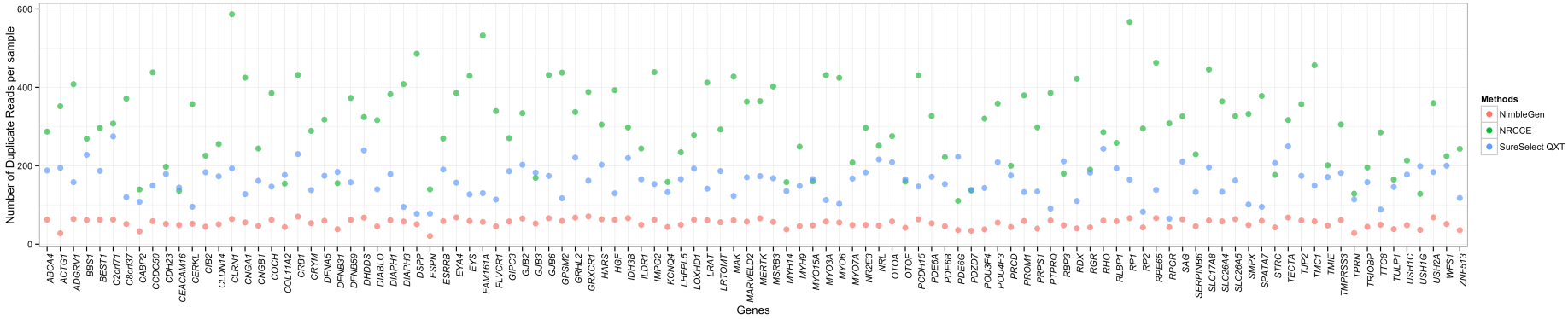
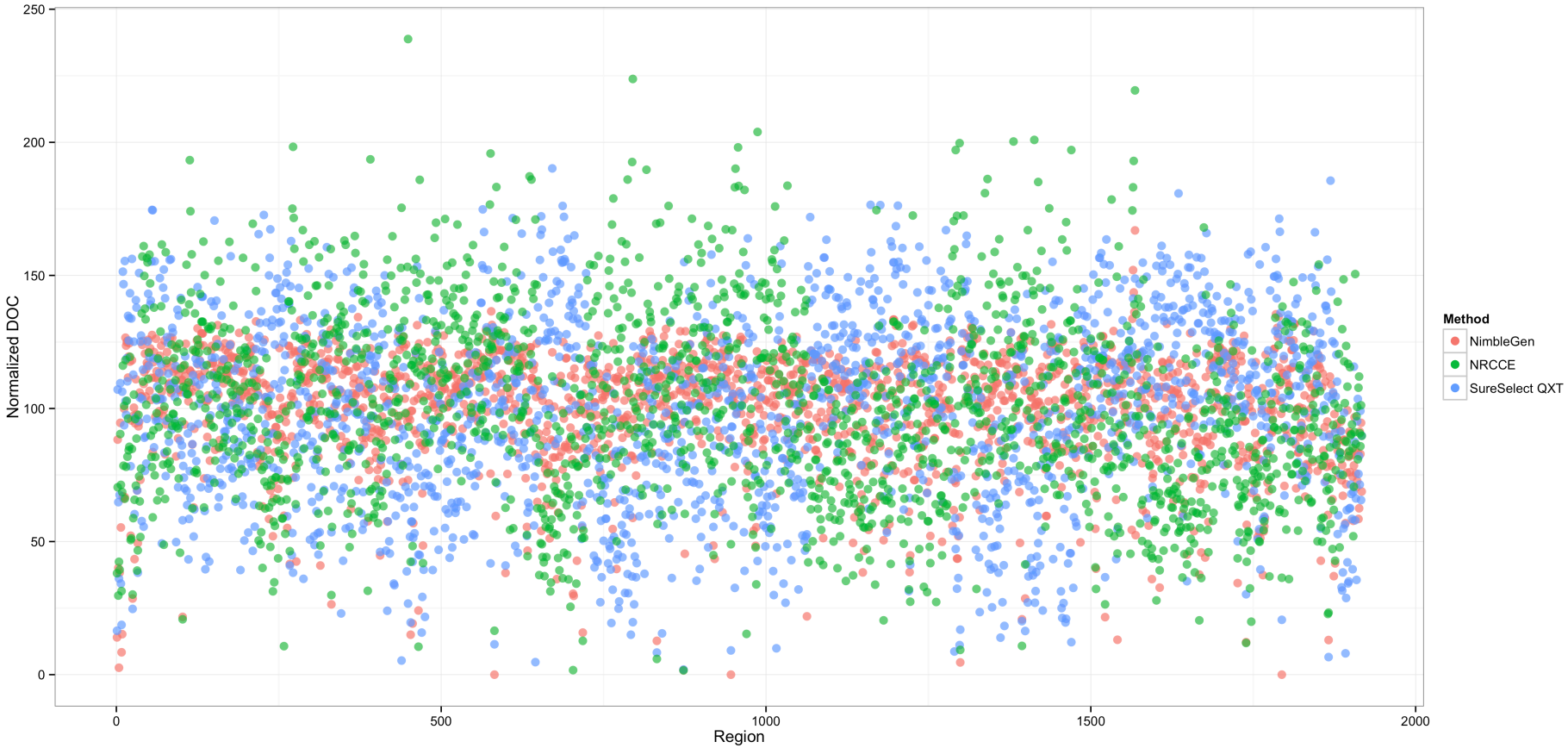
Assessment of the latest NGS enrichment capture methods in clinical context

Gema García-García, David Baux, Valérie Faugère, Mélody Moclyn, Michel Koenig, Mireille Claustres, Anne-Françoise Roux

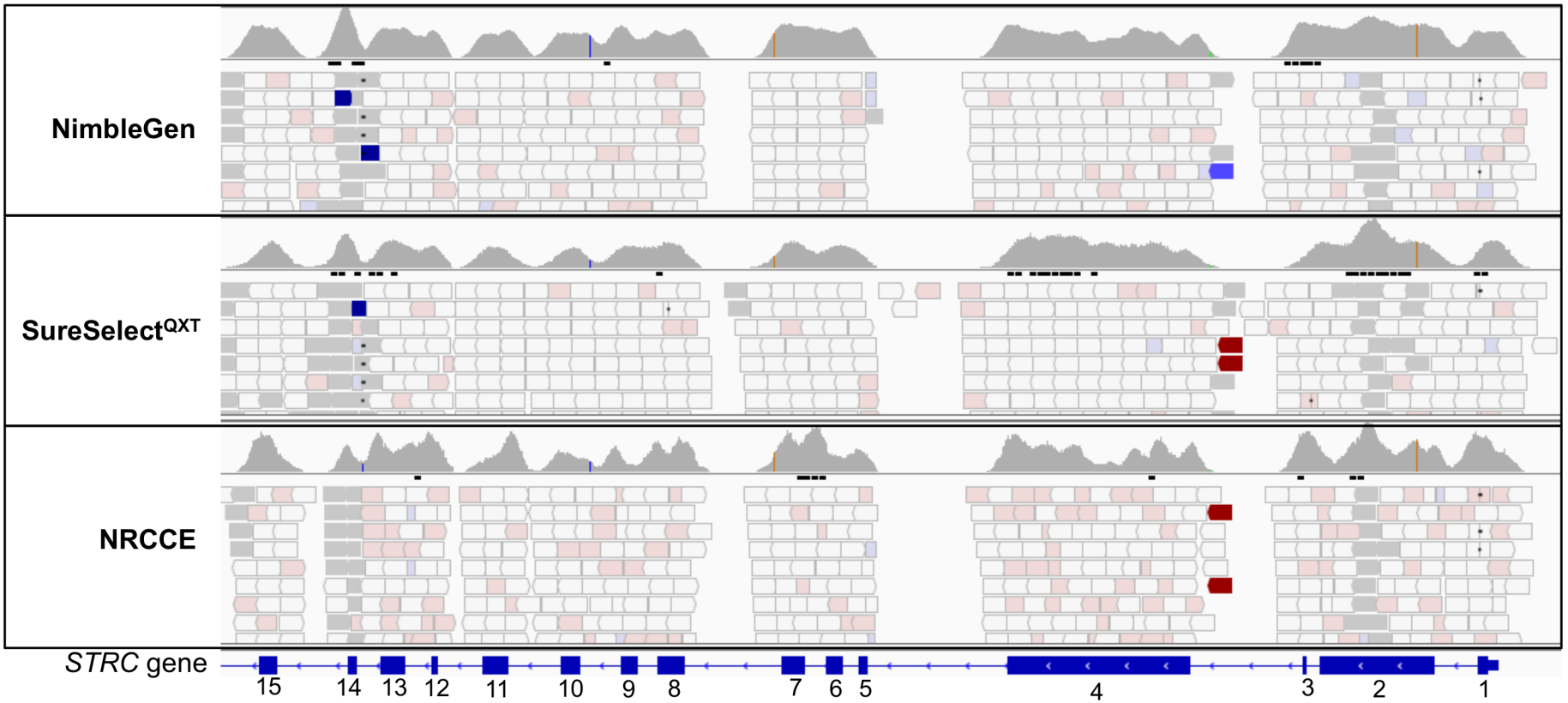
Supplementary Information



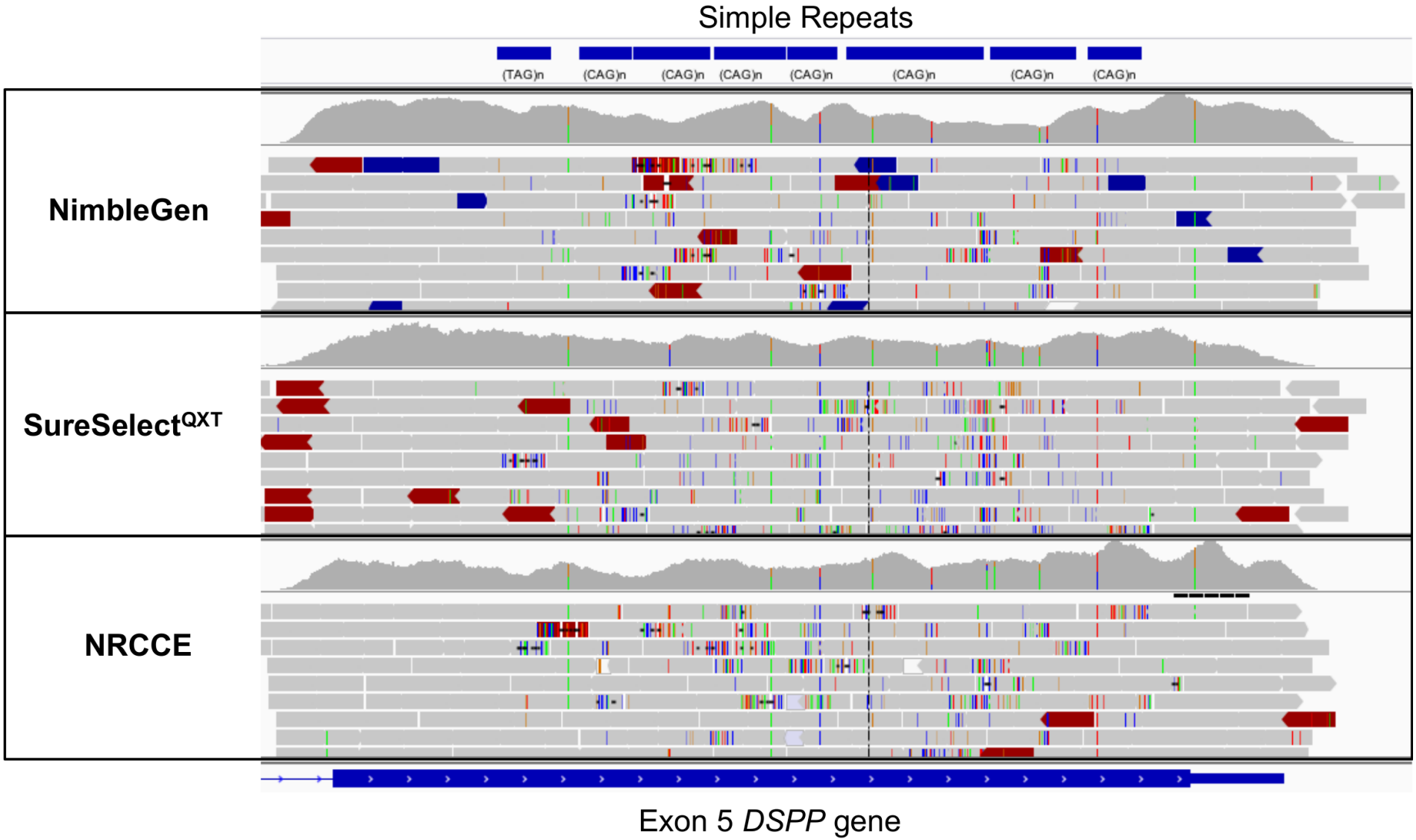
Supplementary Figure S1: Distribution of duplicate reads along the studied genes.



Supplementary Figure S2: Mean normalised DOC of the three methods for the 1,984 common regions.



Supplementary Figure S3: IGV view of *STRC* gene sequence for the three methods. Unfilled reads represents reads of poor genomic specificity.



Supplementary Figure S4: IGV view of *DSPP* sequence of exon 5 for one DNA. The low complexity of the genomic sequence results in poor mapping. Each vertical bar or horizontal mark inside the reads represents alignment mismatches.

	SureSelect QXT	NRCCE	Nimblegen
Company	Agilent	Illumina	Roche
Number of genes	121	112	121
Target size (kb)	781	695	781
Online tool	SureDesign	DesignStudio	NimbleDesign
Bioinformatics support offered	Yes	No	Yes
Probes size	120	80	75.75 (+/-4.62)
Number of probes	30961	12966	NA
Probes density	Overlapping (96bp)	Overlapping (20bp)	Overlapping (NA)
Mean probes per base	5	1	7
Design stringency	Intermediate	Relaxed	Intermediate
Percentage of target covered	99.7	100	99.1

Supplementary Table S1: Main characteristics of the three probe designs
NA: not available

	SureSelect^{QXT}	NRCCE	NimbleGen
Initial DNA amount (ng)	50	50	500
Fragmentation type	Enzymatic	Enzymatic	Mechanic
Number of PCR cycles pre- capture	8	10	7
Pool of patients before hybridization	No	Yes (12 patients / capture)	Yes (4 patients / capture)
DNA Amount of each patient for hybridization (ng)	750	500	250
Hybridization cycles (time)	One (90 min)	Two (2h + o/n)	One (66-72h)
Number of PCR cycles post-capture	14	12	14
Addition of Index	After capture	Before capture	Before capture

Supplementary Table S2: protocol characteristics of the 3 library preparation methods

o/n: overnight

	SureSelect ^{QXT} (A)	NRCCE (I)	Nimblegen (N)	Wilcoxon signed-sum test unilateral hypothesis						A: Agilent; I: Illumina; N: NimbleGen
Library parameters (Means on 2 independant runs- per sample)				H_0	p-value	H_0	p-value	H_0	p-value	
Median Insert Size	224.83	187.50	187.63	I<N	0.9173	I<A	9.64E-06	N<A	9.61E-06	
Insert Size SD	76.58	46.75	35.96	I>N	1.64E-04	I<A	9.66E-06	N<A	9.65E-06	
% of duplicates reads	18.59	41.24	7.77	I>N	5.96E-08	I>A	5.96E-06	N<A	9.69E-06	
Run parameters (Means on 2 independant runs - per run)										
Clusters density (K/mm2)	1090	963	1070							
Bases sequenced (Gb)	5.10	4.80	5.20							
% Q30	89.05	90.15	91.60							
# clusters raw	20197145	18131316	20144285							
% of Clusters passing filter	90.08	91.95	91.53							
% of duplicates clusters	12.88	19.63	3.64							
% of unindexed clusters passing filter	2.77	3.10	2.85							
% of unaligned clusters passing filter	0.78	0.92	1.72							
Run parameters (Means on 2 independant runs - per sample)										
# aligned reads	2885743	2614487	2866327	I<N	0.06776	I<A	0.09375	N<A	0.78880	
% of ontarget reads	75.09	85.78	86.88	I<N	0.01913	I>A	5.96E-08	N>A	9.69E-06	
# aligned bases	415890897	347747806	405126563	I<N	0.01056	I<A	0.00894	N<A	0.70810	
% of on target bases	66.30	75.26	73.95	I>N	0.01548	I>A	5.96E-08	N>A	5.96E-08	
*% nts > 20X**	99.37	99.04	99.32	I<N	0.01070	I<A	0.00318	N<A	0.19720	
*% nts > 50X**	97.36	95.89	98.58	I<N	0.00012	I<A	0.01699	N>A	0.00037	
*Mean DOC (+duplicates)**	350	371	374	I<N	0.31150	I>A	0.34200	N>A	0.06401	
*Mean DOC (-duplicates)**	263	217	349	I<N	4.53E-05	I<A	0.01664	N>A	0.00117	
Variant parameters (Means per sample - AB for variants with filter 'PASS' only)										
*# SNVs**	677	663	674	I<N	0.00033	I<A	9.61E-06	N<A	0.02948	
*Mean AB (SNVs/het)**	0.47	0.48	0.47							
*SD AB (SNVs/het)**	0.07	0.08	0.07							
*Mean AB (SNVs/hom)**	1.00	1.00	1.00							
*SD AB (SNVs/hom)**	0.01	0.01	0.01							
*# Indels**	56	50	56	I<N	2.90E-04	I<A	2.07E-05	N>A	0.99880	
*Mean AB (Indels/het)**	0.45	0.45	0.46							
*SD AB (Indels/het)**	0.10	0.10	0.10							
*Mean AB (Indels/hom)**	0.96	0.96	0.96							
*SD AB (Indels/hom)**	0.05	0.05	0.04							
*Ts/Tv ratio**	2.80	2.82	2.81	I>N	0.004248	I>A	0.16500	N>A	0.97610	
*QUAL score**	4485	3167	5165	I<N	4.17E-06	I<A	0.00033	N>A	0.01243	
*RMSMapping quality**	35.68	33.60	37.75	I<N	0.001572	I<A	8.35E-07	N>A	0.17240	
*RMSMapping quality SD**	1.40	1.27	2.45							
*Evenness(%)**	82.45	81.72	88.42	I<N	9.70E-06	I<A	0.00972	N>A	5.96E-08	
*Enrichment Factor**	3203	4048	3705	I>N	9.70E-06	I>A	9.70E-06	N>A	9.70E-06	

Supplementary Table S3: Parameters analysed and computed in this study with statistical p-values when relevant

SD: Standard deviation
AB: Allele Balance
RMS: Root Mean Square
SNV: Single Nucleotide Variation
**these parameters have been computed considering common regions between the 3 designs only (1984 regions)*
Allele Balances have not been statistically tested as values are substantially equal for each protocol

	Family ID	Gene	#SNV	#indels	indel size	NRCCE	NimbleGen	SureSelectQXT	Validation method
Run 1	**S110**	*GJB2†*	0	1	1	1/1	1/1	1/1	Sanger sequencing
	S661-1	*MYO6†*	1	0	NA	1/1	1/1	1/1	Sanger sequencing
	S661-2	*MYO6†*	1	0	NA	1/1	1/1	1/1	Sanger sequencing
	U1120	*MYO7A**	21	1	22	22/22	22/22	22/22	Sanger sequencing
		*CDH23**	25	0	NA	25/25	25/25	25/25	Sanger sequencing
		*CIB2**	1	0	NA	1/1	1/1	1/1	Sanger sequencing
	U1277	*GJB2**	0	0	NA	0/0	0/0	0/0	Sanger sequencing
		MYO15A†	1	1	1	2/2	2/2	2/2	Sanger sequencing
	S1324	NA							
	U1347	*GJB2†*	0	0	NA	0/0	0/0	0/0	Sanger sequencing
		PCDH15†	1	0	NA	1/1	1/1	1/1	Sanger sequencing
	U110	NA							
	U1389	*USH2A**	14	1	1	15/15	15/15	15/15	Sanger sequencing
		GPR98†	2	0	NA	2/2	2/2	2/2	Sanger sequencing
	U1403	NA							
	U1404	*MYO7A**	11	1	2	11/12	12/12	12/12	Sanger sequencing
		*GJB2**	0	0	NA	0/0	0/0	0/0	Sanger sequencing
	S1188	*CDH23**	23	0	NA	23/23‡	23/23	23/23	Sanger sequencing
Run 2	**S370**	*GJB2**	0	0	NA	0/0	0/0	0/0	Sanger sequencing
		LHFPL5†	1	0	NA	1/1	1/1	1/1	Sanger sequencing
	S432	*GJB2**	0	0	NA	0/0	0/0	0/0	Sanger sequencing
	S634	*GJB2**	1	0	NA	1/1	1/1	1/1	Sanger sequencing
		USH1C†	1	0	NA	1/1	1/1	1/1	Sanger sequencing
		USH2A†	1	0	NA	1/1	1/1	1/1	Sanger sequencing
	S715	*GJB2**	0	0	NA	0/0	0/0	0/0	Sanger sequencing
	S725	*GJB2**	0	0	NA	0/0	0/0	0/0	Sanger sequencing
		MYH9†	1	0	NA	1/1	1/1	1/1	Sanger sequencing
	S731	*GJB2**	1	0	NA	1/1	1/1	1/1	Sanger sequencing
	U1396	*CLRN1**	0	0	NA	0/0	0/0	0/0	Sanger sequencing
		GJB2†	0	1	1	1/1	1/1	1/1	Sanger sequencing
	U1410	*MYO7A**	15	0	NA	15/15§	15/15	15/15	Sanger sequencing
		USH2A†	1	1	1	2/2	2/2	2/2	Sanger sequencing
	U1415-1	*GPR98†*	2	0	NA	2/2	2/2	2/2	Sanger sequencing
	U1415-2	NA							
	U1420	NA							
	U1426	*USH2A†*	0	1	›p (1 exon del	1/1	1/1	1/1	MLPA
		Total	125	8		132/133	133/133	133/133	

Supplementary Table S4: List of patients analysed

*Genes previously analysed by Sanger sequencing

†Pathogenic variants confirmed by sanger sequencing or MLPA

‡1 variant was filtered as LowDepth (7 reads)

§For this genotype NRCCE alone reported a variant chr11:g.76908662T>C with borderline allelic balance (21%, then not filtered out, the limit being 20%) - variant not confirmed by Sanger sequencing