GENE SORT

Solid

Information Sheet

Solid Tumor Profile Test

A comprehensive genomic profiling test for solid tumors

CURRENT GENE LIST*:

ABCA13	*ABL1*	*ABL2*	*AKT1*	*AKT2*	*ALK*	*APC*	*ARID1B*	*ARID2*	*ARNT*
ATM	*AURKA*	*BAP1*	*BIVM*	*BMPR1A*	*BRAF*	*BRCA1*	*BRCA2*	*BRIP1*	*BUB1B*
C15orf56	*CASP8*	*CDH1*	*CDK4*	*CDK12*	*CDKN1B*	*CDKN2A*	*CDKN2B-AS1*	*CDNK2C*	*CHEK2*
CREBBP	*CREM*	*CSF1R*	*CTNNB1*	*DAXX*	*DCTN5*	*DDB2*	*DICER1*	*DNAH5*	*DNMT3A*
EGFR	*EP300*	*ERBB2*	*ERCC2*	*ERCC3*	*ERCC4*	*ERCC5*	*EVI2A*	*EVI2B*	*EZH2*
FAM71B	*FANCC*	*FANCE*	*FANCF*	*FANCG*	*FAT3*	*FBXO11*	*FBXW7*	*FGFR1*	*FGFR2*
FGFR3	*FH*	*FLT3*	*FOXA1*	*GATA2*	*GATA3*	*GPR98*	*HMCN1*	*HOXC13*	*HRAS*
HSCB	*HYDIN*	*IDH1*	*IDH2*	*ITK*	*JAK1*	*JAK2*	*JAK3*	*KCNMB3*	*KIT*
KRAS	*LPAR6*	*LRP1B*	*LRP2*	*LSM3*	*MAP2K1*	*MAP2K2*	*MAP2K4*	*MAP3K1*	*MAP3K13*
MDN1	*MET*	*MIR1281*	*MIR1301*	*MIR641*	*MIR744*	*MLH1*	*MLL*	*MLL2*	*MLL3*
MSH2	*MSH6*	*MUC16*	*MYC*	*MYD88*	*NBR2*	*NCOR1*	*NF1*	*NF2*	*NIN*
NIPA2	*NOTCH1*	*NOTCH2*	*NPM1*	*NRAS*	*NUP214*	*NUP98*	*OMG*	*PAK6*	*PALB2*
PAX5	*PCLO*	*PCM1*	*PDGFRA*	*PGAP2*	*PGAP3*	*PIGL*	*PIK3CA*	*PIK3R1*	*POLE*
PPP2R1A	*PRKAG1*	*PRKAR1A*	*PTEN*	*PTK2B*	*RAD21*	*RB1*	*RECQL4*	*RET*	*ROS1*
RUNX1	*RYR2*	*SETD2*	*SMAD4*	*SMARCA4*	*SMARCB1*	*SMARCD1*	*SMO*	*SMURF2*	*SNAPC5*
SPOP	*SRC*	*STK11*	*TBX3*	*TNFAIP3*	*TP53*	*TTC36*	*TTN*	*UBR4*	*USH2A*
VEGFA	*VHL*	*WRAP53*	*WT1*	*XPA*	*XPC*	*ZNF276*			

*The genes that appear in this list are updated from time to time, and are current as to this version of the information sheet.

OVERVIEW

Personalized medicine does not rely on the “one-size-fits-all” treatment approach but rather takes into account individual patients' genomic uniqueness. Next generation sequencing (NGS) of patients' tumor DNA has greatly enhanced the identification of patient-tumor specific variants, and has enabled oncologists to make more informed personalized treatment decisions for their cancer patients based on their tumor genomic profile.

The GeneSort Solid Profile Tumor Test is a comprehensive genomic profiling diagnostic panel that simultaneously sequences 167 cancer related genes, approximately 1 million bases. These genes have been known to carry variants which are drivers and/or are associated with tumorgenesis. Using NGS technology, the Solid Tumor Test detects base changes, insertions and deletions, and copy number variations within tumor DNA which can then be targeted by matched drug therapies.

The Solid Tumor Test covers, but is not limited to, the following cancer types:

Bladder	Bone	Brain	Breast
Cervical	Colon	Endometrial	Head & Neck
Kidney	Liver	Lung	Ovarian
Pancreatic	Prostate	Rectal	Melanoma (Skin)
Stomach	Thyroid	Uterine	

AID GENOMICS

01-0419
NO.

METHODS

A minimal amount of DNA from the submitted FFPE (formalin fixed paraffin embedded) specimens is enriched for coding regions of the selected cancer-related genes on the panel using a proprietary targeted capture system developed by GeneSort. The captured products are sequenced on an Illumina HiSeq 2500 platform with 2X100 paired-end reads with a median depth of coverage ≥500X. Due to the fact that tumor biopsies usually consist of a mixture of normal and tumor tissue and are likely to contain multiple sub-clones of cancer cells, the deep sequencing (or many reads of the same region) enables characterization of rare tumor variants (≥5% variant allele frequency). Data analysis is performed using a proprietary bioinformatics tools developed in GeneSort. The presence of clinically significant DNA variants, is confirmed where possible (sufficient DNA is available and/or variant allele frequency is ≥20%), by the golden standard Sanger sequencing. In/dels, SNVs, CNVs and fusion are detected.

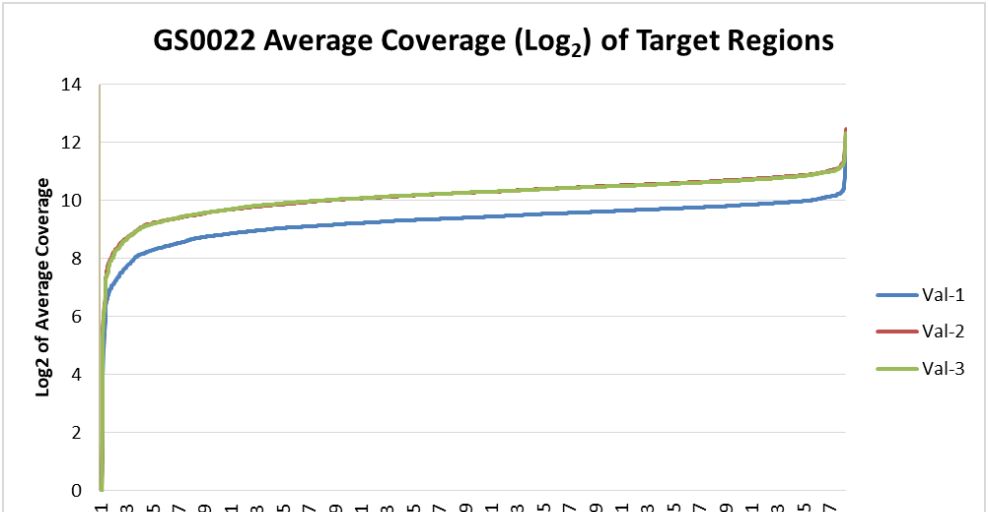
TEST SPECIFICATIONS

Sensitivity, Specificity, and Reproducibility

We confirmed high analytical sensitivity and specificity in a validation study consisting of 16 clinical and four commercial standards samples across 228 classified somatic variants that were duplicated and triplicated to show reproducibility. All targeted regions are fully covered with excellent uniformity spanning 2063 target regions among inter and intra-run replicates (Figure 1). No target region is consistently low enough to require resequencing.

With a threshold of 5% VAF all expected variants were called demonstrating 100% specificity with over 99% reproducibility. Variants were detected as low as 1% VAF. Test specifications are summarized in Table 1.

Figure 1: Log2 mean coverage of two patient samples used in the validation study.



AID GENOMICS

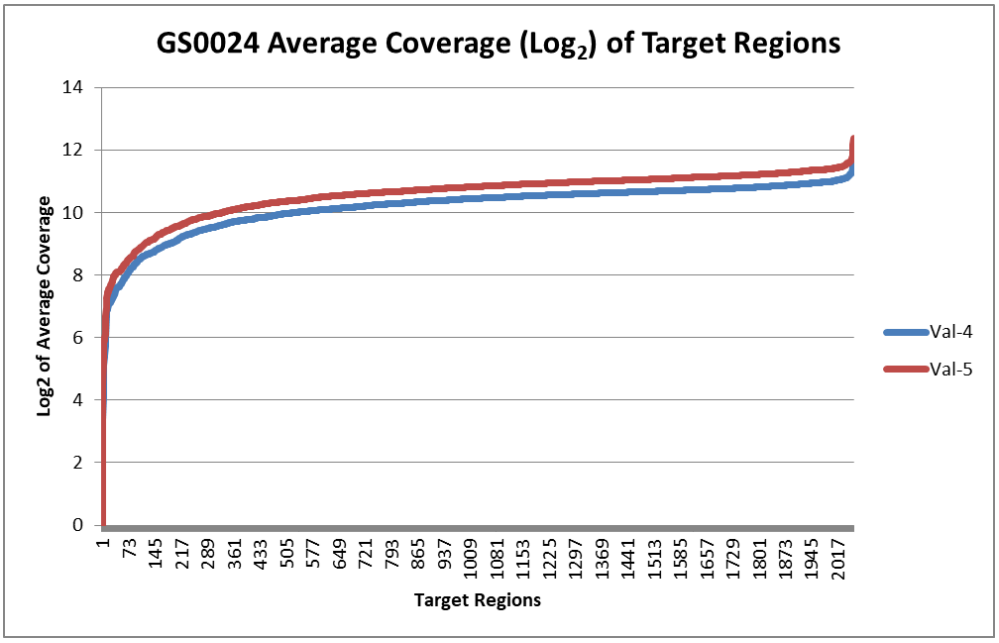


Table 1: Summary of Solid Tumor Profile Test Specifications

Technical Parameters	Test Specifications
Number of Genes	167
Minimum DNA Requirement	2 ng
Genomic Aberrations Detected	SNV, CNV, in/del, Fusions
Analytical Accuracy	99%
Analytical Specificity	100%
Analytical Sensitivity Threshold	≥ 5%
Average Depth of Coverage	≥ 500X
Sample Requirements	FFPE Slides; All Biopsies Accepted
Turnaround Time	< 30 Working Days

AID GENOMICS

THE REPORT

The Solid Profile Test's final report provides a table of variants with possible therapies and relevant technical information regarding sequence depth of coverage, variant frequency and annotation of variants according to clinical databases. In the core of the report, complete information about the genomic alterations is provided with its etiology along with data regarding the possible treatments and detailed references to sustain the results which appear in the report.

Several results outcomes are possible including:

- **Positive result** – Variants with FDA approved drugs for the patients' cancer type or a different cancer type are identified.
- **Positive result** – Variants with ongoing clinical trials are identified.
- **Negative result** – Variants are identified, but there are no approved drugs or clinical trial options.
- **Negative result** – No variants are identified.

REPORTING VARIANTS OF UNKNOWN SIGNIFICANCE (VUS).

VUS represent variants for which clinical decision making is not well defined at the time the report was generated. GeneSort reports include VUS findings so evolving evidence and updated interpretations may be considered for these variants.

REPORTING GERMLINE MUTATIONS

According to the patient's wishes the report can also include germline analysis. In cases where the identified tumor variants are found to be of hereditary origin, genetic counseling is recommended. Genetic counseling and carrier testing for patients' family members can be provided by GeneSort.

REPORTING TUMOR MUTATION BURDEN (TMB)

The Solid Profile Test's report includes TMB index, which is the number of mutations within the coding region of a tumor genome that serves as a quantitative marker shown to correlate with response to immunotherapy treatment. Higher TMB correlates with a higher likelihood of response to immunotherapy, irrespective of the primary tumor site. TMB ranges: low 0.0–10.8 variants/Mb; TMB-high range, 11.7–707.2 variants/Mb.

REPORTING TUMOR MICROSATELLITE INSTABILITY (MSI)

The Solid Profile Test's report includes MSI. Mutations in DNA mismatch repair genes (such as MLH1, MSH2 or MSH6) result in failure to repair errors in repetitive DNA sequences (i.e. microsatellite regions), leading to MSI of the tumors. High MSI, is a predictive marker for better response to immunotherapy.

CONFIRMATION

Confirmation of NGS-identified variants is performed by the golden standard Sanger sequencing. Confirmations are performed when possible (variant allele frequency ≥20% and sufficient DNA is available) on tumor DNA and in germline DNA obtained from patients' saliva samples.

For accurate identification of somatic mutations, the presence of the somatic, tumor reported variants is examined in patient's normal DNA (extracted from saliva sample).

SAMPLE TYPES

(1) Formalin fixed paraffin embedded (FFPE) tumor biopsy - micro-dissected FFPE sections.

(2) Saliva - oral rinse for saliva is collected in 2 ml of mouthwash.

FURTHER INFORMATION

For further information please contact GeneSort by email at info@genesort.com .

SOLID TUMOR PROFILE TEST SELECTED PUBLICATIONS:

(1) Greuber, E. K., Smith-Pearson, P., et al. (2013). *Nat. Rev. Cancer 13*, 559–571.
(2) Kumar, A., Rajendran, V., et al. (2013). *ScientificWorldJournal. 2013*, 756134.
(3) Mure, H., Matsuzaki, K., et al. (2010). *Neuro. Oncol. 12*, 221–232.
(4) Ogawa, S., Takita, J., et al. (2011). *Cancer Sci. 102*, 302–308.
(5) Boursi, B., Sella, T., et al. (2013). *Eur. J. Cancer 49*, 3680–5.
(6) Armaghany, T., Wilson, J. D., et al. (2012). *Gastrointest. Cancer Res. 5*, 19–27.
(7) Lin, C., Song, W., et al. (2014). *Onco. Targets. Ther. 7*, 315–24.
(8) Tecza, K., Pamula-Pilat, J., et al. (2015). *J. Exp. Clin. Cancer Res. 34*, 2.
(9) Mhawech-Fauceglia, P., Wang, D., et al. (2014). *Gynecol. Oncol. 132*, 593–8.
(10) Hathcock, K. S., Padilla-Nash, H. M., et al. (2015). *Blood 126*, 2291–301.
(11) Bolt, J., Vo, Q. N., et al. (2005). *Oral Oncol. 41*, 1013–20.
(12) Park, K., Chen, Z., et al. (2014). *Hum. Pathol. 45*, 2136–43.

(13) Churi, C. R., Shroff, R., et al. (2014). *PLoS One 9*, e115383.
(14) Schindler, G., Capper, D., et al. (2011). *Acta Neuropathol. 121*, 397–405.
(15) Xue, W.-Q., He, Y.-Q., et al. (2014). *Sci. Rep. 4*, 6791.
(16) Magdalena, K., Monika, Z., et al. (2016). *Cancer Med.* 1–7.
(17) Muggia, F., and Safra, T. (2014). *Anticancer Res. 34*, 551–6.
(18) Tian, C. Q., Darcy, K. M., et al. (2013). *Front. Oncol. 3*.
(19) Ma, X. D., Cai, G. Q., et al. (2013). *Biochem. Biophys. Res. Commun. 433*, 232–6.
(20) Bhalla, A., and Saif, M. W. (2014). *JOP 15*, 340–3.
(21) Segev, Y., Iqbal, J., et al. (2013). *Gynecol. Oncol. 130*, 127–31.
(22) Dun, B., Sharma, A., et al. (2013). *Am. J. Transl. Res. 6*, 28–42.
(23) Wong, H., and Yau, T. (2013). *Therap. Adv. Gastroenterol. 6*, 15–31.
(24) Baker, S. J., and Reddy, E. P. (2012). *Genes Cancer 3*, 658–69.
(25) Wu, C.-W., Cheng, Y.-W., et al. (2014). *Mol. Vis. 20*, 1048–56.
(26) Stolz, A., Ertych, N., et al. (2011). *Clin. Cancer Res. 17*, 401–5.
(27) Xu, J., Prosperi, J. R., et al. (2015). *PLoS One 10*, 1–11.
(28) Pan, W.-W., Zhou, J.-J., et al. (2013). *J. Biol. Chem. 288*, 13620–13630.
(29) Schultz, K. A. P., Pacheco, M. C., et al. (2011). *Gynecol. Oncol. 122*, 246–250.
(30) Loghavi, S., Zuo, Z., et al. (2014). *J. Hematol. Oncol. 7*, 74.
(31) Shivarov, V., Gueorguieva, R., et al. (2013). *Leuk. Res. 37*, 1445–50.
(32) Gaidzik, V. I., Schlenk, R. F., et al. (2013) *121*, 4769–4777.
(33) Padfield, E., Ellis, H. P., et al. (2015). *Front. Oncol. 5*, 5.
(34) Gan, H. K., Burgess, A. W., et al. (2012). *Cancer Res. 72*, 2924–2930.
(35) Sun, Z., Shi, Y., et al. (2015). *J. Cell. Mol. Med. XX*, n/a-n/a.
(36) Fanotto, V., Ongaro, E., et al. (2016). *Oncotarget 7*, 69060–69074.
(37) Han, X.-J., Sun, L.-F., et al. (2013). *PLoS One 8*, e75288.
(38) Thomas, L., Spurlock, G., et al. (2012). *Eur. J. Hum. Genet. 20*, 411–9.
(39) Barsotti, A. M., Ryskin, M., et al. (2015). *Oncotarget 6*, 2928–38

(41) Sato, M., Rodriguez-barrueco, R., et al. (2014). *Oncotarget 6*.
(42) Matsuda, Y., Ueda, J., et al. (2012). *Patholog. Res. Int. 2012*.
(43) Kasiri, S., Ansari, K. I., et al. (2013). *RSC Adv. 3*, 3260.
(44) Cathcart-Rake, E., Corless, C., et al. (2014). *J. Thorac. Oncol. 9*, e75–e78.
(45) Membrino, A., Cogoi, S., et al. (2011). *PLoS One 6*.
(46) Cohen, A. L., Holmen, S. L., et al. (2013). *Curr. Neurol. Neurosci. Rep. 13*, 345.
(47) Zhang, C., Moore, L. M., et al. (2013). *Neuro. Oncol. 15*, 1114–1126.
(48) Treliński, J., and Robak, T. (2013). *Curr. Med. Chem. 20*, 1147–61.
(49) Meyer, S. C., and Levine, R. L. (2014). *Clin. Cancer Res. 20*, 2051–2059.
(50) Blandamura, S., Alessandrini, L., et al. (2014). *Pathol. Res. Pract. 210*, 392–6.
(51) Mainetti, L. E., Zhe, X., et al. (2015). *Int. J. cancer 136*, 11–20.
(52) Tang, Y.-L., Fan, Y.-L., et al. (2014). *Oncotarget 5*.
(53) Nobusawa, S., Stawski, R., et al. (2011). *Neuropathology 31*, 583–8.
(54) Parsons, B. L., and Myers, M. B. (2013). *Per. Med. 10*, 191–199.
(55) Karachaliou, N., Mayo, C., et al. (2013). *Clin. Lung Cancer 14*, 205–214.
(56) Deschoolmeester, V., Baay, M., et al. (2010). *Oncologist 15*, 699–731.
(57) Li, B., Liu, H. Y., et al. (2014). *Genet. Mol. Res. 13*, 7513–7518.
(58) Ketscher, A., Jilg, C. A., et al. (2014). *Oncogenesis 3*, e120.
(59) Kim, H. S., Do, S. I., et al. (2015). *Oncol. Lett. 9*, 1261–1265.
(60) Akinleye, A., Furqan, M., et al. (2013). *J. Hematol. Oncol. 6*, 27.
(61) Pham, T. T., Angus, S. P., et al. (2013). *Genes Cancer 4*, 419–26.
(62) Stephens, P. J., Tarpey, P. S., et al. (2012). *Nature 486*, 400–4.
(63) Peterson, E. A., Teffera, Y., et al. (2015). *J. Med. Chem. 58*, 2417–30.
(64) Szabó, D. R., Luconi, M., et al. (2014). *Lab. Invest. 94*, 331–9.
(65) Yamada, N., Noguchi, S., et al. (2014). *Biochim. Biophys. Acta - Mol. Cell Res. 1843*, 590–602.
(66) Lin, F., Ding, R., et al. (2014). *Cancer Cell Int. 14*, 58.
(67) Harkness, E. F., Barrow, E., et al. (2015). *J. Med. Genet. 52*, 553–6.
(68) Angelova, S., Jordanova, M., et al. (2011). *Balk. J. Med. Genet. 14*, 17–24.
(69) Edelbrock, M. A., Kaliyaperumal, S., et al. (2013). *Mutat. Res. Mol. Mech. Mutagen. 743–744*, 53–66.
(70) Giannakouros, P., Comamala, M., et al. (2015). *Am. J. Cancer Res. 5*, 219–30.
(71) Das, S., Rachagani, S., et al. (2015). *Oncotarget 6*.
(72) Felder, M., Kapur, A., et al. (2014). *Mol. Cancer 13*, 129.
(73) Aravalli, R. N., Talbot, N. C., et al. (2015). *World J. Gastroenterol. 21*, 2011–2029.
(74) Avbelj, M., Wolz, O.-O., et al. (2014). *Blood 124*, 3896–3904.

(75) Shimizu, H., Astapova, I., et al. (2015). *Mol. Cell. Biol. 35*, 555–65.
(76) Catic, A., Suh, C. Y., et al. (2013). *Cell 155*, 1380–1395.
(77) Garcia-Becerra, R., Santos, N., et al. (2013). *Int. J. Mol. Sci. 14*, 108–145.
(78) Wang, W., Song, X.-W., et al. (2016). *Cell. Oncol. 39*, 129–137.
(79) Abramowicz, A., and Gos, M. (2014). *Dev. period Med. 18*, 297–306.
(80) Huang, T., Zhou, Y., et al. (2016). *Mol. Cancer 15*, 1–12.
(81) Yuan, Z., Friedmann, D. R., et al. (2012). *J. Biol. Chem. 287*, 34904–34916.
(82) Burgess, M. R., Hwang, E., et al. (2014) *124*, 3947–3955.
(83) Vujic, I., Posch, C., et al. (2014). *Oncotarget 5*, 7936–44.
(84) Zhang, F., Ma, J., et al. (2009). *Curr. Biol. 19*, 524–9.
(85) Medvedovic, J., Ebert, A., et al. (2011). *Adv. Immunol. 111*, 179–206.
(86) Terada, T. (2012). *Prostate 72*, 1150–6.
(87) Siegel, R., Ward, E., et al. (2011). *CA. Cancer J. Clin. 61*, 212–36.
(88) Payne, S. N., Maher, M. E., et al. (2015). *Oncogenesis 4*, e169.
(89) Cizkova, M., Susini, A., et al. (2012). *Breast Cancer Res. 14*, R28.
(90) Yardley, D. A. (2013). *Breast Cancer (Auckl). 7*, 7–22.
(91) Rudd, M. L., Price, J. C., et al. (2011). *Clin. Cancer Res. 17*, 1331–1340.
(92) Wang, S., Cheng, Y., et al. (2016). *Sci. Rep. 6*, 39630.
(93) Milella, M., Falcone, I., et al. (2015). *Front. Oncol. 5*, 24.
(94) Ilmarinen, T., Munne, P., et al. (2017). *Oral Oncol. 73*, 77–82.
(95) Wang, R., Wang, L., et al. (2014). *Clin. Cancer Res. 20*, 4107–4114.
(96) Luo, Y., Tsuchiya, K., et al. (2013). *Oncogene 32*, 2037–2047.
(97) Taskesen, E., Staal, F. J. T., et al. (2015). *BMC Bioinformatics 16 Suppl 4*, S4.
(98) Voorneveld, P. W., Jacobs, R. J., et al. (2015). *Transl. Oncol. 8*, 18–24.
(99) Shtivelman, E., Beer, T. M., et al. (2014). *Oncotarget 5*, 7217–59.
(100) Yang, S., Chen, L., et al. (2017). *Clin. Proteomics 14*, 31.

(102) Fabrizio, D. A., Jr, T. J. G., et al. (2018) *9*, 610–617