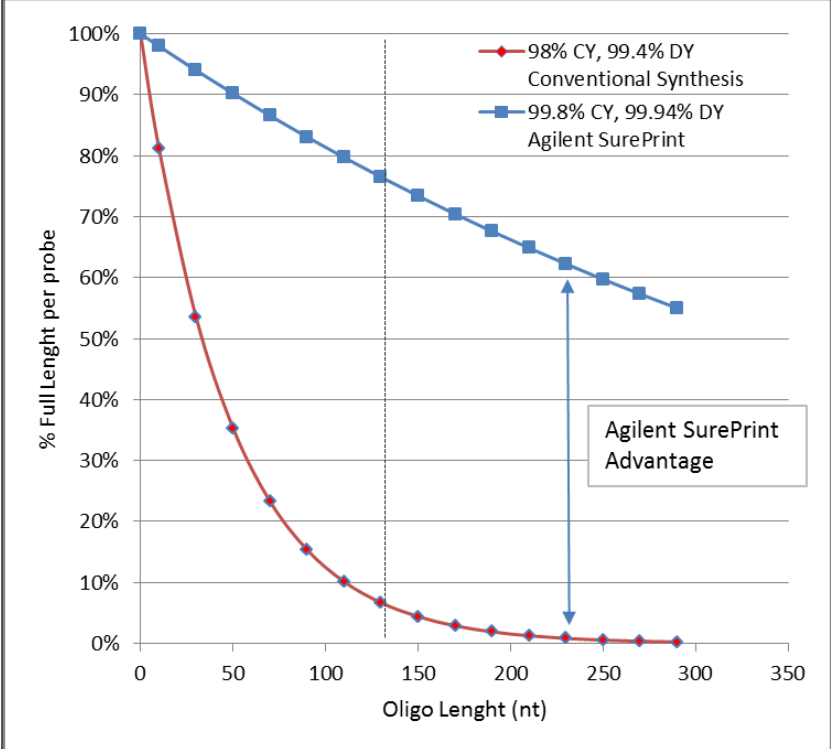


Benefits of Agilent Exomes



Agilent baits are of high quality as shown by the errors per kb

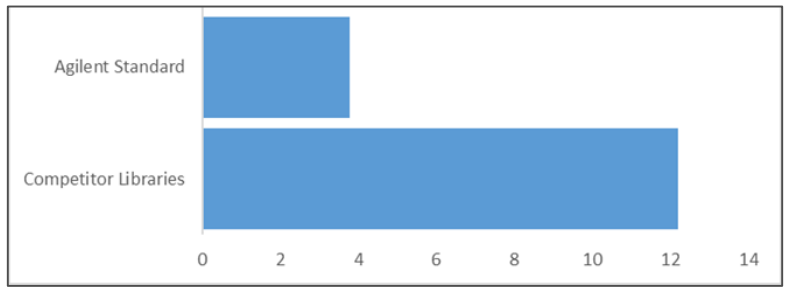
Oligo Synthesis Fidelity



$$\%FL = (CY * DY)^{nt}$$

%FL= %Full Length
 CY=Synthesis Cycle Yield
 DY=Depurination Cycle Yield

Errors per kb



- Agilent baits have ~3x fewer errors per kb and are full length compared to conventional synthesis
- Every Agilent exome lot is going through Quality-Control (QC) to check for coverage, % on-target, dupe rates etc.

No need to QC individual oligos as our bait production is robust and accurate

Agilent exomes provide comprehensive coverage of relevant databases

Vendor ID

RefSeq (34 Mb)	98%
CCDS (32 Mb)	99%
Ensembl (35 Mb)	93%
GENCODE (35 Mb)	96%
Vega (26 Mb)	95%

V6/V6 + COSMIC

RefSeq	99%	99%
CCDS	99%	99%
GENCODE	99%	99%
HGMD cds	99%	99%
OMIM cds	99%	99%
COSMIC		96%

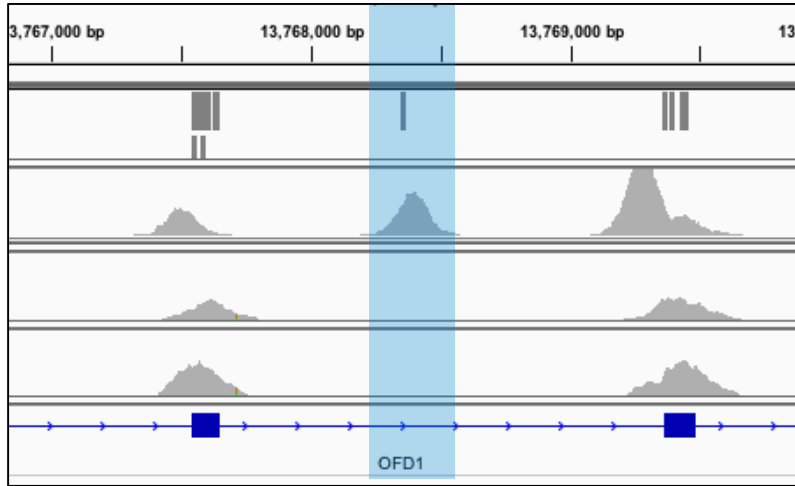
CREv2

Database*	Pe
CCDS	99.77%
GENCODE-v17	99.69%
UCSC Known Genes	99.60%
RefSeq	99.63%
Vega	99.66%
HGMD coding regions	99.86%
OMIM coding regions	99.76%
ClinVar	97.63%
COSMIC	99.76%

With marginally more sequencing, Agilent V6 and CREv2 exomes provide better coverage of critical databases

Agilent exomes are more comprehensive

Agilent exomes target more disease-relevant content



Hum Mol Genet. 2012 Aug 15;21(16):3647-54. doi: 10.1093/hmg/dds194. Epub 2012 May 22.

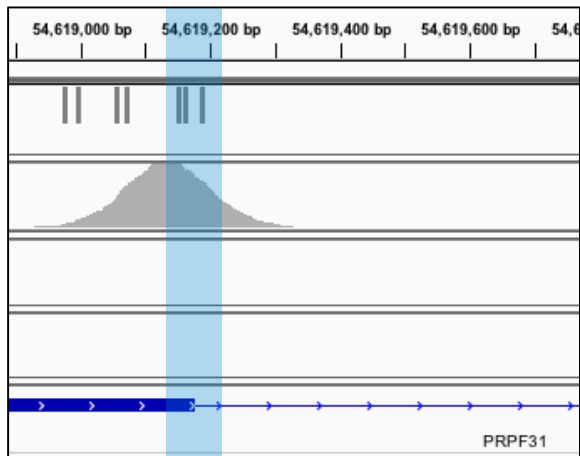
Deep intronic mutation in OFD1, identified by targeted genomic next-generation sequencing, causes a severe form of X-linked retinitis pigmentosa (RP23).

Yabb TR¹, Parfitt DA, Gardner JC, Martinez A, Bevilacqua D, Davidson AE, Zito J, Thiseleton DL, Bessa JH, Aegeri M, Schwarz H, Januga N, Michaelides M, Chaetham ME, Gorin MB, Hardcastle AJ

Author information

Abstract

X-linked retinitis pigmentosa (XLRP) is genetically heterogeneous with two causative genes identified, RPGR and RP2. We previously mapped a locus for a severe form of XLRP, RP23, to a 10.71 Mb interval on Xp22.31-22.13 containing 62 genes. Candidate gene screening failed to identify a causative mutation, so we adopted targeted genomic next-generation sequencing of the disease interval to determine the molecular cause of RP23. No coding variants or



Hum Mutat. 2006 Jul;27(7):644-53.

Variation in retinitis pigmentosa-11 (PRPF31 or RP11) gene expression between symptomatic and asymptomatic patients with dominant RP11 mutations.

Rivolta C¹, McGee TL, Rio Frio T, Jensen RV, Berson EL, Dryja TP

Author information

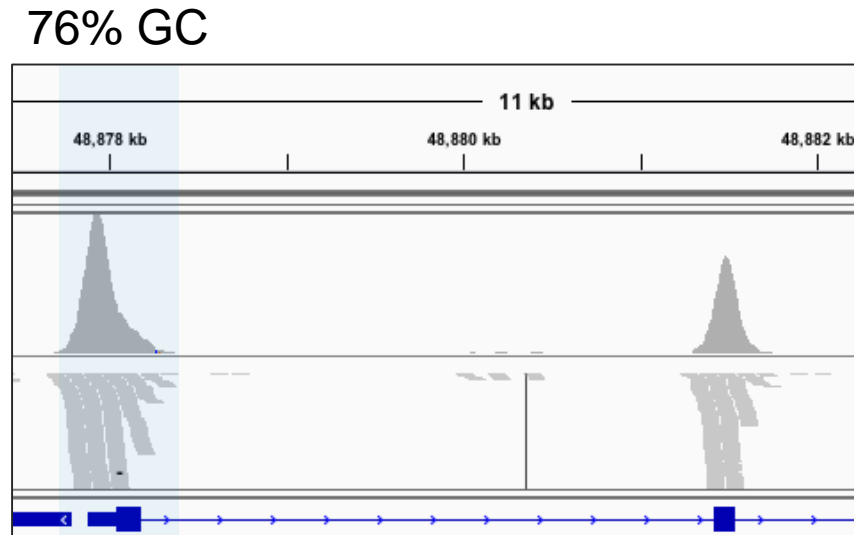
Abstract

Dominant mutations in the mRNA splicing factor gene PRPF31 (RP11) cause retinitis pigmentosa with reduced penetrance. We studied the expression of RP11 in lymphoblast cell lines from 10 patients, including three who were clinically asymptomatic, with six distinct RP11 mutations. Five of the six mutations were characterized and all five created premature nonsense codons or eliminated the normal initiation codon. Semiquantitative RT-PCR indicated

Agilent exomes target more disease-relevant content

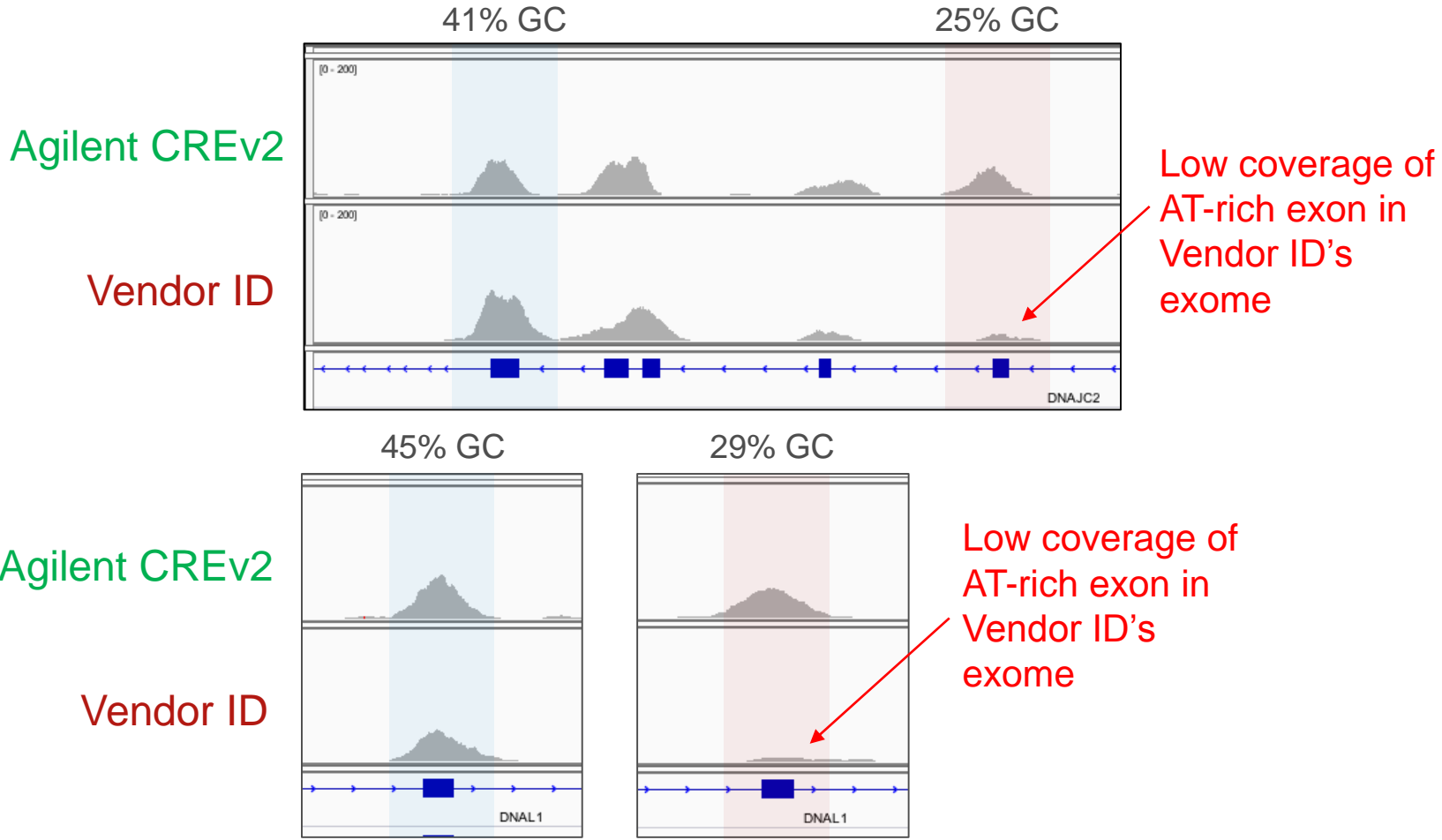
Agilent exomes efficiently capture GC-rich regions

Agilent CREv2



- Vendor ID claims that only Vendor ID can get coverage of high GC exons, but they are only showing the data from older Agilent exome.
- Newer Agilent exomes provide robust coverage of high-GC regions

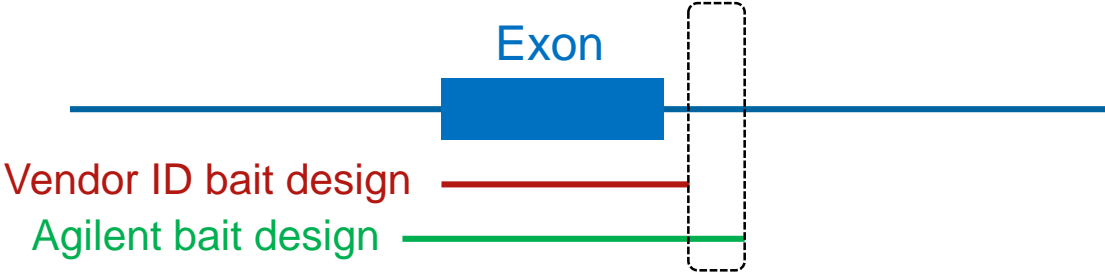
Agilent exomes efficiently capture AT-rich regions



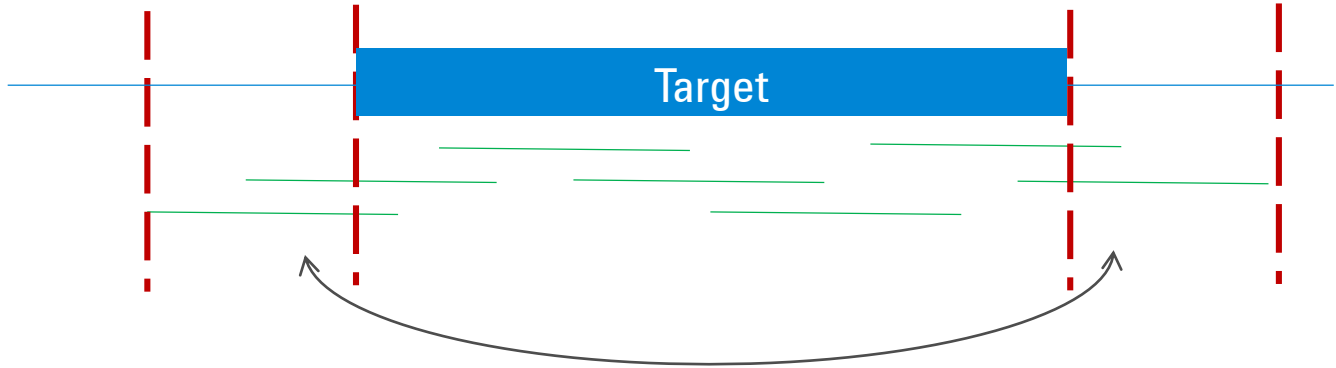
All exomes sequenced to the same average sequencing depth

Why do Agilent exomes have less GC bias?

Difference between Vendor ID and Agilent baits



How do we do our bait design?



Find best region to place the bait for every given target based on: 1. Mappability; 2. GC content

This adds marginally more sequencing footprint but results in less GC bias

Agilent exomes have excellent on-target and duplicate rates

Design	On Target Rate	Duplicate Rate
SureSelect V6	78%	8%
SureSelect CRE V2	76%	10%
Vendor R	62%	17%
Vendor ID	80%	13%

Mean of 8 samples with 100x sequencing; Agilent exomes represent SureSelect XT workflow.

- Vendor ID claims 30% difference in on-target but data shown is from older exomes.
- With new Agilent exomes, the difference is <5%.
- Agilent exomes have better duplicate rates.

High on-target rates and lower duplicate rates ensure maximum use of sequencing data

Agilent exomes provide excellent depth of coverage

Bases covered at	SureSelect V6	SureSelect CREv2	Vendor ID	Vendor R
5x	97%	96%	98%	97%
10x	95%	94%	97%	95%
20x	91%	89%	92%	85%
30x	83%	80%	82%	67%

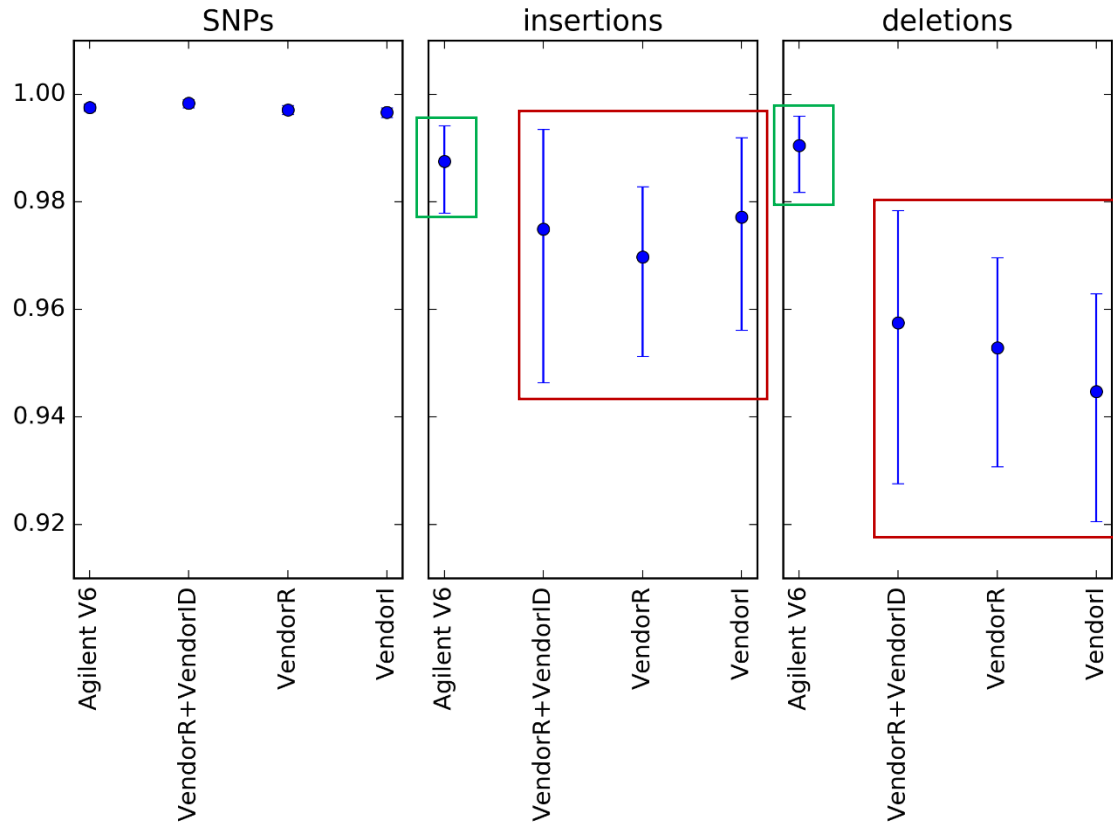
Mean of 8 samples with 100x sequencing; Agilent exomes represent SureSelect XT workflow.

- Vendor ID claims better uniformity. However, older exome data is shown.
- New Agilent exomes provide comparable coverage at 20x and 30x depth of coverage. .

Agilent exomes provide excellent uniformity of coverage throughout the targeted regions

Agilent exomes provide better PPV as indicated by comparative analysis

Positive Predictive Value



- Vendor R library prep Vendor ID exome provides lower indel PPV (higher false positives) than Agilent exomes.
- SNP & indel sensitivity are similar across all exomes

Sensitivity or TruePositive Rate = $TP / (TP + FN)$ and **PPV or Precision** = $TP / (TP + FP)$.
Desired: Lower FN, FP and Higher Sensitivity and PPV

Agilent exomes provide excellent overall solution to target enrichment needs

	Vendor ID	Agilent
Bait Quality	ESI mass spec every probe because they have to.	Our high fidelity DNA printing process obviates the need to QC every oligo. And we robust QC of every lot of exomes ensures consistency.
Sequencing requirements	Needs less sequencing but the trade-off is incomplete disease-associated content and performance across low GC regions	With marginally more sequencing, Agilent exomes provide 1. better content, 2. bait design that helps in extreme high/low GC regions.
On-target and duplicate rates.	Slightly better on-target rates but higher duplicate rates	Excellent on-target and duplicate rates.
Uniformity	Comparable to Agilent exomes.	Excellent coverage and sensitivity/PPV.