Key differences between 13 KRAS mutation detection technologies and their relevance for clinical practice

Sherwood et al

SUPPORTING INFORMATION

Table S1: Description of technologies and platforms assessed in the study, where the data were generated, and regulatory status.

Technology	Regulatory status	Platform	Chemistry	Manufacturer	Data generated by:
therascreen® KRAS RGQ PCR Kit	FDA-approved IVD CE-IVD	Qiagen Rotorgene Q	*therascreen*® KRAS RGQ PCR Kit	Qiagen	AstraZeneca, Cambridge, UK
cobas® KRAS Mutation Test	FDA-approved IVD CE-IVD	Roche cobas z480	cobas® KRAS Mutation Test	Roche Molecular Systems	AstraZeneca, Cambridge, UK
Idylla™ KRAS Mutation Test	CE-IVD	Biocartis Idylla™ platform	Idylla™ KRAS Mutation Test	Biocartis	Biocartis, Mechelen, Belgium
iPLEX® Pro Tests	Research use only	Agena Bioscience MassARRAY® 4	iPLEX® Pro	Agena Bioscience	NewGene Ltd, Newcastle – Upon-Tyne, UK
UltraSEEK™	Research use only	Agena Bioscience MassARRAY® 4,	ULTRASEEK	Agena Bioscience	Agena Bioscience, Inc, CA, USA

ThunderBolts™ Cancer Panel	Research use only	Illumina MiSeq.	RainDrop Source, MiSeq V3	RainDance Technologies Illumina	Bioprocessing Solutions Alliance, IN, USA
Oncomine™ Focus Assay	Research use only	Ion Torrent PGM	Ampliseq,	ThermoFisher	Life Technologies Clinical Services Lab, West Sacramento, CA, USA
Sentosa® SQ NSCLC Panel	CE-IVD	Ion Torrent PGM, Thermofisher	Sentosa® SQ	Vela Diagnostics	Vela Diagnostics, Singapore
Illumina Nextera® Rapid Capture Custom Lung Panel	Research use only	Illumina	Nextera® V3	Illumina	Birmingham Women's Hospital NHS, West Midlands Regional Genetics Laboratory, UK
Ion AmpliSeq™ Cancer Hotspot Panel v2	Research use only	Ion Torrent PGM	Ion Ampliseq Cancer Hotspot Panel v2	ThermoFisher	Addenbrookes, East Anglia NHS Clinical Genetics Laboratory, Cambridge, UK
TruSight® Tumor 15	Research use only	Illumina OGP panel	V3 chemistry	Illumina	Illumina, CA, USA
PrimePCR ddPCR Mutation Assays *KRAS*	Research use only	BioRAD Bio-Rad QX200	Prime PCR *KRAS* mutation assays	BioRad	IMGM Laboratories, Germany
Sanger capillary sequencing	Research use only	3730 capillary sequencer	Big Dye 1.1	Applied Biosystems	IMGM Laboratories, Germany

1 **SUPPLEMENTARY METHODS**

2 *therascreen*® and cobas® KRAS mutation testing was performed at AstraZeneca
3 laboratories, while all other *KRAS* mutation testing methods and assays were performed at
4 10 external partner laboratories where *KRAS* testing is conducted routinely to assist clinical
5 practice, or conducted for research purposes. Participating laboratories were instructed to
6 proceed with the testing process regardless of input requirements as the purpose of this
7 experiment was to assess low copy number samples.

8 **qPCR: *therascreen*® KRAS RQG PCR (Qiagen, Hilden, Germany)**

9 *therascreen*® is based on an Amplification Refractory Mutation System and Scorpions-
10 based allele specific PCR assay. The test consists of 8 assays; 7 *KRAS* mutations and 1
11 control assay. It tests for p.G12A/C/D/V/R/S and p.G13D. *therascreen*® KRAS RGQ PCR kit
12 was used in accordance with the manufacturer's protocol,[1] except for the omission of the
13 sample qualification step due to the need to ensure that the same input of mutant copies
14 was applied to every assay. qPCR was performed on the Rotor-Gene Q instrument and
15 analyzed using the parameters outlined in the kit insert (software version 2.3.1). A 10 µL
16 aliquot of the admixture DNA was diluted 1 in 5 and 5 µL of the subsequent dilution was
17 loaded into each of the 8 mutation assays for every sample. Data analysis used the cut-off
18 criteria defined in the manufacturer's instructions for use. The *therascreen*® assays were
19 performed at AstraZeneca Personalised Healthcare and Biomarkers laboratories
20 (Cambridge, UK).

21 **qPCR: cobas® KRAS Mutation Test (Roche Diagnostics, Mannheim, Germany)**

22 The cobas® KRAS mutation test uses a highly optimized allele-specific asymmetric PCR
23 method which preferentially amplifies the target, using a TaqMelt® fluorescently-labelled
24 hybridization probe.[2] The TaqMelt® probe binds to the target sequence and a melt curve
25 analysis is performed. The probe increases the resolution of wild type sequence from any
26 mutant sequence by approximately 10ºC on the melt curve allowing automated interpretation
27 and reporting of *KRAS* mutation status. A 10 µL aliquot of the admixture DNA was diluted 1

28 in 25 and 25 µL of the subsequent dilution was loaded into each of the two mutation assays
29 for every sample. *KRAS* mutation status was characterized from the admixture DNA using
30 the cobas® KRAS Mutation Test according to the manufacturers' protocol and qPCR was
31 performed using the cobas® z480 instrument. The cobas® KRAS mutation test assays were
32 performed at AstraZeneca Personalised Healthcare and Biomarkers laboratories
33 (Cambridge, UK).

34 **qPCR: Idylla™ (Biocartis NV, Belgium)**

35 The Idylla™ KRAS Mutation Test is a CE-IVD test intended to detect 21 KRAS exon 2/3/4
36 mutations directly from colorectal cancer FFPE tissue (Idylla™ KRAS Mutation Test.
37 Instructions for use). The test is performed in a single-use disposable cartridge containing all
38 reagents necessary for FFPE sample processing and qPCR-based target amplification and
39 detection. The Idylla™ KRAS Mutation Test was performed according to the manufacturers'
40 instructions for use with the modification that a sample of the admixture DNA was mixed with
41 PCR buffer and the Idylla™ instrument was programmed to directly pump this mixture to the
42 Idylla™ KRAS cartridge PCR chambers, such that 1 µl of the sample was analyzed per
43 PCR. After PCR, raw data were processed offline to determine mutation status (Spotfire,
44 TIBCO Software B.V., The Netherlands). The experiment was carried out by Biocartis
45 (Mechelen, Belgium). After PCR, raw data were processed offline using Idylla™ curve
46 processing algorithms and KRAS mutation status was automatically determined (Spotfire,
47 TIBCO Software B.V., The Netherlands).

48 **MALDI-TOF: iPLEX® Pro Tests (Agena Bioscience, San Diego, CA, USA)**

49 The iPLEX® Pro test is a multiplex PCR followed by single base extension combined with
50 MALDI-TOF Mass Spectrometry.[3] Standard iPLEX® Pro chemistry was used and MALDI-
51 TOF detection was performed on the Agena Bioscience MassARRAY® 4 platform using the
52 standard kit protocol.[4] The experiment was carried out by Newgene, Newcastle, UK. The
53 iPLEX® Pro is a high-throughput mutation detection technology and allows multiple
54 mutations to be analyzed in a single investigation using multiplex PCR reactions.[5] The

55 technology uses small (~80 base pairs) PCR product amplification followed by a single base
56 pair extension step at the site of the mutated base of interest with a mass modified ddNTP
57 termination mix. The change in mass is then identified. A 10 μL aliquot of the admixture DNA
58 was diluted 1 in 2 and 2 μL of the subsequent dilution was loaded into each of the multiplex
59 mutation assays for every sample. Primer extension was carried out for 25 activating
60 nucleotide substitution mutations in codons 12, 13, 61 and 146 of the *KRAS* gene and 1
61 activating mutation in *BRAF*. Standard iPlex® Pro chemistry was used and MALDI-TOF
62 detection was performed on the Agena Bioscience MassARRAY® 4 platform using the
63 standard kit protocol.[6] The assays were performed by Newgene Ltd, Newcastle-Upon-
64 Tyne, UK.

65 **MALDI-TOF: UltraSEEK™ (Agena Bioscience, San Diego, CA, USA)**

66 The UltraSEEK™ application is a novel minor variant detection technique able to interrogate
67 multiple informative variants down to 0.1% within a single reaction . UltraSEEK™ is
68 considered different from similar biochemistries by collectively amplifying all variants in a
69 multiplex PCR, followed by a pseudo-enrichment of the minority allele via post-PCR specific
70 targeting of these minor variants with a single base extension step where the wild type allele
71 is omitted. The enrichment is enabled by extending the added analytic primers using
72 biotinylated dideoxy nucleotides specific for the mutant allele. Extended products are
73 subsequently captured to a solid support and washed to eliminate all other components,
74 specifically excess probe. Finally, the eluted products are subsequently dispensed onto
75 bioarrays for detection using MALDI-TOF mass spectrometry. Data analysis was performed
76 using Typer software version 4.0.26.74 (Agena Bioscience). The software acquired raw peak
77 intensity data for all assay products. A linear least squares function was used to fit intensities
78 of the capture control assays and determine the per-well data quality and the normalization
79 factor. Normalized intensity of each assay was determined using the capture control
80 normalization factors. Normalized intensities were used to determine mutation frequencies
81 and their statistical significance.[7] Statistical significance was determined using robust Z-

82 score (median absolute deviation [MAD]-based Z-score). A robust Z-score was calculated for
83 each assay using the median and the MAD values determined from the outlier-trimmed
84 dataset. Samples that exceeded the user-defined assay Z-score cut-off (default of 10) and
85 met the peak quality criteria (adjustable minimum peak intensity and call probability of 0.8 or
86 better) were labeled as containing the mutation by the analysis software and reported
87 accordingly. Manual calls were made by Agena when the Z-score was over the threshold of
88 10, but with peak quality that did not meet the threshold and when samples had a Z-score
89 near the threshold of 10. The assays were performed by Agena Bioscience, San Diego, CA,
90 USA.

91 **NGS: Thunderbolts™ Cancer Panel (RainDance Technologies, Billerica, MA, USA)**

92 The ThunderBolts™ Cancer Panel (RainDance Technologies) is a comprehensive NGS
93 panel for profiling important cancer mutations, which uses 230 single molecule PCR
94 amplicons to target 50 known cancer genes including tumor suppressors, mutation hotspots
95 and drug resistance markers. A 10 µL aliquot of the admixture DNA was diluted 1 in 10 and
96 10 µL of the subsequent dilution was used for library generation using RainDance
97 Technologies' single molecule droplet based PCR technology. Droplets were generated
98 using a RainDance RainDrop Source instrument and library synthesis was carried out using
99 ThunderBolts™ Cancer Panel. Resulting final library quality and quantity analysis was
100 performed using the Agilent BioAnalyzer. Paired end sequencing at 2 x 300 bp was carried
101 out on the Illumina MiSeq instrument using the MiSeq V3 kit. Resulting data were aligned
102 and mapped to the Human genome build 19. The alignment was performed along the length
103 of the amplicon target using a banded Smith-Waterman alignment algorithm and variant calls
104 were generated using the Somatic Variant Caller (v3.2.3). The sequencing was performed at
105 Bioprocessing Solutions Alliance, IN, USA.

106 **NGS: Oncomine™ Focus Assay (ThermoFisher Scientific, Waltham, MA, USA)**

107 Sequencing libraries were generated from each DNA sample using the Oncomine™ Focus
108 Assay, which uses AmpliSeq chemistry to amplify targeted regions from 52 cancer related

109 genes. Duplicate libraries were prepared for each sample and tagged with unique barcodes.
110 After library generation, the Ion Select™ workflow was performed where 6 samples
111 (DNA/RNA pairs) were multiplex templated and sequenced using the Ion Select™ 318 chip
112 on an Ion Torrent PGM instrument. Primary analysis of data was done using IonReporter
113 software. In order to determine and confirm the low allele frequency variants, further manual
114 review was conducted using Integrative Genomics Viewer (IGV) (Broad Institute Cambridge,
115 MA, USA) and compiled with custom script software. Since each sample was sequenced
116 twice, the summary includes an average allele frequency of the duplicate libraries. These
117 experiments were performed at Life Technologies Clinical Services Lab, West Sacramento,
118 CA.

119 **NGS: Sentosa® SQ NSCLC Panel (Vela Diagnostics, Singapore)**

120 The Sentosa® SQ NSCLC Panel is a ready-to-use kit for automated PCR-based NGS library
121 preparation for the detection of 113 mutations in 11 target genes (*BRAF, CTNNB1, EGFR,*
122 *FGFR3, KIT, KRAS, NRAS, PIK3CA, PTEN, RET and TP53*). The minimum coverage for
123 individual mutations must fulfil 1,000X in order to detect 5% variant frequency. The assay
124 may not identify variants that are present in less than 5% in the extracted DNA. The
125 sequencing was performed using Sentosa® SQ 301 (Ion Torrent PGM platform) and the
126 Sentosa® SQ Suite software performed primary analysis (signal processing and base-
127 calling) on raw sequencing data. After primary analysis, the data was transferred to
128 Sentosa® SQ Reporter Server for secondary analysis (alignment and variant calling) and
129 report generation. The assay was carried out at Vela Diagnostics (Singapore).

130 **NGS: Illumina Nextera® Rapid Capture Custom Lung Panel**

131 The Illumina Nextera® Rapid Capture Custom Lung Panel (Cancer Research United
132 Kingdom, London, UK; Illumina, San Diego, California, USA) is a 28 gene NGS panel
133 including *KRAS*, which uses a hybridization-based Illumina Nextera® Rapid Capture
134 Enrichment Protocol, which is then sequenced on an Illumina MiSeq instrument using V3
135 chemistry. The standard protocol recommends a minimum input of 50 ng of DNA in a volume

136 of 10 µL. From the 56 mixtures provided, 49 samples did not meet this concentration
137 requirement, however, they were processed neat without a pre-concentration step which is
138 required to ensure consistency throughout the study. Hence, the hybridisation capture assay
139 gave a suboptimal performance and consequently results have not been included in the
140 analysis. The assay was carried out at Birmingham Women's Hospital, West Midlands
141 Region Genetics Service, NHS.

142 **NGS: Ion AmpliSeq™ Cancer Hotspot v2 (ThermoFisher Scientific, Waltham, MA,**
143 **USA)**

144 Initially, 10 µL of cell-line derived DNA was used for the generation of Ion AmpliSeq™
145 Cancer Hotspot Panel v2 libraries. Following PCR amplification, all samples were processed
146 through, partial primer digestion, adapter ligation using the Ion Xpress Barcode Adapter Kit,
147 final PCR enrichment and quantification using the Quant-IT dsDNA HS Assay (Life
148 Technologies, UK) on Qubit 2.0. Appropriate dilutions were performed, with 20 pM of each
149 individual indexed amplicon library pooled prior to emulsion PCR. Subsequently, 8 samples
150 each were then sequenced on the Ion Torrent PGM platform using the 318 v2 chip (Life
151 Technologies, UK). Sequence reads were mapped against the human reference genome
152 (hg19) with the Torrent Mapping Alignment Program (TMAP), with variants called using the
153 Ion Torrent Variant caller. The experiment was carried out by Addenbrookes, East Anglia
154 NHS Clinical Genetics Laboratory, Cambridge, UK

155 **NGS: TruSight® Tumor 15**

156 TruSight® Tumor 15 uses an amplicon-based NGS library preparation (multiplexed PCR) for
157 detection of 807 somatic mutations that are common in solid tumors in 15 genes.[8] Libraries
158 were sequenced on a MiSeq instrument using V3 chemistry (Illumina, USA). Data were
159 analyzed automatically on-instrument using MiSeq Reporter® v2.6 and the TruSight Tumor
160 15 software module to generate a filtered variant report. VariantStudio data analysis
161 software was used for additional analysis. The experiment was carried out by Illumina, CA,
162 USA.

163 **Droplet digital PCR: PrimePCR ddPCR Mutation Assays KRAS (Bio-Rad Laboratories,**
164 **CA, USA)**

165 With BioRAD PrimePCR ddPCR Mutation Assays *KRAS*, DNA molecules are quantified
166 absolutely by dividing each sample into 10,000 – 20,000 droplets. Due to appropriate
167 dilution, each droplet contains ideally ≥1 (positive) or 0 (negative) target sequence copies. A
168 TaqMan PCR reaction is performed in each of the droplets using a *KRAS* wild type and a
169 mutant assay. Droplets are counted as wild type or mutant positive (containing target DNA)
170 or negative (no DNA) according to their fluorescence signal. Using the number of total
171 droplets, the concentration of positive copies per droplet is calculated as well as the initial
172 absolute DNA amount per sample. Droplet digital PCR (ddPCR) was performed on the
173 QX200™ Droplet Digital PCR system (Bio-Rad Laboratories, Inc.) using human *KRAS*
174 PrimePCR™ ddPCR™ Mutation Detection Assays in a multiplex reaction with corresponding
175 wild type assays with 9 µl DNA in a final reaction volume of 20 µl and these PCR conditions:
176 95°C, 10’; (94°C, 30’’; 55°C, 1’) x 40 cycles, ramp rate: 2°C/second; 98°C, 10’. Data were
177 analyzed with QuantaSoft 1.7.4 software (Bio-Rad Laboratories, Inc.). Target concentration
178 in each sample was expressed as copies/20 µl well. From these data the mutant/wild type
179 ratio was calculated for each sample. The experiment was carried out by IMGM Laboratories
180 GmbH, Martinsried, Germany according to the manufacturer’s instructions.

181 **Sanger capillary sequencing (Applied Biosystems, CA, USA)**

182 Sanger capillary sequencing is based on the incorporation of 2', 3' dideoxyribo-nucleotide-
183 triphosphate (ddNTP) in DNA. A purified PCR product containing the region of interest is
184 introduced into a cycled sequencing reaction, where fluorescently labelled ddNTPs are
185 incorporated which interrupt the reaction. The sequencing products are separated in a highly
186 denaturing polymer matrix according to their size. Fluorescence signals are collected
187 following excitation of a nucleotide-specific reporter dye covalently linked to the terminal
188 dideoxy-analog of the sequence product. The fluorescence of the dyes is analyzed to
189 elucidate the DNA sequence. Sanger capillary sequencing was performed on the Applied
190 Biosystems® 3730 DNA Analyzer using BigDye Version 1.1, human *KRAS* exons 2 and 3

191 primers (*KRAS*E2-V2-for:
192 5’ACACTGACGACATGGTTCTACAGGTGGAGTATTTGATAGTGTA3’; *KRAS*E2-V2-rev:
193 5’TACGGTAGCAGAGACTTGGTCTGGTCCTGCACCAGTAATATGC3’; *KRAS*E2-V3-for: 5’
194 ACACTGACGACATGGTTCTACACTGTGTTTCTCCCTTCTCAG3’; *KRAS*E2-V3-rev: 5’
195 TACGGTAGCAGAGACTTGGTCTAAACTATAATTACTCCTTAATGT3’) and these PCR
196 conditions: 98°C, 2’; (98°C, 15’’; 57°C, 45’’; 72°C, 30’’) x 30 cycles; 72°C, 3’. Amplicon sizes
197 were 242 and 251 bp. Data were analyzed with SeqPilot V4.1.2 software (JSI medical
198 systems GmbH). The experiment was carried out by IMGM Laboratories GmbH, Martinsried,
199 Germany.

200 **Mutation detection technology questionnaire**

201

202

MUTATION DETECTION TECHNOLOGY STUDY VENDOR QUESTIONNAIRE

203

204

Summary

This questionnaire will form the source of data for a tabulated snapshot of the various characteristics of the mutation detection technologies employed on the 56 cell line admixtures screened by your centre. The questionnaire is tick box and drop down box enabled so should take only 5-10 minutes to complete. Thank you in advance of your providing this data as this will be an important table in the proposed manuscript.

205

Mutation Detection Technology Questionnaire

Please answer the questions below and add in more details as necessary in the comments box provided, thank you. The data will be compiled and shown in the Platform Characteristics table in the proposed manuscript. Although the data collected in the questionnaire is reasonably detailed we intend to present relative data in the table.

1. Technology Details:

Technology Name: (Manufacturer/developer, assay, platform: e.g. Roche, cobas KRAS, Roche Light cycler z480)	
Company performing the analysis: (AstraZeneca, Cambridge, UK)	

2. Handling and ease of use:

How many physical handling steps are there from DNA to data generation? (How many times do you need physically move or measure) Please tick "X" in the relevant box. For example: Roche cobas KRAS assay: Step 1. Quantification of DNA, 2. Dilution/normalisation 3. Assay set up 4. Centrifugation 5. PCR cycling	a. ☐1-2 steps b. ☐3-5 steps c. ☐6-8 steps d. ☐9-10 steps e. ☐11-20 steps f. ☐>20 steps
How much "hands on" time does the above data generation process require (wet lab work)? e.g. Roche cobas KRAS assay requires 10 minutes preparing area / 1 hours defrost, 1 hour quantification and normalisation and then 30 minutes set up. Total hands on time =1 hour 40 minutes=1-2 hours.	a. ☐0-30 mins b. ☐31-60 mins c. ☐1-2 hours d. ☐2-5 hours e. ☐5-10 hours f. ☐10-20 hours g. ☐>20 hours
Please provide any additional relevant comments you wish to provide clarification on.	
How many physical handling steps are there from obtaining raw data to generation of a result that can be understood and used by a physician (data interpretation and reporting)? Please tick "X" in the relevant box. For example: Roche cobas KRAS assay: 1. No interpretation, just accept the data that has been generated, a PDF report suitable for physician use is generated.	a. ☐1-2 steps b. ☐3-5 steps c. ☐6-8 steps d. ☐9-10 steps e. ☐11-20 steps f. ☐>20 steps
Please provide any additional relevant comments you wish to provide clarification on.	
How much "hands on" time does the above data interpretation and reporting process require? e.g. Roche cobas KRAS assay requires 5 minutes collection of report, 0 time analysis as it is automated. Total hands on time =5mins= 0-30 minutes.	h. ☐0-30 mins i. ☐30-60 mins j. ☐1-2 hours k. ☐2-5 hours l. ☐5-10 hours m. ☐10-20 hours n. ☐>20 hours

How difficult is the technology to use? Please consider how the assay would be run in a clinical environment, who would be running it: a trained skilled scientist, a technician, a nurse or doctor and what level of knowledge and skill is required.	a. ☐Plug and play, no skill required e.g. insert FFPE tumour sample- result shown automatically. b. ☐Requires simple familiarisation (short training session i.e. 1 day) Basic molecular biology technique required. No interpretation of results required. c. ☐Requires detailed training on software and platform use. Medium length course 1-2 days to be proficient. Basic skills required, some minimal interpretation. d. ☐Requires detailed training over a period of more than 2 days. Requires highly skilled scientist or proficient technician and specialised knowledge to interpret, handle and report data.
Please provide any additional comments if the options do not cover your specific assay/ platform.	

3. Turn- around- time from sample to result

How long does it take to process and provide a result to a physician once a sample has been received? Please provide the actual time it would take to go from DNA to obtaining a simple result that can be reported and understood by a physician. For example: Roche cobas *KRAS* can be set up, PCR cycled and a report for physician is automatically generated in 2-4 hours. A large NGS panel covering multiple genes and requiring manual curation may take 2-3 weeks. (NB work week= 5 days).	a. ☐<1 hour b. ☐1-2 hours c. ☐2-4 hours d. ☐4-6 hours e. ☐1 working day f. ☐1-2 working days g. ☐2-3 working days h. ☐3-4 working days i. ☐5 working days j. ☐5-10 working days k. ☐2-3 work weeks l. ☐3-4 work weeks m. ☐>4 work weeks
Please provide information explaining the turnaround time e.g. Platform is “Point of Care” FFPE sample is inserted and result is obtained in 40 mins, or manual curation of NGS panel results takes 14 days.	

4. Multiplexing Level

How many separate reactions are there for each sample? e.g. Roche cobas KRAS assay requires 2 separate reactions, one for codon 12 and 13 and one for codon 61.	a. ☐1 b. ☐2 c. ☐3-5 d. ☐6-7 e. ☐8+
How many samples can be analysed in parallel on one “run”? e.g. Roche cobas KRAS assay requires separate reactions, one for codon 12 and 13 and one for codon 61.	a. ☐1 b. ☐2-10 c. ☐11-24 d. ☐25-48 e. ☐49-96 f. ☐>96

How many genes are targeted by the assay? e.g. in the case of Roche cobas KRAS assay, one.	a. ☐1 b. ☐2 c. ☐3-5 d. ☐6-10 e. ☐11+
How many codons are assayed by the test? e.g. in the case of Roche cobas KRAS assay, 3 (G12,13 and Q61). In the case of multiple gene panels include all codons.	a. ☐1 b. ☐2 c. ☐3-5 d. ☐6-10 e. ☐11+ f. ☐Direct sequencing, complete coverage g. ☐Direct sequencing, hotspot coverage
How many individual mutations are detectable by the assay (within the assayed region)? e.g. Roche cobas KRAS taqmelt covers all possible mutations in codon 12,13 and 61. Qiagen *therascreen* covers 7, sequencing covers all possible.	a. ☐1 b. ☐2 c. ☐3-5 d. ☐6-10 e. ☐11+ f. ☐All possible

5. DNA input requirements

What is the recommended minimum quantity of DNA? (please use drop down selector)	
How much tissue sectioned off a block is typically needed for the assay? e.g. Roche cobas KRAS requires 5 µm or 1 section (1 x 5 µm section in the vast majority of cases, 10 µm in the event of insufficient DNA being obtained).	a. ☐5 µm b. ☐10 µm c. ☐15-20 µm d. ☐>20 µm
Are there any other pre-analytical requirements that needs to be considered? e.g. Roche cobas KRAS requires that tumour tissue is <1 year old in the case of blocks and <60 days if on slides. Is also requires that the tissue is fixed in 10% Neutral Buffered Formalin and not stained.	
Please include any other information you feel needs to be considered in the manuscript.	

6. Limit of Detection

What is the claimed or validated percentage limit of detection (sensitivity) of the test? E.g. in the case of Roche cobas KRAS assay, 5% is claimed. (please use drop down selector)	5

7. Regulatory status

What is the regulatory status of the platform? Please check all that apply	a. ☐21CFR Establishment Registration b. ☐21CFR Medical Device Listing c. ☐510(k) Premarket Notification d. ☐PMA- Premarket Approval e. ☐Good Manufacturing Practice- 21CFR part 820 f. ☐Research Use Only
What is the regulatory status of the assay? Please check all that apply	a. ☐21CFR Establishment Registration b. ☐21CFR Medical Device Listing c. ☐510(k) Premarket Notification d. ☐PMA- Premarket Approval e. ☐Good Manufacturing Practice- 21CFR part 820 f. ☐Laboratory developed test g. ☐Investigational Use Only commercial kit h. ☐Research Use Only commercial kit i. ☐Test performed in CLIA or equivalent accredited clinical testing laboratory j. ☐CE/IVD
Please provide any other information that may be helpful or any other quality of the technology that makes it stand apart from other technologies. E.g. footprint.	

Once the questionnaire is completed, please save and forward to

Thank you very much for your time.

206 **REFERENCES**

1. Lee S, Cao J, May T, et al. Performance Characteristics of a PCR Assay for the Detection of KRAS Mutations in Formalin-Fixed Paraffin-Embedded Tissue Samples of Non-Small Cell Lung Cancer. *J Mol Biomark Diagn* 2015;6:5.
2. Fumagalli D, Gavin PG, Taniyama Y, et al. A rapid, sensitive, reproducible and cost-effective method for mutation profiling of colon cancer and metastatic lymph nodes. *BMC Cancer* 2010;10:101.
3. Sherwood JL, Muller S, Orr MC, et al. Panel based MALDI-TOF tumour profiling is a sensitive method for detecting mutations in clinical non small cell lung cancer tumour. *PLoS One*. 2014;9(6):e100566.
4. Fumagalli D, Gavin PG, Taniyama Y, Kim SI, Choi HJ, Paik S, et al. A rapid, sensitive, reproducible and cost-effective method for mutation profiling of colon cancer and metastatic lymph nodes. *BMC Cancer*. 2010;10:101.
5. Mosko MJ, Nakorchevsky AA, Flores E, et al. Ultrasensitive Detection of Multiplexed Somatic Mutations Using MALDI-TOF Mass Spectrometry. *J Mol Diagn* 2016;18:23–31.
6. Chan M, Smirnov A, Mulawadi F, et al. A Novel System Control for Quality Control of Diagnostic Tests Based on Next-Generation Sequencing. The Journal of Applied Laboratory Medicine: *An AACC Publication* 2016;1.1:25–35.
7. Pinto P, Rocha P, Veiga I, et al. Comparison of methodologies for KRAS mutation detection in metastatic colorectal cancer. *Cancer Genet* 2011;204:439–46.
8. Illumina. TruSight Tumor 15 Kit Support [28 October 2016].