Supplementary Online Content

Hampel H, Pearlman R, Beightol M, et al. Assessment of tumor sequencing as a replacement for Lynch syndrome screening and current molecular tests for patients with colorectal cancer. *JAMA Oncol*. Published online March 29, 2018. doi:10.1001/jamaoncol.2018.0104

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

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eAppendix. Description of the UW Oncoplex test including a list of the genes included

UW-OncoPlex - Cancer Gene Panel

Questions: **1-800-256-0893**

Background

UW-OncoPlex is a multiplexed gene sequencing panel that detects mutations in tumor tissue in 262 cancer-related genes (listed in the methods below). The panel includes genes related to cancer treatment, prognosis, and diagnosis. The test uses next-generation "deep" sequencing to detect most classes of mutations, including single nucleotide variants, small insertions and deletions (indels), gene amplifications, and selected gene-fusions. In addition, microsatellite instability status is reported for colorectal cancers.

Methods (assay version 5)

The following genes are sequenced on an Illumina instrument to detect single nucleotide variants, small insertions and deletions, gene amplifications, and selected translocations: ABCB1*, ABCC4*, ABCG2*, ABL1, ABL2, AKT1, AKT2, AKT3, ALK**, APC, AR, ARAF (NEW), ARID1A (NEW), ASXL1, ATM, ATRX, AURKA, AURKB, AXL, BAK1, BAP1, BARD1, BCL2, BCL2L11, BCOR, BCORL1 (NEW), BCR*, BRAF**, BRCA1, BRCA2, BRIP1, CALR, CBL, CBLB, CBLC (NEW), CCND1, CCNE1, CDH1, CDK12 (NEW), CDK4, CDK6, CDK8, CDKN1A, CDKN2A, CEBPA, CHD1, CHEK1, CHEK2, CREBBP, CRLF2, CSF1R, CSF3R (NEW), CTNNB1, CUX1 (NEW), CYP1B1*, CYP2C19*, CYP2C8*, CYP2D6*, CYP3A4*, CYP3A5*, DAXX, DDR2, DEPDC5, DNAJB1, DNMT3A, DOCK7, DPYD, EGFR, EIF3A, EIF3E (NEW), EML4*, EPHA3, EPHA5, EPHB2, EPHB6, ERBB2, ERBB3, ERBB4, ERCC2, ESR1 (NEW), ESR2*, ETV6**, EZH2, FAM175A, FANCA (NEW), FBXW7, FCGR1A, FCGR2A, FCGR3A*, FGFR1**, FGFR2**, FGFR3**, FGFR4, FKBP1A (NEW), FLT1, FLT3, FLT4, FOXA1, GAB2, GATA1, GATA2, GATA3, GLI1, GNA11, GNAQ, GNAS, GRIN2A, GRM3, GSTP1*, H3F3A, HDAC4, HIF1A, HNF1A, HRAS, HSPH1*, IDH1, IDH2, IGF1R, IKZF1, IL7R, ITPA*, JAK1, JAK2, JAK3, KDM6A, KDR, KIF5B*, KIT, KRAS, LRP2*, MAN1B1*, MAP2K1, MAP2K2, MAP2K4, MAPK1, MC1R, MCL1, MDM2, MDM4, MED12, MEN1, MET, MIOS, MITF, MLH1**, MLH3, MLL (KMT2A)**, MPL, MRE11A, MSH2**, MSH6**, MTAP, MTHFR*, MTOR (NEW), MUTYH, MYC, MYCL1, MYCN, MYD88 (NEW), NBN, NF1, NF2, NKX2-1, NOTCH1, NOTCH2, NPM1, NPRL2, NPRL3, NQO1*, NRAS, NRP2*, NTRK1**, NTRK2**, NTRK3**, PAK1, PALB2, PAX5, PBRM1, PDGFRA, PDGFRB, PHF6, PIK3CA, PIK3CB (NEW), PIK3R1, PLK1, PLK2, PLK3, PLK4, PML*, PMS2, POLD1, POLE, PRPF40B, PTCH1, PTEN, PTPN11, PTPRD, RAC1 (NEW), RAD21 (NEW), RAD51C, RAD51D, RAF1, RARA**, RB1, RET, RHEB (NEW), RICTOR, ROS1**, RPS14, RPTOR (NEW), RSPO2** (NEW), RSPO3** (NEW), RUNX1, SETBP1 (NEW), SF1, SF3B1, SHH, SLC19A1*, SLC22A2*, SLCO1B3*, SMAD2, SMAD3, SMAD4, SMARCA4, SMARCB1, SMC1A (NEW), SMC3 (NEW), SMO, SOD2*, SPOP, SPRY4, SRC, SRSF2, STAG2 (NEW), STK11, SUFU, SULT1A1*, SUZ12, TACC3, TACSTD2, TET1 (NEW), TET2, TET3 (NEW), TFG*, TGFBR2, TMPRSS2*, TP53, TPMT*, TRRAP, TSC1, TSC2, TYMS*, TYR, U2AF1, (U2AF35), U2AF2, (U2AF65), UGT1A1*, UMPS*, VHL, WT1, ZBTB16, and ZRSR2.

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*Selected regions only sequenced, **Selected introns sequenced in addition to all coding regions

Gene Fusions and Rearrangements Detected (assay version 5)

- ALK fusions (including ALK-EML4, ALK-KIF5B, ALK-TFG, ALK-C2orf44)
- BCR-ABL (common p190 and p210 fusions only)
- BRAF fusions
- DNAJB1- PRKACA fusions
- FGFR3 fusions
- MLL rearrangements
- RET fusions
- ROS1 fusions
- NTRK1 fusions
- NTRK2 fusions (NEW)
- NTRK3-ETV6 fusions (NEW)
- PML-RARA
- RSPO2 fusions (NEW)
- RSPO3 fusions (NEW)
- TMPRSS2 fusions

Microsatellite Instability Analysis

Microsatellite instability (MSI) status is reported for all colorectal cancer cases. MSI is detected using methods described in Salipate et al. 2014 *Clin Chem.* (2014) 60:1192-9.

Turnaround Time

4 - 6 weeks

Shipping Address

- UW MEDICAL CENTER
- LABORATORY MEDICINE - GENETICS LAB
- 1959 NE PACIFIC ST, ROOM NW220
- SEATTLE, WA 98195-7110

Acceptable Specimens

- Formalin-Fixed Paraffin Embedded Tumor Tissue (FFPE)
- Purified DNA

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- Peripheral Blood
- Bone Marrow

Tissue samples (FFPE): Send EITHER (a) slides, OR (b) tissue block: (a) Instructions for slide specimens: 1 slide at 4-micron thickness stained with hematoxylin-and-eosin AND 10 unstained, non-baked slides at 10-micron thickness (a minimum of 5 unstained slides is acceptable). Unstained slides can be on charged or uncharged slides. Note: Sections should contain as much tumor tissue as possible. (b) Instructions for tissue block specimen: Provide complete tissue block containing tumor tissue. If there is more than one tissue block, please provide the block that has the greatest amount of tumor tissue. Tissue block will be returned at completion of testing. Ship at room temperature.

Note: In order to ensure that enough DNA is obtained, the minimum acceptable tissue area is 10 square millimeters when ten 10-micron slides are supplied (1 cubic millimeter of tissue). Tissue sections should contain as much tumor tissue as possible; to ensure detection of all types of mutations, there should be at least 10% tumor cells in the tissue area processed for DNA.

Purified DNA: (Reference hematoxylin-and-eosin stained slide and pathology report required): 5 ug minimum. Ship specimen refrigerated for overnight delivery.

Blood: 6 mL blood in LAVENDER TOP (EDTA) tube. Ship specimen refrigerated for overnight delivery. Specimen can be held for up to 24 hours before shipping if refrigerated.

Bone Marrow: 1 to 2 mL Bone Marrow in LAVENDER TOP (EDTA) tube. Ship specimen refrigerated for overnight delivery. Specimen can be held for up to 24 hours before shipping if refrigerated.

For further details regarding specimen collection, handling and transport, see the Laboratory Medicine Online Test Guide

Requisition Form

- UW Genetics Requisition: http://depts.washington.edu/labweb/referencelab/clinical/TestForms/genetics.pdf
- Select "UW-OncoPlex"
- Indicate on the requisition any previous molecular testing that has been performed on the sample

CPT Codes & Pricing

CPT coding : http://depts.washington.edu/labweb/referencelab/files/OPX.pdf

For pricing information, contact Reference Laboratory Services at **1-800-256-0893**

Reference Range

No mutation detected.

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Further Information

For further information:

- Lab telephone: (206) 598-6429
- Supervisor: Robert Livingston, Ph.D.
- Director: Colin C. Pritchard, M.D., PhD.
- Director: David Wu, M.D., Ph.D.
- Director: Christina Lockwood, Ph.D.
- Director: Eric Konnick, M.D., M.S.
- Director: Brian Shirts, M.D., Ph.D.
- Director: Jonathan Tait, M.D., Ph.D.

References

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2. Pritchard CC, Salipante SJ, Koehler K, Smith C, Scroggins S, Wood B, Wu D, Lee MK, Dintzis S, Adey A, Liu Y, Eaten KD, Martins R, Stricker K, Margolin K, Hoffman N, Churpek J, Tait JF, King MC, Walsh T. Validation and Implementation of Targeted Capture and Sequencing for the Detection of Actionable Mutation, Copy Number Variation, and Gene Rearrangement in Clinical Cancer Specimens. *The Journal of Molecular Diagnostics*(2014) 16:56-67.
3. Salipante SJ, Scroggins SM, Hampel HL, Turner EH, Pritchard CC. Microsatellite instability detection by next generation sequencing. *Clin Chem.*(2014) 60:1192-9.

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eTable 1. Germline mutations in Mismatch Repair Genes detected by Tumor Sequencing

Gene	Nucleotide Change	Protein Change	Detected by MSI followed by BRAF	Detected by IHC followed by BRAF	Detected by tumor sequencing including BRAF	Cohort
MLH1	c.200G>A	p.G67E	Y	Y	Y	Validation
MLH1	c.117-2A>G		Y	Y	Y	Validation
MLH1	c.1210_1211del	p.L404Vfs*12	Y	Y	Y	Validation
MLH1	c.1252_1253del	p.D418Yfs*3	Y	Y	Y	Validation
MLH1	c.1381A>T	K461X	Y	Y	Y	Validation
MLH1	c.1667+1G>A		Y	Y	Y	Prospective
MLH1	c.2041G>A	p.A681T	Y	Y	Y	Prospective
MLH1	c.207+5G>C		Y	Y	Y	Validation
MLH1	c.2252_2253delAA	p.K751Sfs*3	Y	Y	Y	Validation
MLH1	c.589-2A>G		Y	Y	Y	Validation
MLH1	c.677+3A>G		Y	Y	Y	Validation
MLH1	c.791-2A>G		Y	Y	Y	Validation
MSH2	c.2653C>T	p.Q885X	Y	Y	Y	Validation
MSH2	c.1255 C>T	p.Q419X	Y	Y	Y	Validation
MSH2	c.1386+1G>T		Y	Y	Y	Validation
MSH2	c.1477C>T	p.Q493X	Y	Y	Y	Prospective
MSH2	c.1749dupT	p.I584Yfs*14	Y	Y	Y	Validation
MSH2	c.1906G>C	p.A636P	Y	Y	Y	Validation
MSH2	c.2038C>T	p.R680X	Y	Y	Y	Validation
MSH2	c.2096 C>A	p.S699X	Y	Y	Y	Validation
MSH2	c.2131C>T	p.R711X	Y	Y	Y	Validation
MSH2	c.2131C>T	p.R711X	Y	Y	Y	Validation
MSH2	c.2152C>T	p.Q718X	Y	Y	Y	Validation
MSH2	c.229_230del	p.S77Cfs*4	Y	Y	Y	Prospective
MSH2	c.2388delT	p. V797Lfs*15	Y	Y	Y	Validation
MSH2	c.942+3A>T		Y	Y	Y	Prospective
MSH2	c.942+3A>T		Y	Y	Y	Prospective
MSH2	c.942+3A>T		Y	Y	Y	Validation
MSH2	c.942+3A>T		Y	Y	Y	Validation
MSH2	del Exon 1-6		Y	Y	Y	Validation
MSH2	del Exon 1-6		Y	Y	Y	Validation
MSH2	del exon 1-7 + EPCAM		Y	Y	Y	Validation
MSH2	del Exon 3-16		Y	Y	Y	Validation
MSH2	del Exon 3-6		Y	Y	Y	Validation
MSH2	del Exon 5-7		Y	Y	Y	Prospective
MSH2	del Exon 8		Y	Y	Y	Validation
MSH2	del Exon 8		Y	Y	Y	Validation

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MSH6	c.1275_1276del	p.I425Mfs*9	Y	N	Y	Validation
MSH6	c.2504delA	p.Q835Rfs*9	Y	Y	Y	Validation
MSH6	c.2906_2907del	p.Y969Lfs*5	N	N	Y	Validation
MSH6	c.3438+1G>A		Y	Y	Y	Validation
MSH6	c.3516_3517del	p.R1172Sfs*4	Y	Y	Y	Validation
MSH6	c.3840_3846del	p.E1281Lfs*44	Y	N	Y	Validation
MSH6	c.766_767del	p.S256X	Y	Y	Y	Prospective
PMS2	c.1281del	p.H428Tfs*20	N	N	Y	Validation
PMS2	c.137G>T	p.S46I	N†	N†	Y	Prospective
PMS2	c.137G>T	p.S46I	Y	Y	Y	Validation
PMS2	c.1831dup	p.I611Nfs*2	Y	Y	Y	Prospective
PMS2	c.1831dup	p.I611Nfs*2	Y	Y	Y	Validation
PMS2	c.1831dup	p.I611Nfs*2	Y	Y	Y	Validation
PMS2	c.1840A>T	p.K614X	Y	Y	Y	Validation
PMS2	c.1874delT	p.L625X	Y	Y	Y	Prospective
PMS2	c.2113G>A	p.E705K	Y	N	Y	Validation
PMS2	c.736_741del6ins11	p.P246Cfs*3	Y	Y	Y	Prospective
PMS2	c.736_741del6ins11	p.P246Cfs*3	Y	Y	Y	Validation
PMS2	c.765C>A	p.Y255X	N	Y	Y	Validation
PMS2	del Exon 10		Y	Y	Y	Validation
PMS2	del Exon 5-15		N	Y	Y	Validation
Total number of cases missed by each test			**5**	**6**	**0**	

† Would not have had genetic testing because tumor was MSI-high, absent MLH1 and PMS2 and MLH1 was methylated

MSI = microsatellite instability testing

IHC = immunohistochemical staining

Y = yes

N = no

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eTable 2. Non-MMR Pathogenic Potentially Germline Cancer Gene Mutations Identified in the Study

Case #	Gene	Nucleotide Change	Protein Change	Detected by Tumor sequencing	Confirmed by Germline sequencing	Cohort
419	APC	c.3048_3049insTAAT	p.N1017*	Y	N	Prospective
121	APC	c.3734del	p.K1245Rfs*20	Y	NA	Prospective
424	APC	c.3913_3923del	p.A1305Rfs*6	Y	NA	Prospective
91	APC	c.3920T>A	p.I1307K	Y	Y	Prospective
144	APC	c.3927_3931del	p.E1309Dfs*4	Y	NA	Prospective
50	APC	c.3927_3931del	p.E1309Dfs*4	Y	NA	Prospective
388	APC	c.4061_4074del	p.F1354*	Y	N	Prospective
249	APC	c.4114_4128del	p.P1372_Y1376del	Y	NA	Prospective
401	APC	c.4483_4498del	p.S1495Ffs*7	Y	N	Prospective
256	APC	c.994C>T	p.R332X	Y	NA	Prospective
348	APC	c.2805C>A	p.Y935X	Y	NA	Prospective
56	APC	c.4348C>T	p.R1450X	Y	NA	Prospective
76	APC	c.847C>T	p.R283X	Y	NA	Prospective
397	APC	c.4285C>T	p.Q1429X	Y	N	Prospective
103	APC	c.2677G>T	p.E893X	Y	NA	Prospective
120	APC	c.2413C>T	p.R805X	Y	NA	Prospective
60	ATM	c.1564_1565del	p.E522Ifs*43	Y	NA	Prospective
32	ATM	c.8395_8404del	p.Phe2799Lysfs*4	Y	NA	Prospective
253	ATM	c.4245T>G	p.Y1415X	Y	N	Prospective
11	ATM	c.4852C>T	p.R1618X	Y	NA	Prospective
177	BRCA1	c.5503C>T	p.R1853X	Y	N	Prospective
294	BRCA2	c.1755_1759del	p.K585Nfs*3	Y	Y	Prospective
134	BRCA2	c.3860dup	p.N1287Kfs*2	Y	NA	Prospective
442	BRIP1	c.2392C>T	p.R798X	Y	Y	Prospective
420	CHEK2	c.1100del	p.T367Mfs*15	Y	NA	Validation
98	CHEK2	c.1100del	p.T367Mfs*15	Y	N	Validation
107	CHEK2	c.349A>G	p.R117G	Y	NA	Prospective
17	FANCA	del Exon 1-31		Y	NA	Prospective
386	FANCA	del Exon 2-6		Y	NA	Validation
170	MRE11A	c.1222dup	p.T408Nfs*49	Y	NA	Prospective

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465	MRE11A	c.1714C>T	p.R572X	Y	NA	Prospective
35	MRE11A	c.1714C>T	p.R572X	Y	NA	Prospective
57	MUTYH	c.1187G>A	p.G396D	Y	Y	Prospective
108	MUTYH	c.1187G>A	p.G396D	Y	Y	Validation
281	MUTYH	c.1187G>A	p.G396D	Y	Y	Validation
148	MUTYH	c.1187G>A	p.G396D	Y	NA	Prospective
412	MUTYH	c.1187G>A	p.G396D	Y	NA	Prospective
347	MUTYH	c.536A>G	p.Y179C	Y	Y	Prospective
64	MUTYH	c.536A>G	p.Y179C	Y	N	Prospective
237	MUTYH	c.536A>G	p.Y179C	Y	NA	Prospective
216	PALB2	c.758dupT	p.S254Ifs*3	Y	Y	Prospective
456	PTEN	c.803A>T	p.D268V	Y	N	Prospective
378	RAD51D	c.81del	p.V28Wfs*12	Y	NA	Prospective
276	RET	c.1826G>A	p.C609Y	Y	NA	Prospective
446	SMAD4	c.320dup	p.N107Kfs*2	Y	NA	Prospective
183	SMAD4	c.1096C>T	p.Q366X	Y	NA	Prospective
24	SMAD4	c.403C>T	p.R135X	Y	NA	Prospective
113	STK11	c.842del	p.P281Rfs*6	Y	NA	Prospective
227	TP53	c.376T>G	p.Y126D	Y	NA	Prospective

GNGS = germline next-generation sequencing

tumor sequencing = tumor next-generation sequencing

NA = not assessed (i.e. the gene was not included on the test or the case did not undergo germline testing)

N = no

Y = yes

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