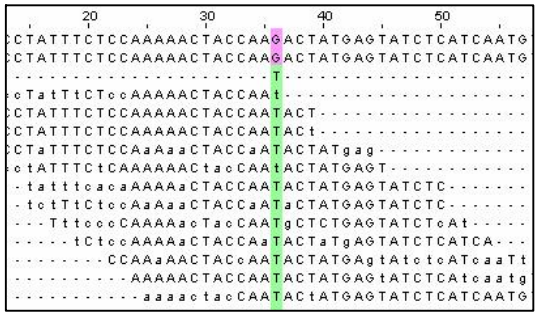
sequencing

data analysis









High Throughput Sequencing in the CGP

1 disease (XLMR)
200 samples
720 X chrom genes

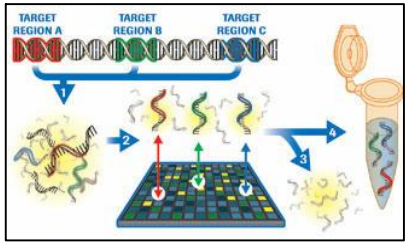
1858 mutations

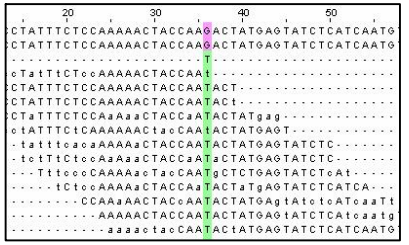
13 diseases
30 samples
920 genes

?

Overview

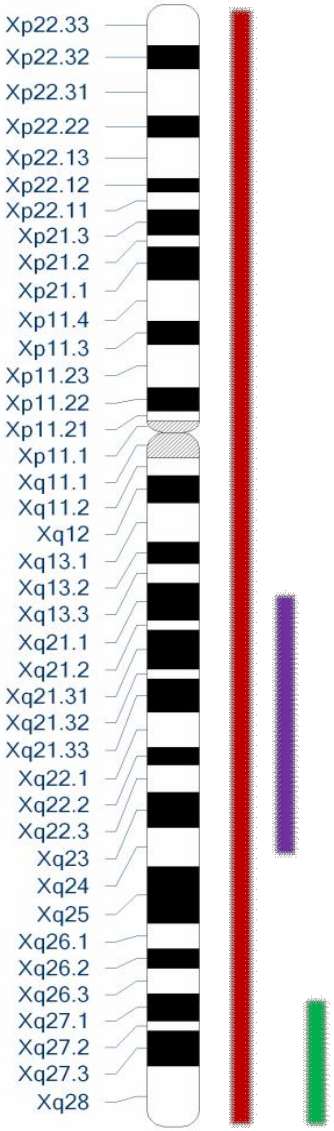
DNA samples





Data

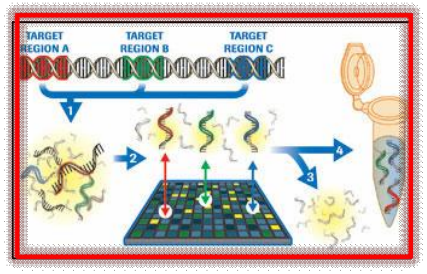
X-linked diseases under investigation

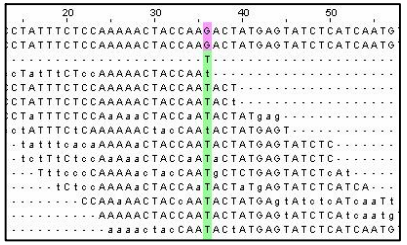


Aicardi	Arena	Lenz microphthalmia
•Affects females (two reported males both XXY) •X-linked dominant (male lethal) •No refined linkage interval •Agenesis of the corpus callosum •Infantile spasms •Lesions “lacunae” of the retina	•Affects males •X-linked recessive •Linked to Xq22-25 •Mental retardation •Spastic paraplegia	•Affects males •X-linked recessive •Linked to Xq27-28 •Microphthalmia •Additional malformations •Ears •Teeth •Fingers •Skeleton •Genitourinary system

Exon Capture on Nimblegen Arrays

DNA samples

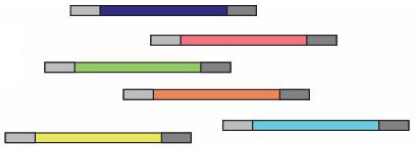




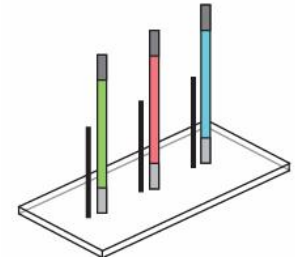
Data

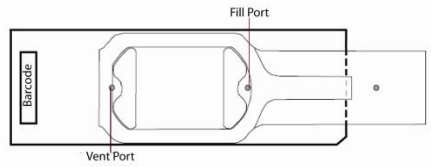
Exon Capture on Nimblegen Arrays

- Shear genomic DNA (~500bp)



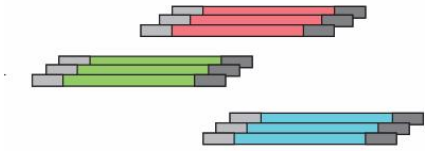
- End repair
- Ligate Solexa adaptors
- Size select (~200bp on gel)





8136 exons
1.5 Mb cds

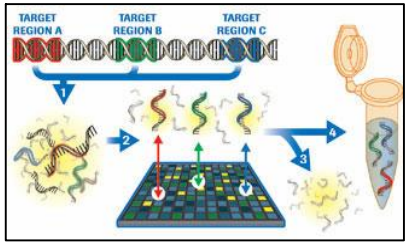
- Hybridise ~10ug adapted DNA
- Wash
- Elute

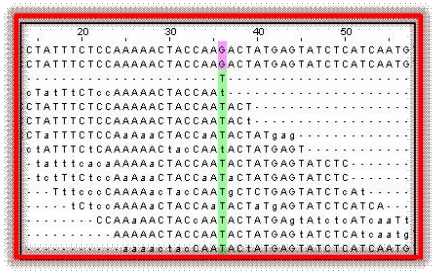


- PCR using Solexa primers
- Sequence

Data Analysis

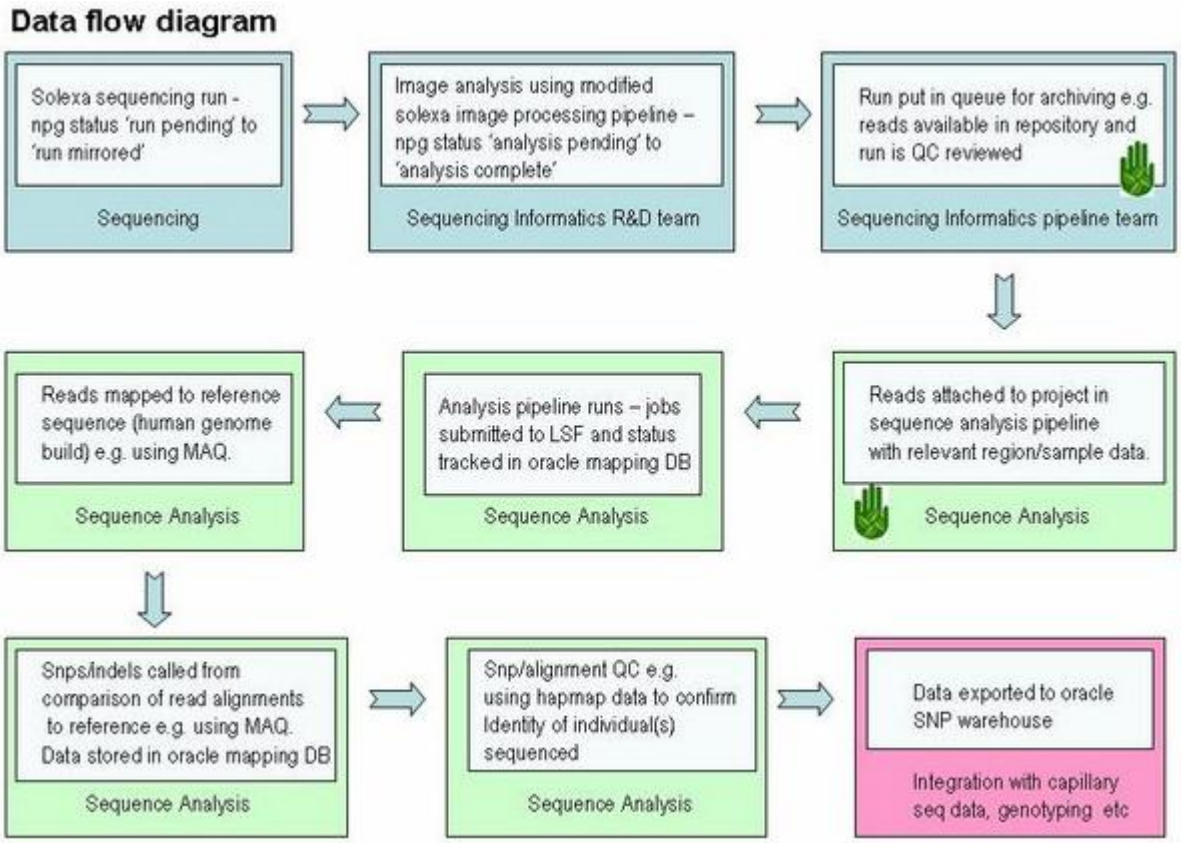
DNA samples



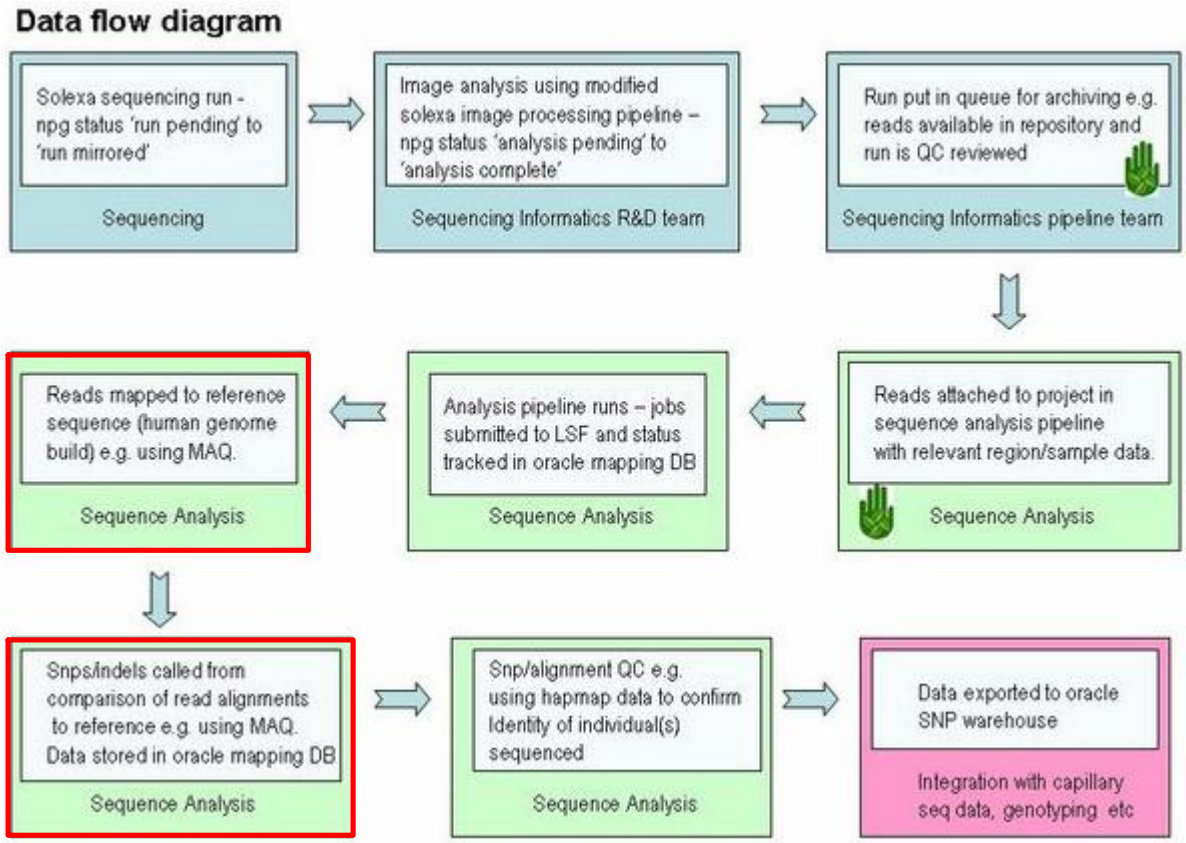


Data

Data Analysis



Data Analysis



Data Analysis

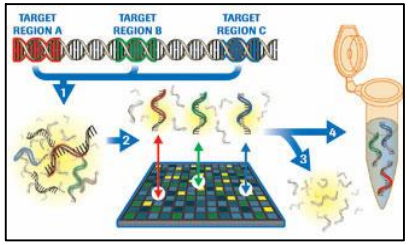
- **Annotated variant**
  nonsense, insdel, splice, missense, silent
- **Read depth**
  Each allele (zygosity)
- **Quality score**
  Each allele
- **Known/unknown (SNPdb)**
- **Flanking sequence window (blat)**

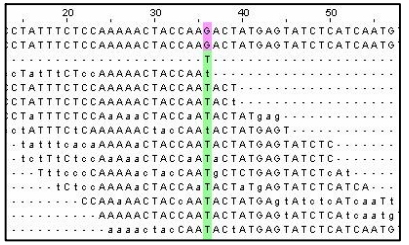
- **Variant ID > Webpage > Pileup Images**

- **Coverage & depth of reads in each gene in each sample**
  - **(%bases >10X depth)**

Results

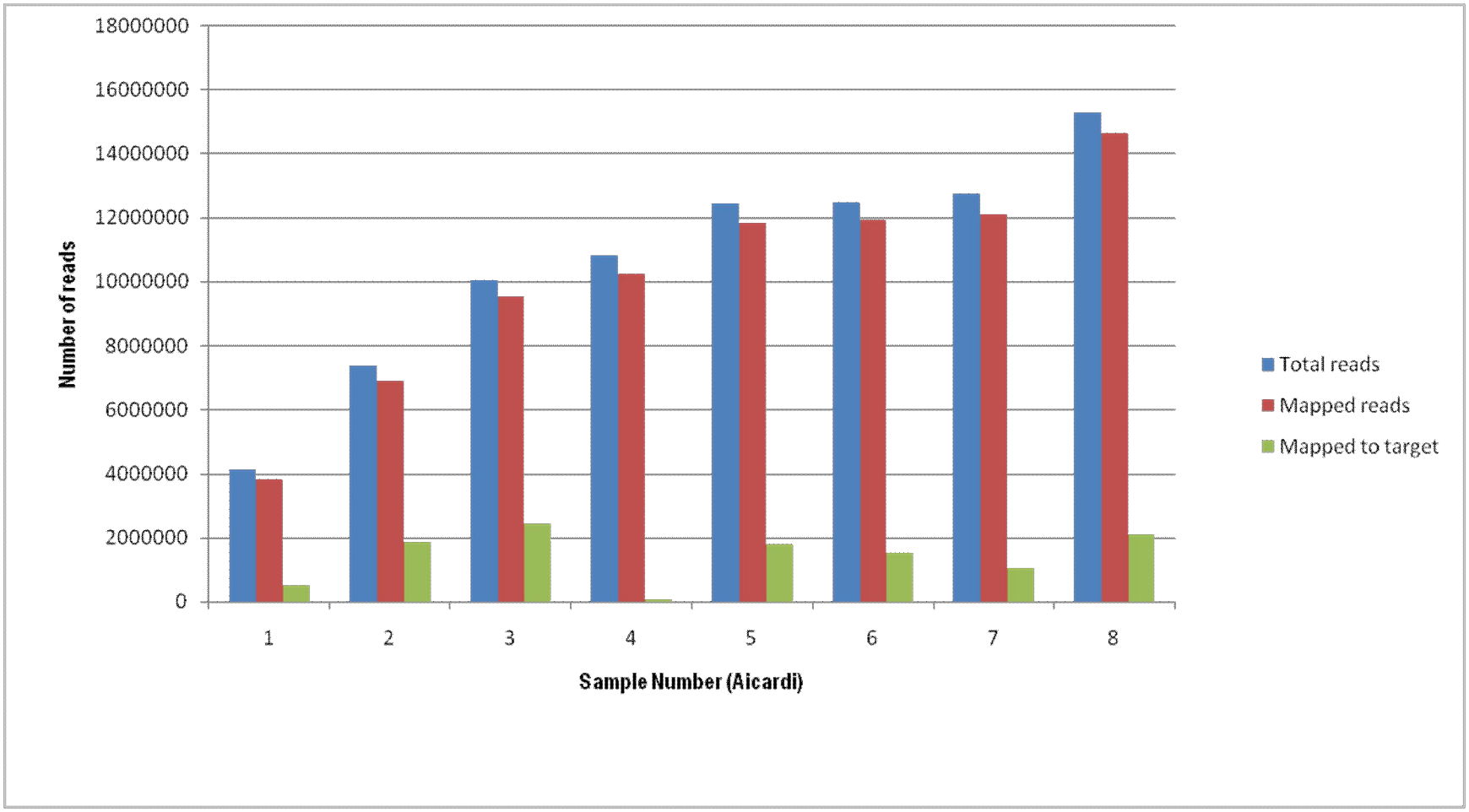
DNA samples



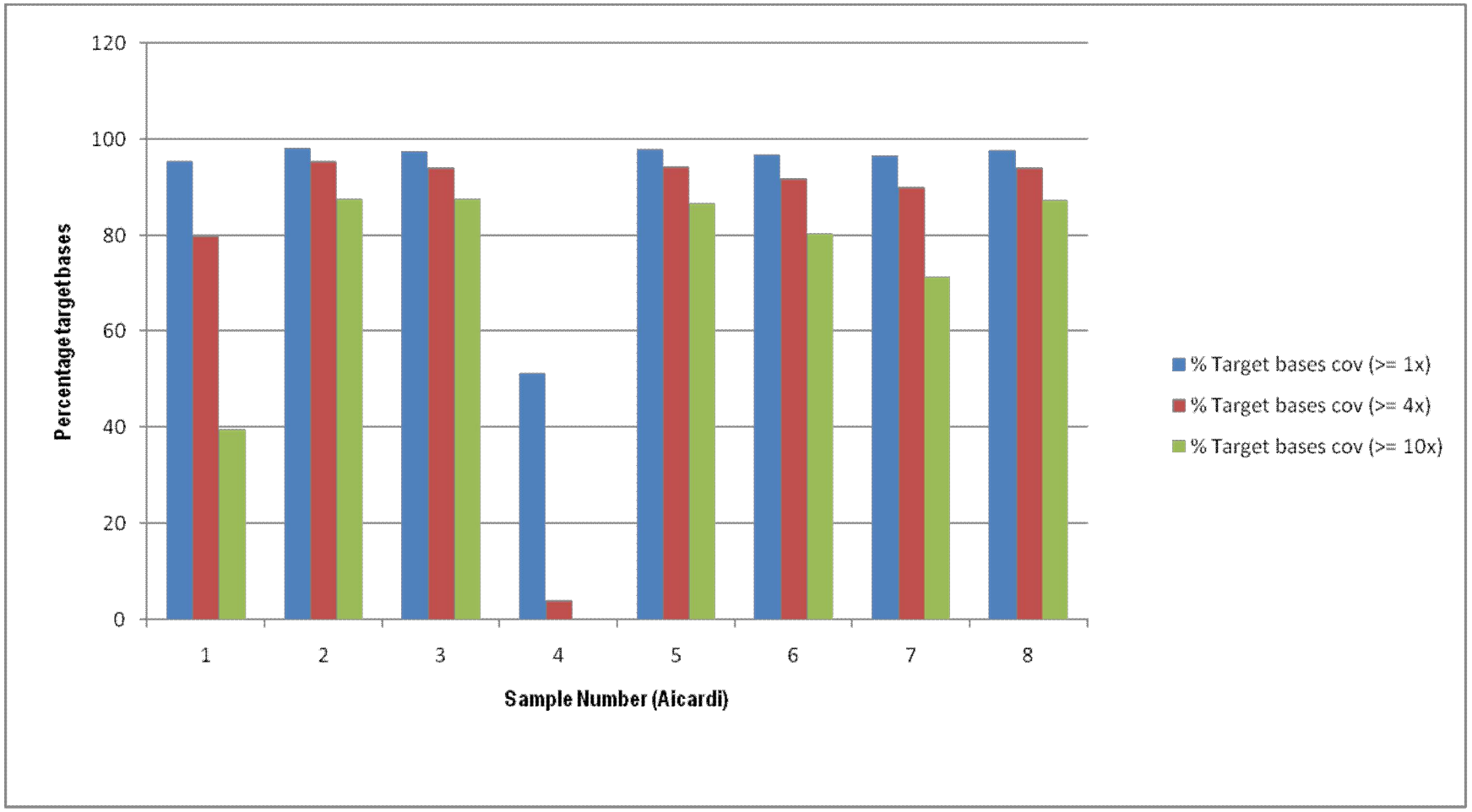


Data

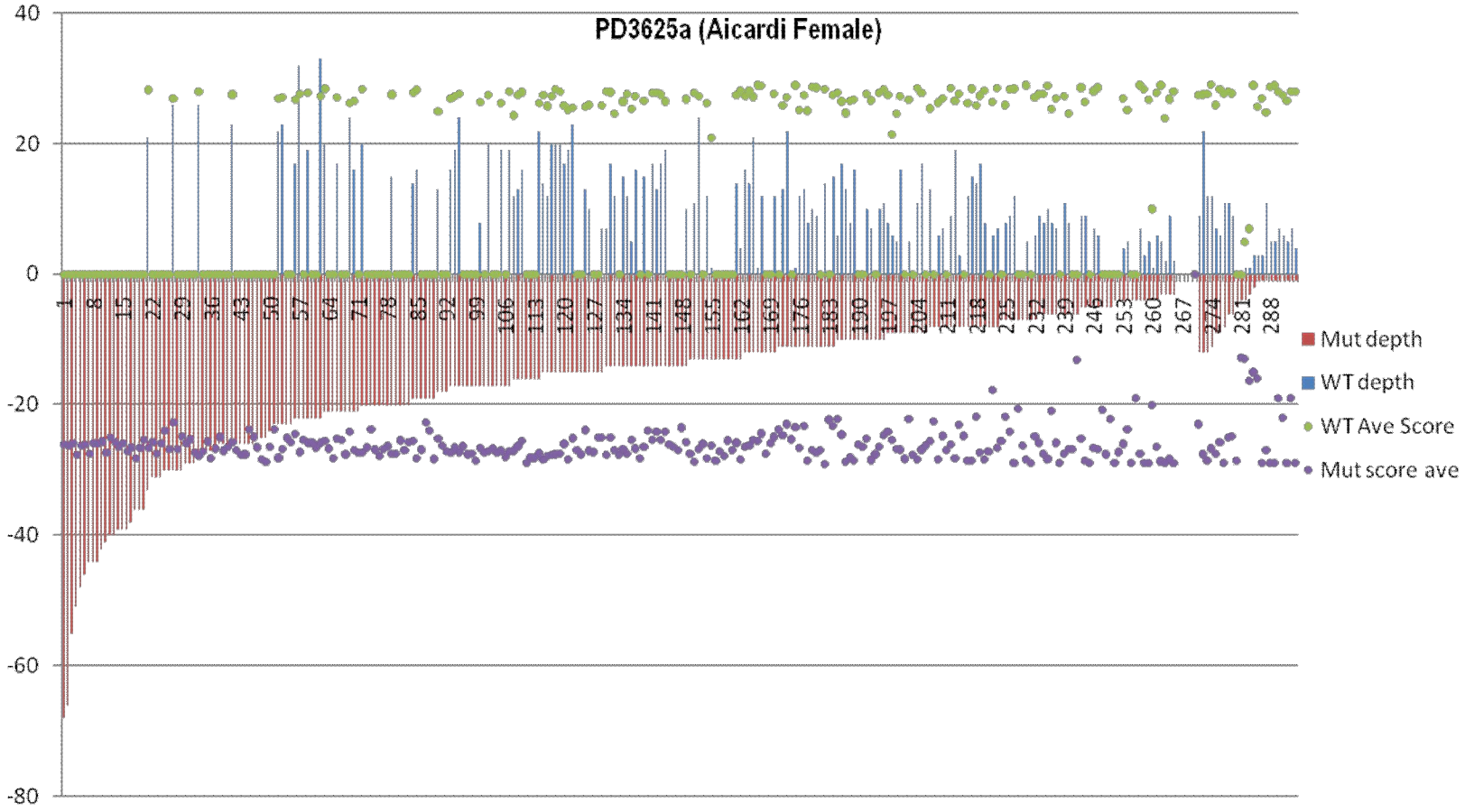
Exonic Capture Performance



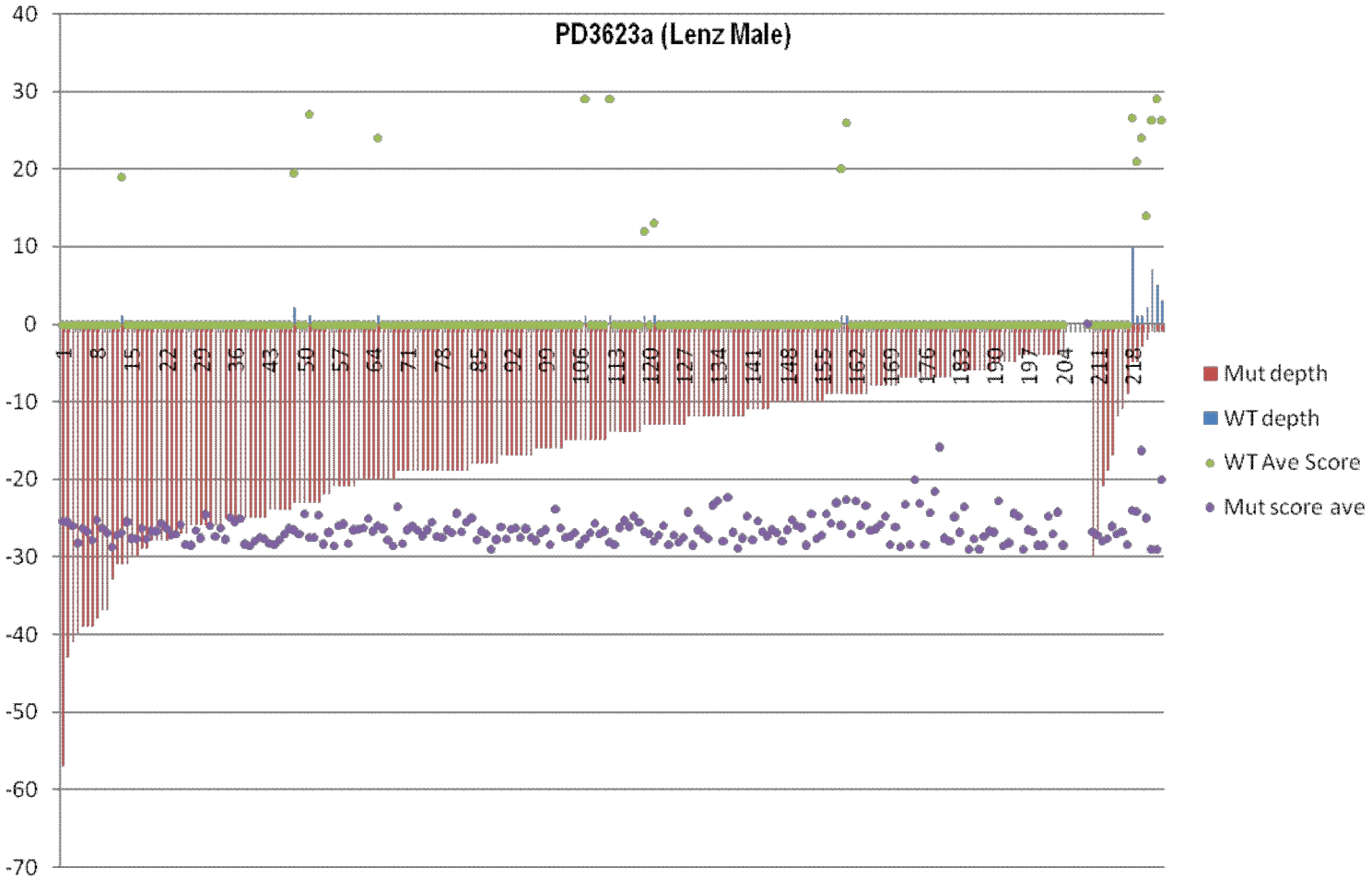
Exonic Capture Performance



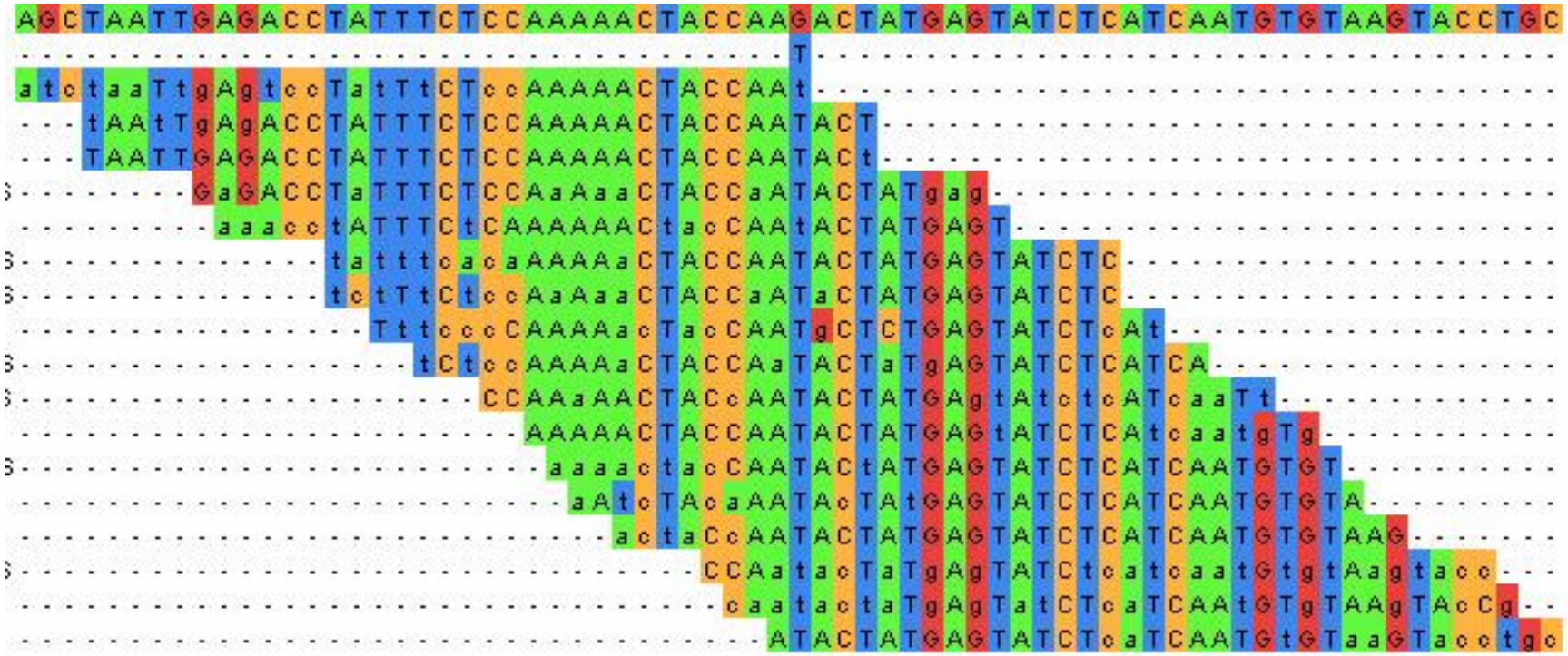
Substitutions (overview)



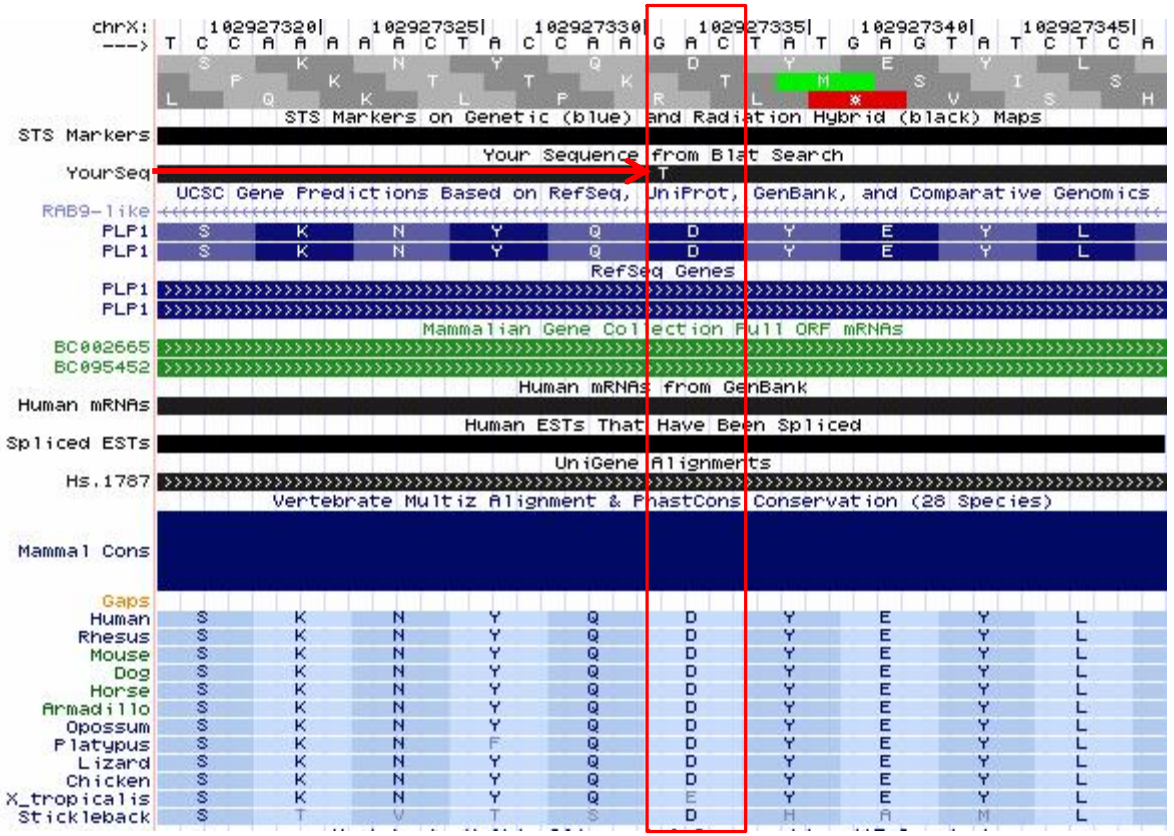
Substitutions (overview)



Arena (Missense)



Arena (Missense)



Arena (Missense)

#312920 GeneTests, Links

SPASTIC PARAPLEGIA 2, X-LINKED; SPG2

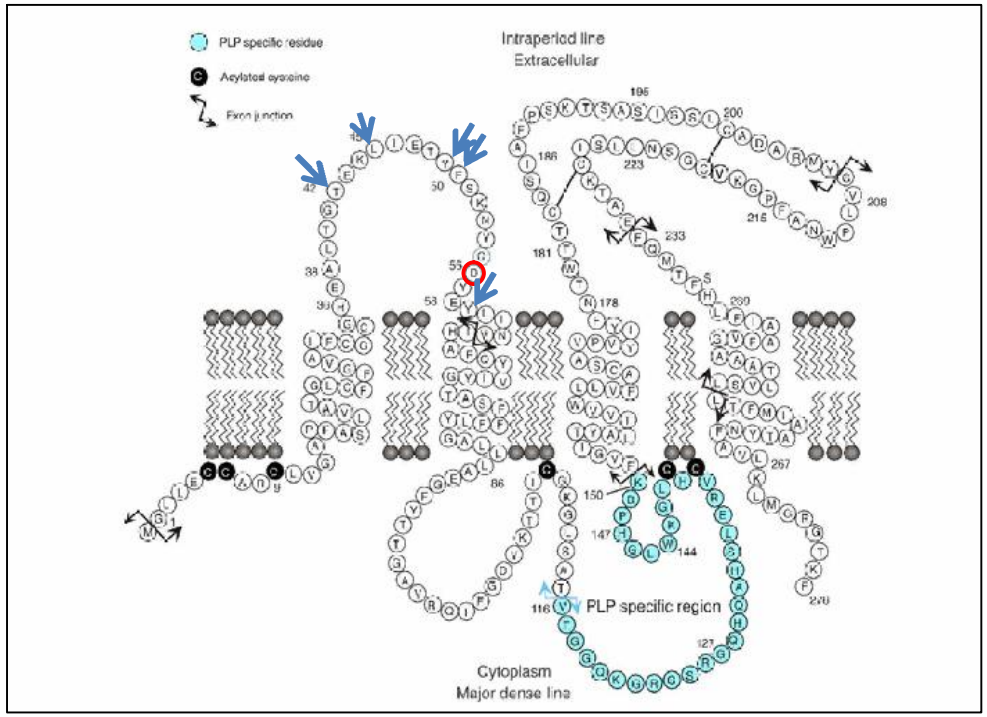
Alternative titles; symbols

SPPX2

Gene map locus Xq22

TEXT

A number sign (#) is used with this entry because spastic paraplegia-2 is caused by mutation in the myelin proteolipid protein gene (PLP1; 300401) and is therefore allelic to Pelizaeus-Merzbacher disease (PMD; 312080).



Summary

Protocol

- **Enrichment variable, however >80% target bases >10X coverage in most samples.**
- **Protocol under continued development, other options available soon (Agilent).**

Analysis

- **Known substitutions identified with high confidence.**
- **False positives relatively low, recognisable.**
- **Small insertions/deletions presently have a high false positive call rate.**

Compared to XLMR screen

- **Analysis easier (with bioinformatics support!)**
- **All data available simultaneously.**
- **Developmental delay good candidate disease to utilise this technology (heterogeneous).**

Team 78: Cancer Genome Project, XLMR Project

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Variation Informatics

Carol Scott

Contribution of Samples

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