MDIC SRS Report: Somatic Variant Reference Samples for NGS

Landscape of Available Reference Samples

A Report from the Landscape Analysis Sub-Group
of the Medical Device Innovation Consortium (MDIC)

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EXECUTIVE SUMMARY

The Landscape Analysis report was created from the work conducted by MDIC's Somatic Reference Sample initiative. The Landscape Analysis subgroup was charged with conducting a thorough analysis of projects related to NGS reference samples to a) avoid duplicative efforts; b) identify gaps where the desired optimum reference samples are not yet developed or available; and c) help define specific gaps and unmet needs with respect to, for example, sample type(s), genes, variants, and so forth. The sub-group has multiple stakeholders including reference sample manufacturers, regulatory agencies, validation study leaders, and end users of reference samples who contributed to this comprehensive summary identifying other efforts for development and evaluation of NGS reference samples which may inform and complement the SRS goals. The report is intended for the use of the diagnostic testing community for a variety of applications, including (but not limited to) assay development, test validation, and quality control.

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DISCLAIMER

This report was completed by the Medical Device Innovation Consortium's Clinical Diagnostics Somatic Reference Samples working group. The views, statements, and opinions presented in this report does not necessarily represent the views or opinions of MDIC and the working group members. This report is intended solely for informational purposes and is not to be construed, under any circumstances, by implication or otherwise, as an endorsement or promotion of any of the products or companies mentioned in this report. Information was obtained from sources believed to be reliable but is in no way guaranteed. MDIC and the working group members do not accept liability for the websites or URL links being accurate, complete, or up-to-date for the contents of external links. MDIC and the work group members expressly distance themselves from the contents of the linked pages, over the structure of which they have no control. This statement is valid for all of the links on the websites and for all contents of the pages to which the links or banners lead. Although the content included in the report was current at the time of release, some projects may have been inadvertently overlooked. The products listed in this report may change over time, please check with the projects listed for products that are currently available.

1. OVERVIEW

The Medical Device Innovation Consortium (MDIC) is the first public-private partnership created with the sole objective of advancing medical device regulatory science for patient benefit. Formed in late 2012, MDIC brings together representatives of the FDA, NIH, CMS, industry, non-profits and patient organizations to improve processes for the development, assessment and review of new medical technologies. Our work is unique and complementary to trade associations such as AdvaMed and the Association of Medical Diagnostics Manufacturers. Members of MDIC share a vision of providing U.S. patients with timely access to high-quality, safe and effective medical devices.

MDIC aims to identify and pursue projects that will improve diagnostic testing and product development using novel regulatory science approaches developed through collaboration among MDIC stakeholders. Providing a predictable path for innovation will help patients benefit through quicker access to more cost-effective advanced diagnostic technologies in less time.

The focus of the MDIC Somatic Reference Samples (SRS) Project is the establishment of a public-private partnership to guide the development of reference samples that can be used to develop and validate NGS-based oncologic tests. Clinical oncology is being transformed by next generation sequencing (NGS)-based diagnostics. This new technology can enable the rapid identification of potentially significant genetic variations across nearly the entire genome and the results are being increasingly used to determine the best course of treatment for oncology patients. However, the absence of well-characterized and community-validated reference samples and data benchmarks create challenges for the efficient development of these critical tests and appropriate clinical use of results. Currently, many commercial manufacturers and clinical laboratories develop their own contrived samples and mixtures for validation of oncology tests because well-characterized reference samples do not exist. This makes it difficult to efficiently develop or effectively compare tests and methodologies. Reference samples which may be used to assess the various components of an NGS test are needed to ensure confidence in the results being provided by different NGS clinical tests and laboratories.

The ultimate goal of the MDIC SRS project is to develop properly consented, widely shareable reference samples that can be made available to the public and mass produced to enable efficient development and improve the accuracy, reliability and transparency of NGS-based oncology tests. These samples will be quality checked and validated, and made available in varying forms (e.g., cells, DNA/RNA, FFPE), to represent most potential variations and allele fractions of interest (e.g., ploidy, fusions, large and small indels, CNVs, homopolymeric regions), and represent tumor/normal matched pairs.

The MDIC SRS subgroup for Landscape Analysis has multiple stakeholders including reference sample manufacturers, regulatory agencies, validation study leaders, and end users of reference samples. The Landscape Analysis subgroup was charged with conducting a thorough analysis of projects related to NGS reference samples to a) avoid duplicative efforts; b) identify gaps where the desired optimum

reference samples are not yet developed or available; and c) help define specific gaps and unmet needs with respect to, for example, sample type(s), genes, variants, and so forth.

This report contains a comprehensive summary identifying other efforts for development and evaluation of NGS reference samples which may inform and complement the SRS goals. It is provided here for the use of the diagnostic testing community for a variety of applications, including (but not limited to) assay development, test validation, and quality control.

2. SYNTHETIC DNA

a. Completed

Project	SeraCare NGS reference Materials - Tumor Profiling
Description	Expert-designed constructs with clinically relevant variants. Highly multiplexed with up to 40 variants or 16 gene fusions in a single reference material. Coverage across broad variant types - SNVs, INDELs, CNVs, and gene fusions. Manufactured in GMP-compliant in ISO9001 and ISO 13485-certified facilities
Reference	https://www.seracare.com/globalassets/seracare-resources/ps-Seraseq-tri-level-tumor-mutation-dna-mix-v2-high-concentration-hc.pdf https://www.seracare.com/globalassets/seracare-resources/ps-Seraseq-tri-level-tumor-mutation-dna-mix-v2-low-concentration-lc.pdf https://www.seracare.com/Seraseq-FFPE-Tumor-Fusion-RNA-Reference-Material-v2-0710-0129/
Genes & Variants	*ABL1, AKT1, ALK, APC, ASXL1, ATM, BRAF, BRCA, CARL, CBL, CD74-ROS1, CEBPA, CSF3R, CTNNB1, EGFR, EML4-ALK, ERBB2, FGFR3, FLT3, FOXL2, GNA11, GNAQ, GNAS, IDH1, JAK2, KIT, KRAS, MET, MPL, MYD88, NCOA4-RET, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, RET, SF3B1, SMAD4, SRSF2, TP53, TPR-ALK, U2AF1*
Reference Sample Type	**Tri-Level Tumor DNA V2**- Multiplexed synthetic DNA fragments "blended" with GM24385 human genomic background, 40 variants in 28 genes; DNA Provided as High and Low Concentration for a range of 10%, 7%, & 4% VAF. **FFPE Fusion RNA V2** - 16 fusion gene transcripts multiplexed "engineered" into GM24385 cells and FFPE. RNA Transcript levels are quantified by digital PCR.
Validation Methods	All internal (SeraCare) validation of variants/allele frequency is performed by digital PCR.
Publicly available	Yes
Contact for additional information	https://seracare.com/Controls---Reference-Materials-NGS-Somatic-Cancer-Tumor-Profiling/

Project	Thermo-Fisher/Acrometrix™ Oncology Hotspot Control
Description	Expert-designed constructs with clinically relevant variants. Highly multiplexed with up to 40 variants or 16 gene fusions in a single reference material. Coverage across broad variant types - SNVs, INDELs, CNVs, and gene fusions. Manufactured in GMP-compliant in ISO9001 and ISO 13485-certified facilities
Reference	https://www.thermofisher.com/order/catalog/product/969056
Genes & Variants	53 genes represented: *ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, FOXL2, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, KRAS, MAP2K1, MET, MLH1, MPL, MSH6, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53, VHL* More than 500 mutations from the Catalogue of Somatic Mutations in Cancer (COSMIC) database. See the AcroMetrix Oncology Hotspot Control Package Insert [EN] at https://www.thermofisher.com/order/catalog/product/969056
Reference Sample Type	A highly-multiplexed, proprietary DNA quality control; mixture of synthetic DNA and genomic DNA in a stabilizing buffered solution. The genomic DNA is derived from the same cell line(GM24385) that is used for the development of a NIST Genome in a Bottle reference material. The synthetic DNA, which is present at low frequencies, introduces hundreds of variants that are frequently found as somatic mutations in cancer.
Validation Methods	Three different library preparation test panels were used to test NGS detection of variants in the AcroMetrix Oncology Hotspot Control: • Ion AmpliSeq™ Cancer Hotspot Panel v2 (CHPv2) on the Personal Genome Machine™ (PGM™) • TruSeq™ Amplicon Cancer Panel (TSACP) on the MiSeq™ • TruSight™ Tumor Panel (TSTP) on the MiSeq™
Publicly available	Yes
Contact for additional information	https://www.thermofisher.com/order/catalog/product/969056

a. In progress

Project	EndoGenus Toolkit
Description	Absolute quantification of multiple DNA tumor markers in plasma using a method that overcomes certain biologic (e.g. inflammation) and technical interferences (leukocyte lysis during blood collection and handling *ex vivo*) that hamper current massive parallel sequencing technology. Applying the Toolkit to mock plasma specimens may yield sensitive, specific, linear and reproducible sequencing results for multiple tumor markers. In blood from active cancer subjects, they will show that the Toolkit helps overcome pre-analytic problems associated with blood storage.
Reference	http://grantome.com/grant/NIH/R21-CA229037-01
Genes & Variants	Not specified
Reference Sample Type	Synthetic DNAs added to human plasma. Also bioinformatic scripts to convert tumor marker levels from fractions to absolute concentrations.
Validation Methods	Co-amplification of synthetic DNAs spiked into plasma before library preparation, and informatic scripts to normalize read counts of each tumor marker that is detected after DNA sequencing. Importantly, tumor marker levels are expressed in copies per mL of plasma to facilitate harmonization with units of measurement in quantitative PCR. Researchers plan to show that this reflects clonal abundance.
Publicly available	Not yet
Contact for additional information	Margaret Gulley, Univ. North Carolina, Chapel Hill

Project	SeraCare TMB Working Group Reference Materials – Tumor Mutational Burden (TMB)
Description	Design, development, and performance of TMB reference materials (RMs) intended to aid in the establishment of performance characteristics and standardization of TMB measurements"
Reference	https://www.seracare.com/globalassets/seracare-resources/ps-Seraseq-tri-level-tumor-mutation-dna-mix-v2-high-concentration-hc.pdf https://www.seracare.com/globalassets/seracare-resources/ps-Seraseq-tri-level-tumor-mutation-dna-mix-v2-low-concentration-lc.pdf https://www.seracare.com/Seraseq-FFPE-Tumor-Fusion-RNA-Reference-Material-v2-0710-0129/ https://www.seracare.com/Controls---Reference-Materials-NGS-Somatic-Cancer-Heme-Malignancy/
Genes & Variants	Cancer Immunotherapy diagnostic assays; WES and large gene panels > 300 genes, variants not yet specified
Reference Sample Type	Format A: Matched tumor normal cancer cell lines, FFPE, TMB H/M/L. Format B: Contrived Reference Materials which consist of known variants (synthetic DNA or plasmids) spiked into GIAB GM24385, gDNA TMB H/M/L.
Validation Methods	NGS; will include inter-laboratory testing
Publicly available	Not yet
Contact for additional information	Russell Garlick, PhD CSO. rgarlick@seracare.com 01-508-244-6435

3. Genomic DNA

a. Completed

Project	American Type Culture Collection (ATCC) Quantitative Reference Standards
Description	DNA isolated from select ATCC cell line quantified for various oncology biomarkers
Reference	https://www.atcc.org/Products/Nucleic_Acid_Proteins_and_Cell_Extracts/Quantitative_Nucleic_Acids/New_quantitated_ACS_DNA.aspx?dsNav=Ro:0
Genes & Variants	*BRAF, EGFR, HER2, KRAS, MET, MYC, NRAS, PTEN, TP53* derived from various cell lines *BRAF* p.V600E; *EGFR* pELREA746del; p.T790M; p.L858R; and p.G719S; *HER 2* amplification; *KRAS* p.G12D; p.G13D; and p.G12V *MET* amplification; *MYC* amplification; *NRAS* p.Q61R *PTEN* p.R130fs; *TP53* pR175H; *p*.R249S ; R248Q; p.G245S; p.R273H
Reference Sample Type	Quantitative Genomic DNA, cell-line derived
Validation Methods	Electrophoresis DNA - M.W. ≥48 kb (or higher than uppermost band of the high MW DNA ladder) Electrophoresis RNA - Content No visible RNA detected in the agarose gel STR Identical STR profile to cell line source DNA Concentration (PicoGreen® method) Report Results Purity (A260/A280) Ratio 1.7 to 2.1 Total DNA amount (PicoGreen® method) ≥ 3 µg Mutation allelic frequency Report results: NGS (Coverage > 10,000X)* Absolute/relative gene copies/ng DNA Report results: ddPCR™ (Average of nine data points)* Electrophoresis DNA - Digestion Verified by restriction enzyme
Publicly available	Yes
Contact for additional information	https://www.atcc.org/Support/Technical_Support.aspx

Project	Horizon Dx gDNA - OncoSpan Reference Standard
Description	OncoSpan is the largest and most extensive cell line-derived Reference Standard to date, featuring 386 variants across 152 key cancer genes. Provided with batch-specific NGS data, giving knowledge and confidence of the background genotype of this cell-line-derived Reference Standard.
Reference	https://www.horizondiscovery.com/reference-standards/all-products/oncospan-reference-standard
Genes & Variants	386 variants across 152 key cancer genes *ABL1, AKT1, AKT2, ALK, APC, AR, ARID1A, ATR, ATRX, AXL, BARD1, BCL6, BLM, BMPR1A, BRAF, BRCA1, BRCA2, BTK, BUB1B, CARD11, CCND1, CCND3, CCNE1, CD79B, CDH1, CDK12, CDK4, CEP57, CFH, CREBBP, CSF1R, CTNNB1, DDR2, DIS3L2, DNMT3A, EGFR, EML4, EP300, EPCAM, ERBB2, ERBB3, ERCC1, ERCC2, ERCC4, ERCC5, ERG, ETS1, ETV4, EWSR1, EXT1, FANCA, FANCD2, FANCE, FANCG, FANCI, FANCM, FBXW7, FGF10, FGF2, FGF3, FGF6, FGFR1, FGFR3, FLCN, FLI1, FLT1, FLT3, FZR1, GATA2, GATA3, GEN1, GNA11, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK1, JAK2, JAK3, KDR, KIT, KRAS, LDLR, MAGI1, MAP2K1, MAP2K2, MAX, MDM4, MED12, MET, MLH1, MLLT3, MMAB, MRE11, MSH2, MSH3, MSH6, MTOR, NBN, NF1, NFE2L2, MOTCH1, NOTCH2, NOTCH3, NRAS, NRG1, NTRK1, NTRK3, PDGFRA, PDGFRB, PIK3CA, PIK3CD, PIK3CG, PIK3R1, PMS2, PPARG, PPP2R2A, PRKAR1A, PROC, PTCH1, PTPN11, RAD51B, RAD54L, RAF1, RB1, RBM45, RECQL4, RET, RHBDF2, ROS1, RPS6KB1, SDHB, SF3B1, SF3B2, SLTM, SLX4, SMARCB1, SMO, SMOX, STK11, TERT, TET2, TFRC, TP53, TP53BP1, TSC1, TSC2, WRN, XPA, XPC, ZNF395* https://info.horizondiscovery.com/oncospan-hd827-ngs-variants-data
Reference Sample Type	Genomic DNA, cell-line derived
Validation Methods	Allelic Frequency = Droplet Digital PCR Genotype = Next Generation Sequencing Quality = Agarose gel electrophoresis Quantification = Spectrophotometry (A260) This includes 249 variants with a COSMIC ID and 30 INDELs (24 deletions and 6 insertions, ranging from 2-16 base pairs). Variants are present between 1-100% allelic frequency (AF), with 52 variants present at ≤ 20% AF for LOD determination of lab's assay. Every batch of OncoSpan DNA has 25 variants confirmed by ddPCR, in addition to being fully exome sequenced by GATC-Eurofins at 500x coverage using Agilent SureSelect Human All Exon V6 kit and Illumina sequencing to ISO 17025. This provides an accurate and reliable truth set for comparison to lab assay's performance.
Publicly available	Yes
Contact for additional information	technical@horizondiscovery.com

Project	Horizon Dx gDNA - Quantitative Multiplex Reference Standard (QMRS)
Description	The Quantitative Multiplex DNA Reference Standard is a highly-characterized, biologically-relevant quality control material used to assess the performance of NGS assays that detect somatic mutations. The QMRS portfolio covers multiple endogenous SNPs, insertions and deletions. The QMRS includes 11 mutations at 0.8-24.5% allelic frequency in genomic DNA, FFPE and Formalin-Compromised DNA format to enable pre-analytical and analytical workflow validation.
Reference	https://www.horizondiscovery.com/reference-standards/all-products/quantitative-multiplex-reference-standard-hd701
Genes & Variants	*BRAF, KIT, EGFR, KRAS, NRAS, PIK3CA; plus ALK, ABL2, APC, AEID1A, BRCA2, CDX1, EP300, FBXW7, FGFR1, FLT3, IDH1, MET, MLH1, NF1, NF2, NOTCH1, NTRK1, PDGFRA* https://www.horizondiscovery.com/reference-standards/all-products/quantitative-multiplex-reference-standard-hd701
Reference Sample Type	Genomic DNA Cell Line Background: HCT116/RKO/SW48
Validation Methods	Allelic Frequency - Droplet Digital PCR™ Genotype - Sanger sequencing of locus specific PCR Quality - D1000 DNA ScreenTape assay Quantification - Qubit dsDNA BR Assay Amplifiability - Droplet Digital PCR™ https://www.horizondiscovery.com/media/resources/data/Reference-standards/certificate-of-analysis_gDNA-multiplex.pdf
Publicly available	Yes
Contact for additional information	technical@horizondiscovery.com

Project	Horizon Dx gDNA - Structural Multiplex Reference Standard
Description	The Structural gDNA Multiplex Reference Standard provides biologically relevant quality control material, which can be used to assess the performance of NGS assays that detect complex structural variants. This product is designed to challenge both molecular and bioinformatic work flows by providing validated copy number variants/amplifications, gene fusions, and large insertions/deletions. Additionally, one may examine the genomic context of variants within regions of specific GC-content (high vs. low). The Structural Multiplex Reference Standard includes 9 variants validated by ddPCR, with most of them at 5% allelic frequency. Includes *RET* and *ROS1* fusion variants, large indels, and *MYC-N* and *MET* focal amplifications
Reference	https://www.horizondiscovery.com/reference-standards/all-products/structural-multiplex-reference-standard-hd753 https://www.horizondiscovery.com/media/datasheets/structural-multiplex-product-info-sheet.pdf
Genes & Variants	*AKT1, BRAF, BRCA2, EGFR, FBXW7, FLT3, GNA11, KRAS, MET, MYC-N NOTCH1, PIK3CA, RET, ROS1*, SNV High GC, SNV low GC, Long Insertion, Long Deletion, Short Deletions (4), Fusion, CNV, SNVs (3). The Structural Multiplex includes 9 ddPCR-validated mutations, with most of them centered at 5% allelic frequency. Highlight features of the Structural Multiplex include RET and ROS1 fusion variants, MYC-N and MET focal amplifications, and a BRCA2 variant. The Structural Multiplex is also available in cfDNA (HD786) and FFPE (HD789) format.
Reference Sample Type	Genomic DNA
Validation Methods	Allelic Frequency - Droplet Digital PCR™ Genotype - Sanger sequencing of locus specific PCR Quality – Agarose gel electrophoresis Quantification – Spectrophotometry (A260) https://www.horizondiscovery.com/media/resources/data/Reference-standards/certificate-of-analysis_gDNA-multiplex.pdf
Publicly available	Yes
Contact for additional information	technical@horizondiscovery.com

Project	Horizon Dx gDNA - Tru-Q Reference Standards – 0, 1, 2, 3, 4, 7
Description	The Tru-Q DNA Reference Standard portfolio covers multiple endogenous SNPs, insertions and deletions mixed to different allele frequencies in multiplex samples. Tru-Q NGS DNA Reference Standards 1 through 4 are manufactured using ten engineered cell lines each, and mixed to generate a 5% Allelic Frequency multiplex sample (5% Tier) for 10 mutations. These may be diluted to even lower allelic frequencies using the Tru-Q 0 Wild Type standard. Furthermore, because the Tru-Q series has 4 different standards at the 5% allelic frequency range, users may rotate these as "blinded" samples for proficiency testing in the laboratory. The Tru-Q 7 Reference Standard is manufactured using forty engineered cell lines and mixed to generate a 1% Allelic Frequency multiplex sample (1.3% Tier).
Reference	https://www.horizondiscovery.com/tru-q-0-100-wildtype-reference-standard-hd752 https://www.horizondiscovery.com/reference-standards/all-products/tru-q-7-1-3-tier-reference-standard-hd734
Genes & Variants	Tru-Q 0 = 100% Wild Type DNA for 40 verified variants found in Tru-Q 1, 2, 3, 4, and Tru-Q 7. Tru-Q 1 = *BRAF, EGFR, FLT3, IDH1, JAK2, KRAS, MEK, NOTCH1, NRAS, PIK3CA* Tru-Q 2 = *ALK, BRAF, EGFR, FGFR2, GNAQ, IDH2, KRAS, NRAS, PIK3CA* Tru-Q 3 = *BRAF, EGFR, FLT3, GNA11, IDH1, KRAS, MET, NRAS, PIK3CA* Tru-Q 4 = *ABL1, BRAF, EGFR, IDH2, KIT, KRAS, NRAS, PDGFRA, PIK3CA* Tru-Q 7 = *ABL1, ALK, BRAF, EGFR, FGFR2, FLT3, GNA11, GNAQ, IDH1, IDH2, JAK2, KIT, KRAS, MEK, MET, NOTCH1, NRAS, PDGFRA, PIK3CA*
Reference Sample Type	Genomic DNA
Validation Methods	EGFR G719S, BRAF V600E and KRAS G13D are checked by ddPCR in every batch of Tru-Q 0 to verify the correct blending ratio of wildtype parental cell lines. **Allelic Frequency -** Droplet Digital PCR™ **Genotype -** Sanger sequencing of locus specific PCR **Quality -** Agarose gel electrophoresis **Quantification -** Spectrophotometry (A260) https://www.horizondiscovery.com/media/resources/data/Reference-standards/certificate-of-analysis_gDNA-multiplex.pdf
Publicly available	Yes
Contact for additional information	technical@horizondiscovery.com

Project	HorizonDx Formalin Compromised (mild to severe) DNA - Quantitative Multiplex Reference Standard
Description	Formalin-Compromised DNA Standards vary in levels of fragmentation and formalin damage allowing challenges to the NGS assay performance. Appropriate for any NGS library preparation including whole-genome, whole-exome, custom capture and targeted amplicon panels to support the development and continued validation of Next Generation Sequencing platforms.
Reference	Formalin Compromised (Mild) https://www.horizondiscovery.com/reference-standards/all-products/quantitative-multiplex-reference-standard-hd798 Formalin Compromised (Moderate) https://www.horizondiscovery.com/quantitative-multiplex-formalin-compromised-moderate-reference-standard-hd799 Formalin Compromised (Severe) https://www.horizondiscovery.com/quantitative-multiplex-reference-standard-hd803
Genes & Variants	*BRAF,* V600E *cKIT,* D816V *EGFR,* L858R, ΔE746 - A750, T790M, G719S *KRAS,* G12D. G13D *NRAS,* Q61K, A59T *PI3KCA,* H1047T, E545K
Reference Sample Type	Formalin Compromised (mild, moderate, severe) genomic DNA
Validation Methods	**Quality** D1000 DNA Screen Tape assay **Allelic Frequency** Droplet Digital™ PCR **Genotype** Sanger sequencing of locus specific PCR **Quantification** Qubit dsDNA BR Assay (post-fragmentation) **Amplifiability -** Droplet Digital PCR™
Publicly available	Yes
Contact for additional information	technical@horizondiscovery.com https://www.horizondiscovery.com/media/resources/data/Reference-standards/HD799_21983.pdf

Project	NIST Gene Copy Number Variation Reference Materials
Description	NIST Standard Reference Material SRM® 2373 was developed to improve the measurements of the *ERBB2/HER2* gene amplification in DNA samples. SRM® 2373 consists of genomic DNA extracted from five breast cancer cell lines (SK-BR-3, MDA-MB-231, MDA-MB-361, MDA-MB-453, and BT-474) with different amounts of amplification of the *ERBB2/HER2* gene. NIST Reference Material (RM) 8366 is intended to harmonize the measurements of ratios of the human epidermal growth factor gene (*EGFR*) and human MET proto-oncogene, receptor tyrosine kinase gene (*MET*) to unamplified reference genes. The six components are genomic DNA materials derived from human cell lines A-431, BT-20, C32, Daoy, Hs 746T, and SNU-5.
Reference	SRM2373 References: https://europepmc.org/abstract/pmc/pmc4906140 https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5397679/ RM 8366: https://www-s.nist.gov/srmors/view_detail.cfm?srm=8366
Genes & Variants	*ERBB2/HER2*; *EGFR*; *MET*
Reference Sample Type	Human Genomic DNA, cell-line derived
Validation Methods	For SRM 2373, the copy numbers of the *ERBB2/HER2* gene and selected reference genes (*DCK*, *EIF5B*, *RPS27A*, and *PMM1*) were measured using quantitative PCR and digital PCR assays. The certified values are the ratios of the *ERBB2/HER2* gene copy numbers to the reference gene copy numbers. For RM8366, the copy numbers of the *EGFR* and *MET* and selected reference genes (*DCK*, *EIF5B*, *RPS27A*, and *PMM1*) were measured by digital PCR assays. The reference values are the ratios of the *EGFR* or *MET* gene copy numbers to the reference gene copy numbers.
Publicly available	Publicly available as NIST SRM 2373 and RM 8366: http://www.nist.gov/srm/
Contact for additional information	Kenneth D. Cole, National Institute of Standards and Technology (NIST) https://www.nist.gov/people/kenneth-d-cole

Project	Platinum Genomes
Description	A reference data set of 5.4 million phased human variants validated by genetic inheritance from sequencing a three-generation 17-member pedigree. NOTE: germline variants not somatic, but may be mixed to mimic somatic variant fractions
Reference	Eberle, MA, Fritzilas, E, Krusche, P, et al. Genome Research 27: 157-164, 2017.
Genes & Variants	5.4 million phased variants- 4.7 million SNVs + 0.7 million small indels (1-50 bp)
Reference Sample Type	Genomic DNA, raw sequence data for a full pedigree is available from dbGAP. https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs001224.v1.p1
Validation Methods	Illumina (San Diego and UK), Wellcome Trust Center for Human Genetics, Bid Data Institute. HiSeq 2000 50X-200X, data fro CGI, six informatics pipelines 97.0% of autosomes are covered, 92.5% of chromosome X is covered. Also have data from HiSeqX and NovaSeq.
Publicly available	Yes, from Coriell
Contact for additional information	http://www.genome.org/cgi/doi/10.1101/gr.210500.116

Project	Thermo-Fisher/Acrometrix™ Oncology Hotspot Control
Description	A highly-multiplexed, proprietary DNA quality control; mixture of synthetic DNA and genomic DNA in a stabilizing buffered solution. The genomic DNA is derived from the same cell line (GM24385) that is used for the development of a NIST Genome in a Bottle reference material. The synthetic DNA, which is present at low frequencies, introduces hundreds of variants that are frequently found as somatic mutations in cancer.
Reference	AcroMetrix Oncology Hotspot Control Package Insert https://www.thermofisher.com/order/catalog/product/969056
Genes & Variants	53 genes represented: *ABL1, AKT1, ALK, APC, ATM, BRAF, CDH1, CDKN2A, CSF1R, CTNNB1, EGFR, ERBB2, ERBB4, EZH2, FBXW7, FGFR1, FGFR2, FGFR3, FLT3, FOXL2, GNA11, GNAQ, GNAS, HNF1A, HRAS, IDH1, IDH2, JAK2, JAK3, KDR, KIT, KRAS, MAP2K1, MET, MLH1, MPL, MSH6, NOTCH1, NPM1, NRAS, PDGFRA, PIK3CA, PTEN, PTPN11, RB1, RET, SMAD4, SMARCB1, SMO, SRC, STK11, TP53, VHL* over 500 mutations from the Catalogue of Somatic Mutations in Cancer (COSMIC) database
Reference Sample Type	Genomic DNA plus synthetic DNA
Validation Methods	To test how many variants on the AcroMetrix Oncology Hotspot Control could be detected by NGS, three different library preparation test panels were used: the Ion AmpliSeq™ Cancer Hotspot Panel v2 (CHPv2) on the Personal Genome Machine™ (PGM™), the TruSeq™Amplicon Cancer Panel (TSACP) on the MiSeq™, and the TruSight™ Tumor Panel (TSTP) on the MiSeq
Publicly available	Yes
Contact for additional information	https://www.thermofisher.com/document-connect/document-connect.html?url=https://assets.thermofisher.com/TFS-Assets/CDD/manuals/MAN0010820-AMX-Oncology-Hotspot-Ctrl-EN.pdf&title=AcroMetrix%20Oncology%20Hotspot%20Control%20Package%20Insert%20[EN]

Project	SeraCare NGS reference Materials - Heme Malignancy
Description	Expert-designed constructs with clinically relevant variants Highly multiplexed - 23 DNA variants and nine RNA fusions Broad variant types - SNVs, INDELs, and gene fusions Manufactured under cGMP and ISO 13485; customizable and flexible content
Reference	https://seracare.com/Controls---Reference-Materials-NGS-Somatic-Cancer-Heme-Malignancy/
Genes & Variants	**Gene List - DNA Mix**: *ABL1, ASXL1, BRAF, CALR, CBL, CEBPA, CSF3R, FLT3, IDH1, JAK2, MPL, MYD88, CPM1, SF#B1, SRSF2, U2AF* **Gene List – RNA Fusions:** *BCR-ABL1, ETV6 – ABL1 (transcript 1), ETV6 – ABL1 (transcript 2), FIP1L1 – PDGFRA, MYST3 – CREBBP, PCM1 – JAK2, PML – RARA, RUNX1 – RUNX1T1, TCF3 – PBX1*
Reference Sample Type	Seraseq Myeloid Mutation DNA Mix in GM24385 cell line DNA background Seraseq Myeloid Fusion RNA Mix
Validation Methods	All internal validation of variants/allele frequency is done by digital PCR. Technical product report or CofA is available for the Seraseq products.
Publicly available	Yes
Contact for additional information	https://seracare.com/Controls---Reference-Materials-NGS-Somatic-Cancer-Heme-Malignancy/ https://www.seracare.com/Seraseq-Myeloid-Mutation-DNA-Mix-0710-0408/ https://www.seracare.com/Seraseq-Myeloid-Fusion-RNA-Mix-0710-0407/

Project	NIBSC Reference Materials for Cancer Genomics
Description	World Health Organization/ National Institute for Biological Standards and Control (WHO/NIBSC); WHO standards have been evaluated in international collaborative studies, encompassing as many different methods as possible (i.e. a WHO standard should not be method specific, where multiple methods exist)
Reference	www.nibsc.org/science_and_research/advanced_therapies/genomic_reference_materials.asp
Genes & Variants	***Currently available****:* *BCR-ABL1* (WHO) - p210 b3a2 *JAK2* (WHO) - p.V617F *KRAS* codons 12 and 13 (WHO) – p.G12A, p.G12C, p.G12D, p.G12R, p.G12S, p.G12V, p.G13D Lynch-HNPCC (*MLH1-MSH2*: CE) ***Expected early 2020***: *TP53* – p.306* *; PTEN* – p.K267fs*9 *; MAP2K1/MEK1*- p.D67N*; NRAS* – p.G12C*; PIK3CA* – p.E545K For quantification/calibration, structural variants and other variants in a "cancer typical" genome for NGS based diagnostics. Have pre-approval from WHO to prepare standards for *HER2/ERBB2* and *BRAF*, will seek WHO pre-approval for ctDNA (EGFR), microsatellite instability, *PIK3CA* (multiple variants) and further "broad cancer genome" standards
Reference Sample Type	Freeze-dried human genomic DNA prepared from cell lines established from patients, NOT genetically modified cell lines.
Validation Methods	**BCR-ABL:** reverse transcriptase QPCR by reference to BRC, ABL and GUSB (10 labs) **JAK2:** allele-specific (AS)-QPCR, digital PCR, allelic discrimination-based endpoint PCR (including AS-PCR, Amplification Refractory Mutation System-PCR (ARMS-PCR) and Matrix Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometric analysis) and next-generation sequencing (29 labs) **KRAS**: next-generation sequencing (NGS), Sanger sequencing, real-time PCR, pyrosequencing, digital PCR (dPCR), Matrix Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometric analysis (MassARRAY®), KRAS StripAssay®, high resolution melt analysis (HRM), Amplification Refractory Mutation System-PCR (ARMS-PCR), PCR-Reverse Sequence Specific Oligonucleotide probe technique (PCR-rSSO), minisequencing, and restriction fragment length polymorphism analysis (RFLP) (56 labs) **MLH1/MSH2:** Exon Copy Number genotyping using MLPA technology. Point mutations, deletions, and amplifications are included. Mutations were confirmed by direct sequencing and MLPA-based genotyping of *MLH1* and *MSH2*.
Publicly available	Yes
Contact for additional information	Dr. Jennifer Boyle jennifer.boyle@nibsc.org

Project	WHO *BCR-ABL* Reference Panel
Description	The 1st World Health Organization International Genetic Reference Panel for quantitation of *BCR-ABL* for use as primary standards to support cancer genomic diagnostics in the calibration of diagnostic assays, kits, and secondary standards for *BCR-ABL*.
Reference	http://apps.who.int/iris/bitstream/handle/10665/70141/WHO_BS_09.2106_eng.pdf?sequence=1&isAllowed=y White HE, Matejtschuk P, Rigsby P, *et al*. Establishment of the first World Health Organization International Genetic Reference Panel for quantitation of BCR-ABL mRNA. Blood. 2010;116(22):e111-7. doi: 10.1182/blood-2010-06-291641.
Genes & Variants	*BCR-ABL1* (WHO); Reciprocal chromosomal translocation t(9;22)(q34;q11) resulting in the aberrant fusion gene *BCR-ABL1.*
Reference Sample Type	Four freeze-dried human cell line materials for RNA extraction, each material with a different defined value for *BCR-ABL1* as a percentage of total reference gene (*ABL*, *BCR*, or *GUSB*), corresponding to approximately 10%, 1%, 0.1%, and 0.01%, as aligned to the International Scale.
Validation Methods	The panel was validated in an international collaborative study involving 10 laboratories performing reverse transcriptase QPCR.
Publicly available	Yes (NIBSC product code 09/138). Distribution is typically restricted to laboratories calibrating secondary standards or kits/assays to be used by others (not intended for the calibration of in-house assays for local use only).
Contact for additional information	https://www.nibsc.org/science_and_research/advanced_therapies/genomic_reference_materials/bcr-abl_(who).aspx

Project	WHO *JAK2* V617F Reference Panel
Description	The World Health Organization 1st International Reference Panel for genomic *JAK2* V617F for use as primary standards to support cancer genomic diagnostics in the calibration of diagnostic assays, kits, and secondary standards for Janus Kinase 2 (*JAK2*) mutation p.Val617Phe (c. 1849G>T; commonly abbreviated to V617F).
Reference	https://www.who.int/biologicals/ECBS_2016_BS2293_JAK2_WHO_reference_panel.pdf?ua=1
Genes & Variants	*JAK2* V617F (WHO); NM_004972.3 (JAK2) c.1849G>T (p.Val617Phe, commonly abbreviated to V617F), plus wild-type JAK2 material
Reference Sample Type	Seven freeze-dried human genomic DNA materials produced from cell lines, each material with a different defined value for *JAK2* V617F as a percentage of total *JAK2*: 100% *JAK2* V617F, 89.5%, 29.6%, 10.8%, 1.00%, 0.03%, and 0%.
Validation Methods	The panel was validated in an international collaborative study involving 29 laboratories and shows suitability as standards in allele-specific (AS)-QPCR, digital PCR, allelic discrimination-based endpoint PCR (including ASPCR, Amplification Refractory Mutation System-PCR (ARMS-PCR) and Matrix Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometric analysis) and next-generation sequencing.
Publicly available	Yes (NIBSC product code 16/120)
Contact for additional information	https://www.nibsc.org/science_and_research/advanced_therapies/genomic_reference_materials/jak2_v617f_(who).aspx

Project	WHO *KRAS* Reference Panel
Description	The World Health Organization (WHO) 1st International Reference Panel for genomic *KRAS* codons 12 and 13 mutations can be used as primary standards to support cancer genomic diagnostics in the calibration of diagnostic assays, kits, and secondary standards for the seven most-common *KRAS* mutations.
Reference	http://www.who.int/biologicals/expert_committee/BS2317_KRAS_WHO_reference_panel_WHO_BS_2017.pdf?ua=1
Genes & Variants	*KRAS* codons 12 and 13 (WHO); NM_033360.3 (KRAS) c.35G>C (p.Gly12Ala), c.34G>T (p.Gly12Cys), c.35G>A (p.Gly12Asp), c.34G>C (p.Gly12Arg), c.34G>A (p.Gly12Ser), a c.35G>T (p.Gly12Val), c.38G>A (p.Gly13Asp), plus wild-type KRAS codons 12 and 13 material
Reference Sample Type	Eight freeze-dried human genomic DNA materials produced from cell lines covering the seven most-common CRC-associated *KRAS* mutations, as found in codons 12 and 13, plus a wild-type *KRAS* standard (and diluent). Each material has assigned consensus mutation percentage, and mutant and total KRAS copy numbers. The materials may be diluted to produce further standards at a range of KRAS consensus mutation percentages.
Validation Methods	The panel was validated in an international collaborative study involving 56 laboratories and shows suitability as standards in next-generation sequencing (NGS), Sanger sequencing, real-time PCR, pyrosequencing, digital PCR (dPCR), Matrix Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) mass spectrometric analysis (MassARRAY®), KRAS StripAssay®, high resolution melt analysis (HRM), Amplification Refractory Mutation System-PCR (ARMS-PCR), PCR-Reverse Sequence Specific Oligonucleotide probe technique (PCR-rSSO), minisequencing, and restriction fragment length polymorphism analysis (RFLP)
Publicly available	Yes (NIBSC product code 16/250)
Contact for additional information	https://www.nibsc.org/science_and_research/advanced_therapies/genomic_reference_materials/kras_(who).aspx

Project	Onco-Ref™ Genomic DNA Reference Standards - SeraCare
Description	Clinically-relevant variants which may be directly incorporated into Sanger, qPCR, and digital PCR sample processing workflows (post-extraction step) to optimize NGS protocols, evaluate assay sensitivity and specificity, and analyze the impact of workflow changes on downstream analysis. Built with proprietary Footprint-Free™ technology, Onco-Ref™ reference standards are free of genomic scars found in other cell line-based materials that interfere with downstream genetic analysis.
Reference	https://www.seracare.com/Controls---Reference-Materials-Sanger---qPCR-Genomic-DNA/
Genes & Variants	*ABL1, AKT1, ALK, APC, BRCA1, BRCA2, BRAF, CDH1, CDX2, CTNNB1, EGFR, ERBB2, ESR1, FBXW7, FGFR2, FGFR3, FLT3, GNAQ, GNAS, HRAS, IDH2, JAK2, KIT, KRAS, MAP2K1, MET, MLH1, NOTCH1, NRAS, PDGFRA, PIK3CA, PIK3R1, PTEN, RB1, RET, ROS1, SMAD4, STK11, TP53* Over 250 clinically-relevant variants available as genomic DNA.
Reference Sample Type	Genomic DNA created by precise and efficient CRISPR/Cas9 genome editing using footprint-free technology. Isogenically-paired mutant and wild-type cell lines that can be blended as required.
Validation Methods	Variants quantitated with digital PCR and confirmed by Sanger sequencing. Manufactured in ISO 13485-certified facilities. Technical product report or CofA is available for the Seraseq products.
Publicly available	Yes
Contact for additional information	https://www.seracare.com/Controls---Reference-Materials-Sanger---qPCR-Genomic-DNA/

b. In progress

Project	Somatic Mutation Working Group of the SEQC2 Consortium (FDA-led)
Description	Multiple sequencing centers and multiple platforms of WGS, WES, RNASeq, single cell sequencing, and Hi-C of a paired HCC1395 and HCC1395BL cell lines from ATCC. Designed to be a benchmark for technologies. No prioritization was given to any particular variant, but the goal is to maximize completeness for regions coverable by short-read technologies
Reference	"Achieving reproducibility and accuracy in cancer mutation detection with whole-genome and whole-exome sequencing", Nature Biotechnology (under review)
Genes & Variants	Whole genome sequencing to find true somatic SNV/INDEL/SV/CNV in regions covered by short-read technologies. ~ 40,000 somatic SNVs and ~ 2000 somatic INDELs in the whole genome
Reference Sample Type	Freeze-dried human genomic DNA prepared from cell lines established from patients, not genetically modified cell lines.
Validation Methods	Targeted sequencing of randomly selected 450 SNV and 21 INDEL sites, captured by AmpliSeq and sequenced on NextSeq 500 to depths of 2000x. WES captured by SureSelect All Exon + UTR V6 sequenced on Ion Torrent Ion S5 XL to tumor-normal depths of 34x/47x. Orthogonal method, i.e., AmpliSeq by Abbvie. And Ion Torrent by EATRIS.
Publicly available	NCBI SRA accession: SRP162370 (to be released)
Contact for additional information	Wenming Xiao (FDA) wenmingxiao@fda.hhs.gov

Project	Oncopanel Working Group of the SEQC2 Consortium (FDA-led)
Description	With over 200 participants from academia, government agencies, and industry, the SEQC2 Oncopanel Working Group evaluates emerging gene sequencing-based diagnostic tests for cancers including liquid biopsy. Genomic DNA from 10 human cancer cell lines (Universal Human Reference RNA -UHRR: liver, liposarcoma, brain, skin, breast, testis, cervix, T-lymphocyte, B-lymphocyte, macrophages) are mixed of equal mass to create an oncopanel reference sample that contain variants with VAF as low as 2.5%. This sample is then mixed into Agilent Male DNA Control Sample (from a normal human cell line) to produce a set of dilution samples. Currently, this set of reference samples is used in a study to assess the analytical performance of 8 pan-cancer oncopanels and 5 ctDNA liquid biopsy panels across 30 testing laboratories. The project aims to provide recommendation in support for FDA's mission in regulatory oversight of such diagnostic tests. The reference samples are for technology benchmarking purposes.
Reference	Under construction
Genes & Variants	Whole Exome
Reference Sample Type	fresh frozen DNA; contrived samples (with and without synthetic plasma) to mimic cell-free DNA
Validation Methods	Multiple whole exome sequencing and whole genome sequencing datasets have been generated to determine the true variants and in-variant positions in the individual UHRR cell lines. 400 variants have been chosen for ddPCR validation.
Publicly available	Not yet (to the public upon completion)
Contact for additional information	Dr. Joshua Xu (Zhihua.xu@fda.hhs.gov)

4. Cell-free DNA

a. Completed

Project	SeraCare NGS Reference Materials - Liquid Biopsy
Description	The Seraseq ctDNA Mutation Mix v2 reference standards and cancer patient plasma samples have *comparable* post-sequencing molecular diversity of barcoded molecules relative to mass input by Qubit™ (330 cps/ng) [J Larsen, et. al., Poster#: 5574, 2018 AACR Meeting, Chicago, IL]. Molecular diversity is plotted for all amplicons in all samples; the median number of unique MBCs across all amplicons >100% of expectation based on input DNA quantities, indicating a highly efficient workflow.
Reference	https://seracare.com/Controls---Reference-Materials-NGS-Somatic-Cancer-Liquid-Biopsy/
Genes & Variants	*AKT1* *CTNNB1* *FLT3* *GNAS* *KRAS* *NRAS/CSDE1* *RET* *APC* *EGFR* *FOXL2* *IDH1* *MPL* *PDGFRA* *SMAD4* *ATM* *ERBB2* *GNA11* *JAK2* *NCOA4-RET* *PIK3CA* *TP53* *BRAF* *FGFR3* *GNAQ* *KIT* *NPM1* *PTEN* *TPR-ALK* Broad variant types - SNVs, INDELs, CNVs, SVs, and gene fusions
Reference Sample Type	cfDNA - Multiple formats - purified ctDNA or full-process in plasma-like matrix in a genomic DNA background of GM24385. Offered in numerous dilutions for Allele Frequencies from 5% to 0.1%
Validation Methods	All internal validation of variants/allele frequency is done by digital PCR and orthogonally validated by NGS. Technical product report or Certificate of Analysis is available for the Seraseq products
Publicly available	Yes
Contact for additional information	https://seracare.com/Controls---Reference-Materials-NGS-Somatic-Cancer-Liquid-Biopsy/

Project	HorizonDx cfDNA - EGFR Multiplex Reference Standard
Description	A cell line-derived, clinically relevant control that can be used to assess the performance of cfDNA assays that detect somatic resistance mutations in EGFR. Supplied at 5%, 1%, 0.1% and 0% (EGFR Multiplex wild type) allelic frequencies and covers ten EGFR variants implicated in the responsiveness to EGFR tyrosine kinase inhibitors (EGFR-TKIs) and anti-EGFR monoclonal antibodies. This product covers clinically relevant SNPs, insertions and deletions in EGFR and can be used to optimize, validate or routinely monitor assay performance.
Reference	https://www.horizondiscovery.com/reference-standards/all-products/egfr-multiplex-cfdna-reference-standard-hd825 https://www.horizondiscovery.com/media/resources/data/Reference-standards/HD825_28695_PI_v2.pdf
Genes & Variants	*EGFR* variants include L858R, ΔE746 - A750, T790M, V769 - D770insASV, L861Q, G719S, C797S, S464L, G465R, S768I. EGFR Q787Q, EGFR L844L confirmed in parent cell line.
Reference Sample Type	Cell-free DNA
Validation Methods	**Fragmentation Size** D1000 DNA Screen Tape assay **Allelic Frequency** Droplet Digital™ PCR **Quantification** Qubit dsDNA BR Assay (post-fragmentation) Certificate of Analysis: https://www.horizondiscovery.com/media/resources/data/Reference-standards/HD825_28695.pdf
Publicly available	Yes
Contact for additional information	technical@horizondiscovery.com

Project	HorizonDx cfDNA - Multiplex I Reference Standard
Description	https://www.horizondiscovery.com/media/resources/data/Reference-standards/HD816_25278_PI.pdf
Reference	cfDNA: https://www.horizondiscovery.com/multiplex-i-cfdna-reference-standard-set-hd780 cfDNA in synthetic plasma: https://www.horizondiscovery.com/reference-standards/all-products/multiplex-i-cfdna-reference-standard-set-synthetic-plasma-hd816
Genes & Variants	*EGFR*: L858R, ΔE746 - A750, T790M, V769 - D770insASV *KRAS*: G12D *NRAS*: Q61K, A59T *PI3KCA*: E545K
Reference Sample Type	Cell-free DNA fragmented to an average length of 160 - 170 bp
Validation Methods	**Fragmentation Size** D1000 DNA Screen Tape assay **Allelic Frequency** Droplet Digital™ PCR **Quantification** Qubit dsDNA BR Assay (post-fragmentation) **Certificates of Analysis:** cfDNA: https://www.horizondiscovery.com/media/resources/data/Reference-standards/HD780_16922.pdf cfDNA in synthetic plasma: https://www.horizondiscovery.com/media/resources/data/Reference-standards/certificate-of-analysis_cfDNA-plasma.pdf
Publicly available	Yes
Contact for additional information	technical@horizondiscovery.com

Project	HorizonDx cfDNA – Structural Multiplex Reference Standard
Description	This product is designed to challenge both molecular and bioinformatic work flows by providing validated copy number variants/amplifications, gene fusions, and large insertions/deletions. Additionally, one may examine the genomic context of variants within regions of specific GC-content (high vs. low). The Structural Multiplex cfDNA Reference Standard includes 9 variants validated by ddPCR, with most of them at 5% allelic frequency. Includes *RET* and *ROS1* fusion variants, large indels, and *MYC-N* and *MET* focal amplifications.
Reference	https://www.horizondiscovery.com/reference-standards/all-products/structural-multiplex-cfdna-reference-standard-hd786
Genes & Variants	*AKT1, BRCA2, EGFR, FBXW7, FLT3, GNA11, KRAS, MET, MYC, NOTCH1, PIK3CA, RET, ROS1* SNV High GC, SNV low GC, Long Insertion, Long Deletion, Short Deletions (4), Fusion, CNV, SNVs (3). https://www.horizondiscovery.com/media/datasheets/structural-multiplex-product-info-sheet.pdf
Reference Sample Type	cfDNA fragmented to an average length of 160 bp
Validation Methods	**Quality -** D1000 DNA Screen Tape assay **Allelic Frequency -** Droplet Digital™ PCR **Quantification -** Qubit dsDNA BR Assay (post-fragmentation) **Genotype** – Sanger sequencing of locus specific PCR
Publicly available	Yes
Contact for additional information	https://www.horizondiscovery.com/media/resources/data/Reference-standards/HD786_18046.pdf technical@horizondiscovery.com

b. In progress

Project	FNIH Cell free circulating tumor DNA (ctDNA) Quality Control Material
Description	The Foundation for the National Institutes of Health (US; FNIH) supported collaborative effort involves academia, private industry, professional organizations including ASCO, CAP and AMP, and the FDA to develop quality control materials for circulating tumor DNA (ctDNA), a component of circulating cell-free DNA (cfDNA) in cancer patient blood. The project team has developed plans for the development, performance evaluation and qualification of the reference materials for use with assays designed to detect and report the presence of cancer-related mutations found in ctDNA. The effort is intended to be performed in a pre-competitive environment with materials manufactured by three commercial vendors. In a Phase II clinical pilot, the FNIH materials will be sent to approximately 10 laboratories for blinded comparative testing. Procedures used for "fit for purpose" testing will be made available through publications and may serve as a roadmap for other reference material generation.
Reference	M.K. Williams, G.R. Oxnard, C. Karlovich, R. McCormack, K.D. Cole, J.C. Barrett, and C. Paweletz. Circulating Tumor DNA: A Unique Cross-Section Initiative to Validate Reference Materials. DIA Global Forum (2017) Volume 9, August Issue, 8-10. https://www.diaglobal.org/_GlobalForum/2017/August2017/index.html
Genes & Variants	*AKT1* E17K*; ALK* G1202R*; EML4-ALK* EML4-ALKv1*,* EML4-ALKv3 (translocations); *BRAF* V600E*; BRCA1* K654fs*47, BRCA2* R2645fs*3 (del fs*); *EGFR* L858R*,* T790M*,* E746_A750 (del del in frame); *ERBB2* A775_G776insYVMA (ins in frame)*; ERBB2 (*amplification CNV*); KRAS* G12D*; CD74-ROS1* (TBD translocation); *PIK3CA* H1047R
Reference Sample Type	Cell-free DNA quality control materials (contrived samples) for actionable biomarkers
Validation Methods	ddPCR, NGS (probe and amplicon) at 4 laboratory sites
Publicly available	Not yet
Contact for additional information	Robert McCormack, Co-PI Mickey Williams, Co-PI, Frederick National Labs, Dana Connors, FNIH https://fnih.org/what-we-do/biomarkers-consortium/programs/ctdna-reference-materials

Project	International Quality Network for Pathology (IQN Path)
Description	Cell-free (circulating tumor) DNA pilot External Quality Assessment (EQA); uses Acrometrix reference samples
Reference	https://bmccancer.biomedcentral.com/articles/10.1186/s12885-018-4694-x
Genes & Variants	*KRAS* p.(G12D), *NRAS* p.(G12D), *EGFR p*.(L858R), p.(T790)/exon 19 del; 2 diff. allelic frequencies
Reference Sample Type	10 plasma samples with ctDNA for EQA pilot; Acrometrix reference samples
Validation Methods	Six different methodologies (NGS, ddPCR, Idylla, OncoBEAM, Therascreen, Cobas); NB not all methods were suitable for all variants . Five reference laboratories participated
Publicly available	Not yet
Contact for additional information	Zandra C. (Sandi) Deans at UK NEQAS for Molecular Genetics, Department of Laboratory Medicine, Royal Infirmary of Edinburgh, Little France Crescent, Edinburgh, EH16 4SA, UK

Project	Blood Profiling Atlas in Cancer (BloodPAC)
Description	For the first ctDNA pilot (JFDI study), the Thermo-Fisher/Acrometrix™ Oncology Hotspot Control panel was used. Consortium managed by the Center for Computational Science Research, Inc (CCSR). Three working groups: Data, Technology Applications, and Sample (essential standards for blood sample collection)
Reference	https://www.bloodpac.org/
Genes & Variants	Thermo-Fisher/Acrometrix™ Oncology Hotspot Control
Reference Sample Type	Liquid biopsy, cfDNA.
Validation Methods	Amplicon-based NGS, hybrid capture NGS, and Digital PCR. Six laboratory participants. Thermo-Fisher/Acrometrix™ Oncology Hotspot Control was tested in the labs of various BloodPAC members. Preanalytical variables for minimal technical data elements for collection and inclusion in the BloodPac data commons. Eleven have been accepted by FDA and CAP. Generic analytical protocol developed.
Publicly available	Not yet
Contact for additional information	Lauren Leiman (Exec. Dir) Kelli Bramlett (liquid biopsy)

5. Human Cell lines

a. Completed

Project	ATCC Human Tumor Cell Lines, including Genetic Alteration Panels; Tissue Specific Tumor Panels
Description	Collection of human tumor cell lines annotated with gene mutation information from the Sanger Institute COSMIC database; organized by tissue or according to gene of interest and molecular signature. Provides information for each line about the specific mutation, predicted protein sequence, zygosity, and tumor histology.
Reference	https://www.atcc.org/~/media/pdfs/culture%20guides/cell_lines_by_gene_mutation.ashx https://www.atcc.org/~/media/PDFs/Culture%20Guides/TumorCellPanelsBrochure.ashx https://www.atcc.org/en/Documents/Learning_Center/~/media/210D071CAF32424BADF98CE953A56D11.ashx https://www.atcc.org/en/Documents/Learning_Center/~/media/5F7B1CCACF724E3398BE56BFBEE3EFE4.ashx http://atcc.org/Products/Cells_and_Microorganisms/By_Disease__Model/Cancer/Tumor_Cell_Panels/Panels_by_Molecular_Signature.aspx http://atcc.org/Products/Cells_and_Microorganisms/By_Disease__Model/Cancer/Tumor_Cell_Panels/Cell_lines_by_genetic_mutation.aspx
Genes & Variants	SNVs: *APC, BRAF, CDKN2A, CTNNB1, EGFR, ERBB2, ERK, KRAS, MAPK1, MAPK3, MYC, NRAS, PIK3CA, PIK3R1, PTEN, RB1, SMAD4, TP53* Amplifications: *AKT, EGFR, ERBB2, FGFR1, FGFR2, MET, MYC* Deletions: *CTNNB1, PIK3R1, PTEN, RB1, SMAD4* Gene Fusions: *AML1-ETO, BCL2-IGH, EML4-ALK, ETV6-RUNX1, EWS-ATF1, EWSR1-FLI1, FGFR3-BAIAP2L1, FGFR3-TACC, FIG-ROS1, MLL-AF9, MLL/MLLT2(AF4), TMPRSS2-ERG*
Reference Sample Type	Human cell lines established from tumor tissues of multiple cell lineage types
Validation Methods	STR profiling, and depending on specific cell line could include targeted NGS sequencing, qPCR, genetic alterations, protein expression, and cell functionality
Publicly available	Yes
Contact for additional information	https://www.atcc.org/Support/Technical_Support.aspx tech@atcc.org

Project	Somatic Reference Sample Standard for Cancer Genome Sequencing
Description	University of British Columbia, TGen, Illumina and reference to a previously published somatic sequence analysis performed by Sanger Centre. https://www.nature.com/articles/srep24607
Reference	Craig, D. W. et al. A somatic reference standard for cancer genome sequencing. Sci. Rep. 6, 24607; doi: 10.1038/srep24607 (2016)
Genes & Variants	35,989 somatic alterations including: 35,543 SNVs, 446 small indels. CNV affecting 6,586 genes Clinically actionable mutations BRAF V600E SNV; PTEN 12kb focal deletion; TERT dinucleotide block substitution; CDK2NA 2bp small deletion
Reference Sample Type	Human cell lines; ATCC COLO829 & COLO29BL
Validation Methods	Sanger sequencing
Publicly available	Yes
Contact for additional information	David W. Craig davidwcr@usc.edu

Project	Genome in a Bottle (GIAB) Consortium
Description	Current GIAB samples are EBV-immortalized cell lines and not somatic, though they have been used as negative controls, and mixtures of GIAB cell lines and commercial products are available for somatic testing that use GIAB cell lines as a background. GIAB members are pursuing development of tumor-normal cell lines that are broadly consented for fully public WGS and commercial redistribution.
Reference	https://www.nature.com/articles/nbt.2835 (2014) https://doi.org/10.1101/281006 (also in press in Nature Biotechnology, 2018)
Genes & Variants	Currently >3 million benchmark germline SNVs, small indels, and reference regions in 7 genomes. New draft benchmark for large insertions and deletions, and currently characterizing more challenging variants and regions of the genome
Reference Sample Type	Human germline ref standard - GIAB genomes are from the Personal Genome Project (PGP), an Ashkenazim Jewish (AJ) mother-father-son trio whose DNA is in NIST RMs 8391 and 8392 and the son of a Chinese trio whose DNA is NIST RM 8393.
Validation Methods	Integration of WGS from multiple short, linked, and long read technologies
Publicly available	Publicly available as NIST DNA RMs, cell lines and DNA from Coriell, and as derived products from Horizon, SeraCare, and Acrometrix. Current samples listed in this table: https://www.nature.com/articles/sdata201625/tables/2
Contact for additional information	Justin Zook of National Institute of Standards and Technology (NIST) http://www.genomeinabottle.org

b. In progress

Project	Sustainable Predictive Oncology Therapeutics and Diagnostics (SPOTDx) Diagnostic Quality Assurance Pilot
Description	Labs will demonstrate their ability to accurately analyze reference samples for a variety of DNA variants using both wet lab (FFPE) and dry lab (*in silico*) samples. Report will include findings of clinical decision points for the targeted therapy.
Reference	https://www.tapestrynetworks.com/our-work/healthcare/diagnostic-quality-assurance-pilot
Genes & Variants	*KRAS & NRAS*; multiple SNVs and variant allele fractions
Reference Sample Type	Wet Lab challenge: Human cell lines engineered by CRISPR technology with specific variants. Cells propagated, formalin-fixed, and cell pellets paraffin-embedded. *In silico* (dry) lab challenge: Pre-defined variant profiles introduced by a computerized process into each participating lab's own BAM and/or FASTQ files (customized files)
Validation Methods	Targeted NGS with Illumina and IonTorrent platforms; *in silico* file import and analysis. Performance standards specifications of Illumina Companion Diagnostic Extended RAS Panel CDx for a targeted colorectal cancer therapy - FDA approved June 2017
Publicly available	Not yet
Contact for additional information	https://www.tapestrynetworks.com/our-work/healthcare/diagnostic-quality-assurance-pilot

Project	Tumor Mutational Burden (TMB) Harmonization Project -**Stage 2**
Description	Three project stages; Stage 1 - *In silico* data analysis to "Identify sources of variability between TMB calculated using WES & various targeted panels used in the clinic" Stage 2 - Empirical analysis of TMB in cell lines derived from human tumors Stage 3 - Clinical Analysis of data from clinical samples
Reference	https://www.focr.org/tmb
Genes & Variants	>300 genes for Cancer Immunotherapy diagnostic assays; targeted oncology gene panels with large number of genes represented
Reference Sample Type	Human cell lines derived from human tumors; 10 matched pairs of human cancer cell lines (ATCC) for 2 breast and 8 lung (with preliminary TMB values) plus matched genomic DNA from peripheral blood mononuclear cells (PBMC); contrived samples may also be used from SeraCare
Validation Methods	Whole exome sequencing (WES; various assays) and Tumor mutation burden measurement; "Identify sources of variability after alignment of TMB scores from targeted panels to the reference standard"
Publicly available	Not yet
Contact for additional information	https://www.focr.org/tmb

6. Tissue/Formalin-fixed paraffin-embedded (FFPE)

a. completed

Project	HorizonDx FFPE - EGFR or KRAS Gene-Specific Multiplex Reference Standard (1 or 5% VAF)
Description	These Gene-Specific Reference Standards cover EGFR or KRAS-specific mutations. Standards are available specifically in either an EGFR Multiplex (1 and 5% allelic frequency range) or a KRAS Multiplex (5% allelic frequency range).
Reference	https://www.horizondiscovery.com/reference-standards/all-products/egfr-gene-specific-multiplex-reference-standard-hd850 https://www.horizondiscovery.com/reference-standards/all-products/kras-gene-specific-multiplex-reference-standard-hd301
Genes & Variants	*EGFR*: L861Q, ΔE746 - A750, L858R, T790M, and G719S *KRAS*: G12D, G13D, Q61H, A146T *NRAS*: G12V, Q61K
Reference Sample Type	FFPE DNA Reference Standard (15 or 20 µm sections) 4% formalin fixed. Approx. 3.5 x 10^5 cells per section. Expect ≥ 400 ng DNA. Cell line background is SW48.
Validation Methods	**Genotype** Sanger sequencing of locus specific PCR **Quality** Agarose gel electrophoresis **Quantification** Quantifluor™ Certificate of Analysis: https://www.horizondiscovery.com/media/resources/data/Reference-standards/certificate-of-analysis_FFPE-DNA.pdf
Publicly available	yes
Contact for additional information	technical@horizondiscovery.com

Project	HorizonDx FFPE - Quantitative Multiplex Reference Standard (QMRS)
Description	The Quantitative Multiplex Reference Standard (QMRS) portfolio covers multiple endogenous SNPs, insertions and deletions. The QMRS includes 11 mutations at 0.8-24.5% allelic frequency in FFPE DNA format (see Reference for details).
Reference	https://www.horizondiscovery.com/reference-standards/all-products/quantitative-multiplex-reference-standard-hd200
Genes & Variants	*BRAF;* V600E *KIT;* D816V *EGFR;* ΔE746 - A750, L858R, T790M, and G719S *KRAS;* G12D, G13D *NRAS;* G12V, Q61K *PIK3CA;* H1047R, E545K
Reference Sample Type	FFPE DNA Reference Standard (15 µm sections) 10% formalin fixed; Approx. 3.5 x 10^5 cells per section. Expect > 400 ng DNA. Cell lines as background are HCT116, RKO, SW48.
Validation Methods	**Genotype** Sanger sequencing of locus specific PCR **Quality** Agarose gel electrophoresis **Quantification** Quantifluor™
Publicly available	Yes
Contact for additional information	https://www.horizondiscovery.com/media/resources/data/Reference-standards/certificate-of-analysis_FFPE-DNA.pdf technical@horizondiscovery.com

Project	HorizonDx FFPE - Structural Multiplex Reference Standard
Description	The Structural Multiplex FFPE DNA Reference Standard includes 9 digital PCR-validated variants with allelic frequencies ranging from 3.5% to 9.7% and CNVs at 4.5x and 8.5x amplification. Includes *RET* and *ROS1* fusion variants, *MYC-N* and *MET* focal amplifications.
Reference	https://www.horizondiscovery.com/reference-standards/all-products/structural-multiplex-ffpe-dna-reference-standard-hd789
Genes & Variants	*AKT1, BRCA2, EGFR, FBXW7, FLT3, GNA11, KRAS, MET, MYC, NOTCH1, PIK3CA, RET, ROS1* SNV High GC, SNV low GC, Long Insertion, Long Deletion, Short Deletions (4), Fusion, CNV, SNVs (3). https://www.horizondiscovery.com/media/datasheets/structural-multiplex-product-info-sheet.pdf
Reference Sample Type	FFPE DNA Reference Standard (15 µm sections) 10% formalin fixed; Approx. 3.5 x 10^5 cells per section. Expect > 400 ng DNA using Promega Maxwell LEV Plus Extraction kit
Validation Methods	**Allelic Frequency -** Droplet Digital™ PCR **Genotype -** Sanger sequencing of locus specific PCR **Quality -** Agarose gel electrophoresis **Quantification -** Quantifluor™
Publicly available	Yes
Contact for additional information	https://www.horizondiscovery.com/media/resources/data/Reference-standards/certificate-of-analysis_FFPE-DNA.pdf technical@horizondiscovery.com

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Genes

Variant types

Contact information

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