

Table SI. Targeted regions analysed by the RNA-based sequencing FusionPlex Lung Panel.

| Genes        | Transcript (GRCh37) | Fusions/exon skipping                                                              | SNVs and indels                                           | RNA expression |
|--------------|---------------------|------------------------------------------------------------------------------------|-----------------------------------------------------------|----------------|
| <i>ALK</i>   | NM_004304.5         | Exons 2, 4, 6, 10, 16, 17, 18, 19, 20, 21, 22, 23, 26 (5')                         | Exons 22, 23, 25 (T1151-C1156, F1174, L1196-S1206, G1269) | Yes            |
| <i>BRAF</i>  | NM_004333.6         | Exons 2, 7, 8, 9, 10, 11, 12, 15, 16 (5'); exons 1, 3, 7, 8, 10, 13 (3')           | Exon 15 (V600)                                            | Yes            |
| <i>EGFR</i>  | NM_005228.5         | Exons 7, 8, 9, 16, 19, 20 (5'); exons 1, 24, 25 (3'); exon 2-7 skipping (EGFRvIII) | Exons 18-21 (E709-G719, E746-L760, V774-G796, L858-L861)  | Yes            |
| <i>FGFR1</i> | NM_015850.4         | Exons 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 17 (5'); exons 12, 17 (3')                   | -                                                         | Yes            |
| <i>FGFR2</i> | NM_000141.5         | Exons 2, 5, 7, 8, 9, 10 (5'); exons 16-17 (3')                                     | -                                                         | Yes            |
| <i>FGFR3</i> | NM_000142.5         | Exons 3, 5, 8, 9, 10 (5'); exons 16-18 (3')                                        | -                                                         | Yes            |
| <i>KRAS</i>  | NM_004985           | -                                                                                  | Exons 2-3 (G12-G13, Q61)                                  | Yes            |
| <i>MET</i>   | NM_000245.4         | Exons 2, 4, 5, 6, 13, 14, 15, 16, 17, 21 (5'); exons 2, 13 (3'); exon 14 skipping  | -                                                         | Yes            |
| <i>NRG1</i>  | NM_013957.5         | Exons 1, 8 (5')                                                                    | -                                                         | Yes            |
| <i>NRG1</i>  | NM_004495.4         | Exons 1, 2, 3, 4, 6 (5')                                                           | -                                                         | Yes            |
| <i>NRG1</i>  | NM_013962.3         | Exon 1 (3')                                                                        | -                                                         | Yes            |
| <i>NTRK1</i> | NM_002529.4         | Exons 2, 4, 6, 8, 10, 11, 12, 13 (5')                                              | -                                                         | Yes            |
| <i>NTRK2</i> | NM_006180.6         | Exons 5, 7, 9, 11, 12, 13, 14, 15, 16, 17 (5')                                     | -                                                         | Yes            |
| <i>NTRK3</i> | NM_002530.4         | Exons 4, 7, 10, 12, 13, 14, 15, 16 (5')                                            | -                                                         | Yes            |
| <i>NTRK3</i> | NM_001007156.3      | Exon 15 (5')                                                                       | -                                                         | Yes            |
| <i>NTRK3</i> | NM_002530.4         | Exons 13-15 (3')                                                                   | -                                                         | Yes            |
| <i>RET</i>   | NM_020630.6         | Exons 2, 4, 6, 8, 9, 10, 11, 12, 13, 14 (5')                                       | Exons 15-16 (A883, M918)                                  | Yes            |
| <i>ROS1</i>  | NM_002944.3         | Exons 2, 4, 7, 31, 32, 33, 34, 35, 36, 37 (5')                                     | Exon 38 (G2032)                                           | Yes            |

indels, insertions-deletions; SNV, single nucleotide variation.

Table SII. Targeted regions analysed by the custom 51-gene DNA-based sequencing Solid Tumor Solution panel.

| Genes           | Transcript (GRCh37) | SNVs and indels                                                                                                                                                                                                                                                                                                   | CNV |
|-----------------|---------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| <i>AKT1</i>     | NM_001014431.2      | Exon 3                                                                                                                                                                                                                                                                                                            | -   |
| <i>ALK</i>      | NM_004304.5         | Exons 21-25                                                                                                                                                                                                                                                                                                       | Yes |
| <i>ARID1A</i>   | NM_006015.6         | chr1:27056211-27056211_C_T; chr1:27106878-27106878__C; chr1:27105725-27105725_A_G; chr1:27106002-27106002_C_G; chr1:27023008-27023008__GGC; chr1:27087548-27087548_C_T; chr1:27100973-27100973_G_A; chr1:27102119-27102119_C_A; chr1:27099947-27099947_C_T; chr1:27105931-27105931_G_; chr1:27106667-27106667__TT | -   |
| <i>BRAF</i>     | NM_004333.4         | Exons 11, 15                                                                                                                                                                                                                                                                                                      | Yes |
| <i>BRCA1</i>    | NM_007294.3         | Exons 2-23                                                                                                                                                                                                                                                                                                        | -   |
| <i>BRCA2</i>    | NM_000059.3         | Exons 2-27                                                                                                                                                                                                                                                                                                        | -   |
| <i>CDK4</i>     | NM_000075.3         | Exon 2                                                                                                                                                                                                                                                                                                            | Yes |
| <i>CDKN2A</i>   | NM_000077.4         | Exons 1-3                                                                                                                                                                                                                                                                                                         | Yes |
| <i>CTNNB1</i>   | NM_001098209.2      | Exon 3                                                                                                                                                                                                                                                                                                            | -   |
| <i>DDR2</i>     | NM_001014796.3      | Exon 18                                                                                                                                                                                                                                                                                                           | -   |
| <i>DICER1</i>   | NM_001195573.1      | Exons 24-25                                                                                                                                                                                                                                                                                                       | -   |
| <i>EGFR</i>     | NM_005228.5         | Exons 18-21                                                                                                                                                                                                                                                                                                       | Yes |
| <i>ERBB2</i>    | NM_001005862.2      | Exons 8, 17, 20                                                                                                                                                                                                                                                                                                   | Yes |
| <i>ERBB4</i>    | NM_001042599.1      | Exons 10, 12                                                                                                                                                                                                                                                                                                      | -   |
| <i>ESR1</i>     | NM_001122742.1      | Exons 2-8 (excluding UTR3 regions)                                                                                                                                                                                                                                                                                | -   |
| <i>FBXW7</i>    | NM_001013415.2      | Exons 8-12                                                                                                                                                                                                                                                                                                        | Yes |
| <i>FGFR1</i>    | NM_001174063.2      | Exons 13, 15                                                                                                                                                                                                                                                                                                      | Yes |
| <i>FGFR2</i>    | NM_000141.5         | Exons 7, 12, 14                                                                                                                                                                                                                                                                                                   | Yes |
| <i>FGFR3</i>    | NM_000142.4         | Exons 7, 9, 14, 16                                                                                                                                                                                                                                                                                                | Yes |
| <i>FOXL2</i>    | NM_023067.4         | Exon 1                                                                                                                                                                                                                                                                                                            | -   |
| <i>GNA11</i>    | NM_002067.5         | Exons 4-5                                                                                                                                                                                                                                                                                                         | -   |
| <i>GNAQ</i>     | NM_002072.5         | Exons 4-5                                                                                                                                                                                                                                                                                                         | -   |
| <i>GNAS</i>     | NM_016592.4         | Exon 8                                                                                                                                                                                                                                                                                                            | -   |
| <i>H3F3A</i>    | NM_002107.5         | Exon 2                                                                                                                                                                                                                                                                                                            | -   |
| <i>H3F3B</i>    | NM_005324.5         | Exon 2                                                                                                                                                                                                                                                                                                            | -   |
| <i>HIST1H3B</i> | NM_003537.4         | Exon 1                                                                                                                                                                                                                                                                                                            | -   |
| <i>HRAS</i>     | NM_001130442.2      | Exons 2-4                                                                                                                                                                                                                                                                                                         | Yes |
| <i>IDH1</i>     | NM_005896.3         | Exon 4                                                                                                                                                                                                                                                                                                            | -   |
| <i>IDH2</i>     | NM_002168.4         | Exon 4                                                                                                                                                                                                                                                                                                            | -   |
| <i>KIT</i>      | NM_000222.3         | Exons 8-11, 13, 17, 18                                                                                                                                                                                                                                                                                            | Yes |
| <i>KMT2A</i>    | NM_001197104.2      | chr11:118348829-118348829; chr11:118342559-118342559                                                                                                                                                                                                                                                              | -   |
| <i>KMT2D</i>    | NM_003482.3         | chr12:49431880-49431880; chr12:49443567-49443567; chr12:49425386-49425386; chr12:49445095-49445095; chr12:49436060-49436060                                                                                                                                                                                       | -   |
| <i>KRAS</i>     | NM_004985.5         | Exons 2-4                                                                                                                                                                                                                                                                                                         | Yes |
| <i>MAP2K1</i>   | NM_002755.4         | Exons 2-3                                                                                                                                                                                                                                                                                                         | -   |
| <i>MAP2K2</i>   | NM_030662.4         | Exons 2-7                                                                                                                                                                                                                                                                                                         | -   |
| <i>MET</i>      | NM_000245.4         | Exons 2, 14-20                                                                                                                                                                                                                                                                                                    | Yes |
| <i>MTOR</i>     | NM_004958.4         | Exons 46-52                                                                                                                                                                                                                                                                                                       | -   |
| <i>MYOD1</i>    | NM_002478.5         | Exon 1                                                                                                                                                                                                                                                                                                            | Yes |
| <i>NRAS</i>     | NM_002524.5         | Exons 2-4                                                                                                                                                                                                                                                                                                         | Yes |

|               |                |                                                                                                                                                                                                                                              |     |
|---------------|----------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----|
| <i>PDGFRA</i> | NM_006206.6    | Exons 12, 14, 18                                                                                                                                                                                                                             | Yes |
| <i>PIK3CA</i> | NM_006218.4    | Exons 1, 2, 4, 5, 7, 9, 20                                                                                                                                                                                                                   | Yes |
| <i>PTPN11</i> | NM_002834.5    | Exon 3                                                                                                                                                                                                                                       | -   |
| <i>RAC1</i>   | NM_006908.5    | Exon 3                                                                                                                                                                                                                                       | -   |
| <i>RAF1</i>   | NM_002880.3    | Exons 7, 10, 12, 13, 14, 15                                                                                                                                                                                                                  | Yes |
| <i>RET</i>    | NM_020630.5    | Exons 11, 13, 15, 16                                                                                                                                                                                                                         | Yes |
| <i>ROS1</i>   | NM_002944.2    | Exons 38, 41                                                                                                                                                                                                                                 | Yes |
| <i>SF3B1</i>  | NM_012433.4    | Exons 15-17                                                                                                                                                                                                                                  | Yes |
| <i>SMAD4</i>  | NM_005359.6    | Exons 8-12                                                                                                                                                                                                                                   | -   |
| <i>TERT</i>   | NM_001193376.2 | Promoter regions and exons 1, 8, 9, 13                                                                                                                                                                                                       | Yes |
| <i>TGFBR2</i> | NM_001024847.2 | chr3:30691872-30691872_A_; chr3:30691872-30691872_AA; chr3:30732969-30732969_C_T; chr3:3071347830713481_CAGA_; chr3:30732969-30732969_C_T; chr3:30729932-30729933_GA_; chr3:3071359430713611_TTCCTGACGGCTGAGGAG_; chr3:30713724-30713724_T_C | -   |
| <i>TP53</i>   | NM_000546.5    | Exons 2-11                                                                                                                                                                                                                                   | Yes |

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CNV, copy number variation; indels, insertions-deletions; SNV, single nucleotide variation; UTR3, 3' untranslated region.

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Table SIII. Targeted regions analysed by the Hedera Profiling 2 circulating tumor DNA panel.

| Genes         | Transcript (GRCh37) | Fusions/exon skipping            | SNVs and indels               | CNV |
|---------------|---------------------|----------------------------------|-------------------------------|-----|
| <i>AKT1</i>   | NM_001382430.1      | -                                | Exon 4                        | -   |
| <i>ALK</i>    | NM_004304.5         | Yes                              | Exons 1-29                    | Yes |
| <i>BRAF</i>   | NM_004333.6         | -                                | Exons 1-18                    | Yes |
| <i>EGFR</i>   | NM_005228.5         | Yes/exon 2-7 skipping (EGFRvIII) | Exons 1-28 + part of intron 1 | Yes |
| <i>ERBB2</i>  | NM_004448.4         | -                                | Exons 1-23, 25-27             | Yes |
| <i>ESR1</i>   | NM_000125.4         | -                                | Exons 5-8                     | -   |
| <i>FGFR1</i>  | NM_023110.3         | Yes                              | Exons 2-18                    | Yes |
| <i>FGFR2</i>  | NM_000141.5         | Yes                              | Exons 2-18                    | Yes |
| <i>FGFR3</i>  | NM_000142.5         | Yes                              | Exons 2-18                    | Yes |
| <i>FGFR4</i>  | NM_213647.3         | -                                | Exons 2-18                    | -   |
| <i>GNA11</i>  | NM_002067.5         | -                                | Exons 4-5                     | -   |
| <i>GNAQ</i>   | NM_002072.5         | -                                | Exons 4-5                     | -   |
| <i>GNAS</i>   | NM_000516.7         | -                                | Exons 8-9                     | -   |
| <i>HRAS</i>   | NM_005343.4         | -                                | Exons 2-5                     | Yes |
| <i>IDH1</i>   | NM_005896.4         | -                                | Exon 4                        | -   |
| <i>IDH2</i>   | NM_002168.4         | -                                | Exons 4-5                     | -   |
| <i>KEAP1</i>  | NM_203500.2         | -                                | Exons 2-6                     | -   |
| <i>KIT</i>    | NM_000222.3         | -                                | Exons 1-21                    | -   |
| <i>KRAS</i>   | NM_004985.5         | -                                | Exons 2-5                     | Yes |
| <i>MAP2K1</i> | NM_002755.4         | -                                | Exons 2-6, 8                  | -   |
| <i>MET</i>    | NM_000245.4         | Yes/exon 14 skipping             | Exons 2-21                    | Yes |
| <i>NRAS</i>   | NM_002524.5         | -                                | Exons 2-5                     | Yes |
| <i>NTRK1</i>  | NM_002529.4         | Yes                              | Exons 8-17                    | -   |
| <i>NTRK2</i>  | NM_006180.6         | -                                | Exons 14-19                   | -   |
| <i>NTRK3</i>  | NM_001012338.3      | -                                | Exons 15-20                   | -   |
| <i>PDGFRA</i> | NM_006206.6         | -                                | Exons 12, 18                  | -   |
| <i>PIK3CA</i> | NM_006218.4         | -                                | Exons 2-21                    | Yes |
| <i>PTEN</i>   | NM_000314.8         | -                                | Exons 1-9                     | -   |
| <i>RET</i>    | NM_020975.6         | Yes                              | Exons 1-20                    | Yes |
| <i>ROS1</i>   | NM_001378902.1      | Yes                              | Exons 32-42                   | -   |
| <i>STK11</i>  | NM_000455.5         | -                                | Exons 1-9                     | -   |
| <i>TP53</i>   | NM_000546.6         | -                                | Exons 2-11                    | Yes |

CNV, copy number variation; indels, insertions-deletions; SNV, single nucleotide variation.

Table SIV. Detailed description and metadata of the next-generation sequencing datasets deposited in the National Center for Biotechnology Information BioProject database (<https://www.ncbi.nlm.nih.gov/bioproject/>) under accession number PRJNA1416035.

| Accession number                                                                       | Methodology | Sample source         | Collection date |
|----------------------------------------------------------------------------------------|-------------|-----------------------|-----------------|
| SRX31979638                                                                            | DNaseq      | Tissue biopsy         | August 2024     |
| SRX31979637                                                                            | STS         | Blood (liquid biopsy) | May 2024        |
| SRX31979636                                                                            | RNASeq      | Tissue biopsy         | October 2023    |
| SRX31979635                                                                            | STS         | Blood (liquid biopsy) | October 2023    |
| SRX31979634                                                                            | RNASeq      | Tissue biopsy         | September 2023  |
| DNaseq, DNA-based sequencing; RNASeq, RNA-based sequencing; STS, Solid Tumor Solution. |             |                       |                 |