Supplementary Online Content

Swaminathan SS, Field MG, Sant D, et al. Molecular characteristics of conjunctival melanoma using whole-exome sequencing. *JAMA Ophthalmol*. Published online November 9, 2017. doi:10.1001/jamaophthalmol.2017.4837

This supplementary material has been provided by the authors to give readers additional information about their work.

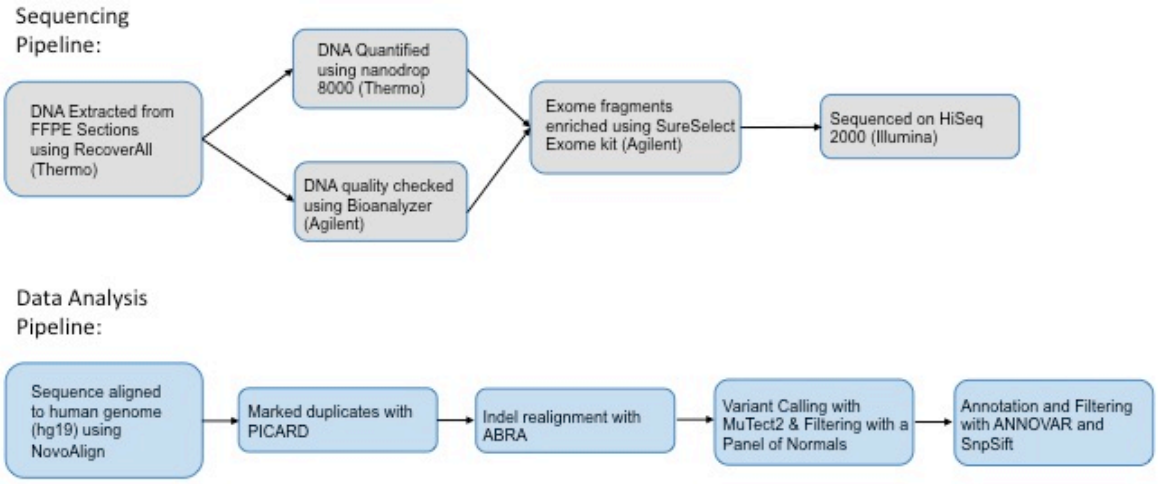
eAppendix. Additional Methods

Genomic DNA from FFPE sections was purified by RecoverAll Total Nucleic Acid Isolation Kit (Thermo, Rockland, DE) following the manufacturer's instructions. The quantity and quality of DNA was evaluated by a nanodrop 8000 spectrophotometer (Thermo, Rockland, DE) and a Bioanalyzer 2000 (Agilent, Palo Alto, CA), respectively. Whole exome sequencing was conducted in the Sequencing Core facility at the John P. Hussman Institute for Human Genomics in the University of Miami. Briefly, DNA samples were sheared using an E210 sonicator (Covaris, Woburn, MA) and the whole exome was captured using the SureSelect XT Human All Exon V5 kit (Agilent, Palo Alto, CA). To sequence the enriched 50Mb exomes, a 3-plex strategy per lane was conducted on the HiSeq 2000 sequencer (Illumina, San Diego, CA) using 125-bp paired-end reads, which yielded an average ~100× coverage depth at targeted regions.

Sequences were aligned to the human genome hg19 using NovoAlign (http://www.novocraft.com/products/novoalign/). Quality control and file manipulation was performed with FastQC, PICARD and SAMtools.[1] Variant calling was performed using MuTect2.[2] Variants predicted to be germline by the algorithms in MuTect2 or present in a panel of normal samples (n = 117, samples from blood to represent germline) were removed. To minimize artifact introduced by the specimen archiving process,[3] we filtered out all variants present in less than 20% of sequencing reads. Additionally, variants were excluded if they were outside of coding or splicing regions, had fewer than 3 alternate reads, were present in greater than 0.5% of the population, or were predicted to be non-damaging using ANNOVAR.[4]

1. Li H, Handsaker B, Wysoker A, et al. The Sequence Alignment/Map format and SAMtools. Bioinformatics 2009; 25(16):2078-9.
2. McKenna A, Hanna M, Banks E, et al. The Genome Analysis Toolkit: a MapReduce framework for analyzing next-generation DNA sequencing data. Genome Res 2010; 20(9):1297-303.
3. Wong SQ, Li J, Tan AYC et al. Sequence artefacts in a prospective series of formalin-fixed tumours tested for mutations in hotspot regions by massive parallel sequencing. BMC Medical Genomics 2014; 7:23.
4. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 2010; 38(16):e164.

eFigure 1. Schematic diagram of the customized sequencing and data analysis pipeline.



eTable 1. Broad copy number aberrations (Broad Regions) regions identified by GISTIC analysis with a q-value significance score < 0.2. Chromosome 6p gain was noted in all samples.

Arm	# Genes	Amp frequency	Amp frequency score	Amp z-score	Amp q-value
1q	1955	0.4	0.4	1.97	0.155
6p	1173	1	1	5.1	7.43E-06
6q	839	0.6	0.6	2.44	0.0532
8q	859	0.6	0.6	2.46	0.0532

Arm	# Genes	Del frequency	Del frequency score	Del z-score	Del q-value
16p	872	0.6	0.6	2.47	0.0502
16q	702	0.6	0.6	2.36	0.0573
19q	1709	0.4	0.4	1.78	0.182
21p	13	1	1	4.13	0.000264
21q	509	0.6	0.6	2.25	0.0672

eTable 2. Specific amplified (Amp_Genes) regions identified by GISTIC analysis with a q-value significance score < 0.2. Chromosome 6p gain was noted in all samples.

cytoband	1p36.13	4p15.33	5q13.2	6p22.1	6p22.1	8q12.1	9p13.1	11q13.1	12p13.31	15q26.3	16p11.2	17q21.2
q value	4.43E-05	0.12939	0.003965	0.00032494	0.00032494	0.03588	0.14622	4.43E-05	4.43E-05	0.02189	0.00053898	0.10191
residual q value	4.43E-05	0.12939	0.003965	0.00032494	1	0.03588	0.14622	4.43E-05	4.43E-05	0.02189	0.00053898	0.10191
wide peak boundaries	chr1:16853469-17100800	chr4:14884836-15076917	chr5:68856260-70096675	chr6:27765466-27870329	chr6:1-171115067	chr8:59404889-59555610	chr9:39010498-40513014	chr11:65164168-65273902	chr12:94469559-9605858	chr15:102291831-102355571	chr16:29321829-29641162	chr17:39174123-39353070

eTable 3. List of specific amplified genes identified by GISTIC analysis in eTable 2.

cytoband	1p36.13	4p15.33	5q13.2	6p22.1	6p22.1	8q12.1	9p13.1	11q13.1	12p13.31	15q26.3	16p11.2	17q21.2
	MST1P2	CPEB2	SMN1	HIST1H1B	hsa-mir-1913	CYP7A1	CNTNAP3	hsa-mir-612	DDX12P	OR4F6	SULT1A3	KRTAP1-3
	MST1P9	LOC441009	SMN2	HIST1H2AI	hsa-mir-1202	SDCBP	FAM74A1	FRMD8	LOC642846		SLX1B	KRTAP1-1
	NBPF1		SERF1A	HIST1H2AK	hsa-mir-1273c	NSMAF	ZNF658B	NEAT1			SLC7A5P1	KRTAP4-6
	CROCCP2		SMA4	HIST1H2AJ	hsa-mir-3145		FAM75A2	MALAT1			LOC388242	KRTAP2-1
	ESPNP		SMA5	HIST1H2AL	hsa-mir-548a-2		FAM75A1	MIR612			SNX29P2	KRTAP4-12
	MIR3675		GUSBP3	HIST1H2AM	hsa-mir-588		LOC653501				LOC440354	KRTAP1-5
			GTF2H2B	HIST1H2BL	hsa-mir-3144						SULT1A4	KRTAP4-4
			GTF2H2C	HIST1H2BN	hsa-mir-548b						SLX1A	KRTAP4-1
			SERF1B	HIST1H2BM	hsa-mir-587						BOLA2	KRTAP4-5
			GTF2H2D	HIST1H2BO	hsa-mir-2113						LOC606724	KRTAP4-3
			GUSBP9	HIST1H3I	hsa-mir-4282						BOLA2B	KRTAP4-2

			LOC100170939	HIST1H3J	hsa-mir-30a						SLX1A-SULT1A3	KRTAP2-4
			LOC100272216	HIST1H3H	hsa-mir-30c-2						SLX1B-SULT1A4	KRTAP4-11
				HIST1H4K	hsa-mir-548u							KRTAP4-8
				HIST1H4J	hsa-mir-133b							KRTAP2-2
				HIST1H4L	hsa-mir-586							KRTAP9-1
					hsa-mir-1275							LOC730755
					hsa-mir-219-1							KRTAP4-9
					hsa-mir-1236							KRTAP4-7
					hsa-mir-877							
					hsa-mir-3143							
					hsa-mir-548a-1							
					ABCF1							
					ACAT2							
					CRISP1							
					AGER							
					AIF1							
					AIM1							
					AMD1							
					ARG1							
					ATP6V1G2							
					BAI3							
					BAK1							
					BCKDHB							
					CFB							
					PRDM1							
					BMP5							
					BMP6							
					DST							
					BPHL							
					BTN1A1							
					BYSL							
					C2							
					C4A							
					C4B							
					DDR1							
					RUNX2							
					CCNC							
					CCND3							
					CDC5L							
					CDKN1A							
					CDSN							
					CGA							
					CLIC1							
					CLPS							
					CCR6							
					CNR1							
					COL9A1							

					COL10A1							
					COL11A2							
					COL12A1							
					COL19A1							
					COX7A2							
					ATF6B							
					MAPK14							
					CSNK2B							
					CTGF							
					CYP21A2							
					CYP21A1P							
					DAXX							
					DNAH8							
					DOM3Z							
					DSP							
					E2F3							
					EDN1							
					EEF1A1							
					SERPINB1							
					SLC29A1							
					EPB41L2							
					EPHA7							
					EYA4							
					ESR1							
					F13A1							
					FABP7							
					FANCE							
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					FOXO3							
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					FUCA2							
					FYN							
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					GABRR2							
					GCNT2							
					GJA1							
					GCLC							
					GLO1							
					GLP1R							
					GMDS							
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					GPX5							
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					GSTA2							
					GSTA3							
					GSTA4							
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					GUCA1B							
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					HIST1H1D							
					HIST1H1E							
					HIST1H1B							
					HIST1H1T							
					HIST1H2AE							
					HIST1H2AD							
					HIST1H2BD							
					HIST1H2BB							
					HIST1H1A							
					HCRTR2							
					HDAC2							
					HFE							
					HIVEP1							
					HIVEP2							
					HLA-A							
					HLA-B							
					HLA-C							
					HLA-DMA							
					HLA-DMB							
					HLA-DOA							
					HLA-DOB							
					HLA-DPA1							
					HLA-DPB1							
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					HLA-DQB2							
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					HSPA1L							
					HSP90AB1							
					HTR1B							
					HTR1E							
					ID4							
					IFNGR1							
					IGF2R							
					IL17A							
					IMPG1							
					IRF4							
					ITPR3							
					JARID2							
					KIFC1							
					KIF25							
					KPNA5							
					LAMA2							
					LAMA4							
					LPA							
					LTA							
					LTB							
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					MAS1							
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					MDFI							
					ME1							
					MEA1							
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					MAP3K5							
					MEP1A							
					MICB							
					MLN							
					MLLT4							
					MOCS1							
					MOG							
					MSH5							
					MUT							
					MYB							
					MYO6							
					RPL10A							
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					NEU1							
					NFKBIE							
					NFKBIL1							
					NFYA							
					NMBR							
					NQO2							

					NOTCH4							
					NT5E							
					OPRM1							
					PARK2							
					PBX2							
					PCMT1							
					PDCD2							
					ENPP1							
					ENPP3							
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					PGK2							
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					PHF1							
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					SERPINB9							
					PIM1							
					PKHD1							
					PLAGL1							
					PLG							
					PLN							
					POLH							
					POU3F2							
					POU5F1							
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					PPP1R10							
					PPP2R5D							
					PREP							
					PRIM2							
					PKIB							
					MAPK13							
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					PSMB1							
					PSMB8							
					PSMB9							
					PTK7							
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					REV3L							
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					RHAG							
					RING1							
					BRD2							
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					ROS1							
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					RXRB							
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					SIM1							
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					SOX4							
					SRF							
					SRPK1							
					SSR1							
					ELOVL4							
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					THBS2							
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					TNF							
					TNFAIP3							
					TNXA							
					TNXB							
					TPBG							
					TPD52L1							
					TPMT							

					CRISP2							
					TSPYL1							
					TTK							
					TUBB2A							
					TULP1							
					UTRN							
					VARS							
					VEGFA							
					EZR							
					VIP							
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					HIST1H2AL							
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					HIST1H2AB							
					HIST1H2AM							
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					HIST1H2BC							
					HIST1H2BO							
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					HIST1H3D							
					HIST1H3C							
					HIST1H3E							
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					HIST1H3J							
					HIST1H3H							
					HIST1H3B							
					HIST1H4A							
					HIST1H4D							
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					HIST1H4K							
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					HIST1H4C							
					HIST1H4H							
					HIST1H4B							
					HIST1H4E							
					HIST1H4L							
					HIST1H4G							
					STX7							
					CMAHP							
					DHX16							
					SUPT3H							
					PEX3							
					GCM1							
					DDO							
					RNASET2							
					KCNK5							
					STX11							
					B3GALT4							
					SNX3							
					RNGTT							
					RIPK1							
					CD164							
					SYNGAP1							
					WISP3							
					STK19							
					IER3							
					SYNJ2							
					VNN2							
					VNN1							
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					WASF1							
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					HIST1H2AG							
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					MAP7							
					TBX18							
					LATS1							
					GCM2							
					WDR46							
					ZBTB22							
					TAAR2							
					TAAR3							
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					HMGN3							
					PPT2							
					CDYL							
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					MED23							
					QKI							
					LY86							
					FHL5							
					AKAP7							
					ATG5							
					MED20							
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					BAG2							
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					SNAP91							
					FIG4							
					NUP153							
					CASP8AP2							
					RANBP9							
					SLC17A4							
					UST							
					TRIM10							
					FLOT1							
					RCAN2							

					SLC17A2							
					HCG9							
					PRSS16							
					CRISP3							
					TRDN							
					CITED2							
					BTN3A3							
					BTN2A2							
					ECI2							
					PFDN6							
					HMGN4							
					TRIM38							
					CAP2							
					SYNCRIP							
					UBD							
					AGPAT1							
					SLC35A1							
					SCGN							
					C6orf108							
					C6orf10							
					FARS2							
					FUT9							
					CNPY3							
					TRAF3IP2							
					HBS1L							
					SLC17A3							
					RPP40							
					FRS3							
					PDE10A							
					SLC22A7							
					HCP5							
					EHMT2							
					SMPDL3A							
					APOBEC2							
					PNRC1							
					ASCC3							
					RAB32							
					TRIM31							
					KATNA1							
					FGFR1OP							
					BTN3A2							
					BTN3A1							
					BTN2A1							
					CAPN11							
					BVES							
					NUDT3							
					SEC63							
					NRM							
					STK38							
					SCAF8							

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					ICK							
					ENPP4							
					ANKRD6							
					RIMS1							
					DOPEY1							
					ZNF292							
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					TAB2							
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					SASH1							
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					DAAM2							
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					DDAH2							
					HEBP2							
					ORC3							
					CD2AP							
					BRD7P3							
					MTCH1							
					ZNF318							
					SPDEF							
					MTO1							
					ASF1A							
					YIPF3							
					USP49							
					CCDC28A							
					MTHFD1L							
					PNISR							
					IBTK							
					MOXD1							
					IPCEF1							
					ZNF451							
					SENP6							
					OR2B6							
					TIAM2							
					FBXL4							
					C6orf123							
					FAM50B							
					FBXO9							
					FBXO5							

					SLC17A5							
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					OR11A1							
					RGS17							
					OR2W1							
					OR2J2							
					OR2H1							
					SNORD52							
					SNORD50A							
					SNORD48							
					PDE7B							
					FILIP1							
					BRPF3							
					GNMT							
					TNFRSF21							
					SESN1							
					TINAG							
					DLL1							
					OSTM1							
					MRPS18B							
					TMEM14A							
					MRPL18							
					NDUFAF4							
					C6orf15							
					MYLIP							
					ABT1							
					DSE							
					PRICKLE4							
					PACSIN1							
					ZNRD1							
					CLDN20							
					NOX3							
					DEF6							
					C6orf48							
					SLC35B3							
					HDDC2							
					GMNN							
					MRPL2							
					TFB1M							
					CYB5R4							
					TUBE1							
					C6orf203							
					TBC1D7							
					NRN1							
					CYP39A1							
					CDC40							
					RWDD1							
					AIG1							
					NOL7							
					SNX9							

					FAM8A1							
					UBE2J1							
					DCDC2							
					ETV7							
					TMEM14C							
					VTA1							
					LGSN							
					TDP2							
					CUTA							
					PPIL1							
					BRP44L							
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eTable 4. Specific deleted gene (Del_Genes) regions identified by GISTIC analysis with a q-value significance score < 0.2. Chromosome 6p gain was noted in all samples.

cytoband	3q11.1	12q14.2	16q24.2	21q11.2	Xp22.33
q value	0.0029269	0.089361	0.19893	0.1887	0.0048605
residual q value	0.0029269	0.089361	0.19893	0.1887	0.0048605
wide peak boundaries	chr3:89521354-93709932	chr12:63544058-64181655	chr16:88104957-88524995	chr21:11095865-14991078	chrX:1652540-2533608
genes in wide peak	PROS1	DPY19L2	ZNF469	hsa-mir-3156-3	ASMT
cytoband				ANKRD30BP2	AKAP17A
q value				MIR3156-3	ZBED1
residual q value					DHRSX
wide peak boundaries					LINC00102