

Liquid biopsy monitoring uncovers acquired RAS-mediated resistance to cetuximab in a substantial proportion of patients with head and neck squamous cell carcinoma

Supplementary Material

Supplementary Table 1: Illumina-sequencing primers.*

| PCR | Primer name | Primer sequence 5'-3' |
|---------|--------------------|--|
| 1st PCR | Seq_Exon 12_I_fw | ACACTCTTCCCTACACGACGCTCTTCCGATCTacattgttttataattttcaccac |
| | Seq_Exon 12_II_fw | ACACTCTTCCCTACACGACGCTCTTCCGATCTcgctccctcaaggagataag |
| | Seq_KRAS_Exon 2_fw | ACACTCTTCCCTACACGACGCTCTTCCGATCTgctctgctgaaaatgactgaa |
| | Seq_KRAS_Exon 3_fw | ACACTCTTCCCTACACGACGCTCTTCCGATCTccagactgtttctcccttc |
| | Seq_KRAS_Exon 4_fw | ACACTCTTCCCTACACGACGCTCTTCCGATCTggactctgaagatgtacatgg |
| | Seq_NRAS_Exon 2_fw | ACACTCTTCCCTACACGACGCTCTTCCGATCTgttctgtggtgtgaaatgac |
| | Seq_NRAS_Exon 3_fw | ACACTCTTCCCTACACGACGCTCTTCCGATCTattgaactccctccctccc |
| | Seq_NRAS_Exon 4_fw | ACACTCTTCCCTACACGACGCTCTTCCGATCTtcccgtttttagGGAGCAGA |
| | Seq_HRAS_Exon 2_fw | ACACTCTTCCCTACACGACGCTCTTCCGATCTggcaggagaccctgtagga |
| | Seq_HRAS_Exon 3_fw | ACACTCTTCCCTACACGACGCTCTTCCGATCTctgagccctgtcctcctg |
| | Seq_Exon 12_I_rv | TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTtggtttctgaccggaggt |
| | Seq_Exon 12_II_rv | TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTgaccattagaaccaactcca |
| | Seq_KRAS_Exon 2_rv | TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTagaatggtcctgcaccagtaa |
| | Seq_KRAS_Exon 3_rv | TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTtccctcattgcactgtactcc |
| | Seq_KRAS_Exon 4_rv | TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTcagtggttactacctgtctgt |
| | Seq_NRAS_Exon 2_rv | TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTaagtgttctgattagctgga |
| | Seq_NRAS_Exon 3_rv | TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTtggctctcatggcactgt |
| | Seq_NRAS_Exon 4_rv | TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTgcaaaactctgcacaaatgc |
| | Seq_HRAS_Exon 2_rv | TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTagccctatcctggctgtgt |
| | Seq_HRAS_Exon 3_rv | TGACTGGAGTTCAGACGTGTGCTCTTCCGATCTcaaacacacacaggaagccc |
| 2nd PCR | Linker_Seq_fw | AATGATACGGCGACCACCGAGATCTACACTCTTCCCTACACGAC |
| | Linker_Bar_Seq_rv | CAAGCAGAAGACGGCATAACGAGAT(N ₆₋₇)GTGACTGGAGTTCAGACGTGTG |

* Capital letters NGS specific sequences; lower case letters gen-specific sequences

Supplementary Table 2: EGFR and RAS reference exon sequences.

| Exon name | Reference sequences 5'-3' |
|--------------|---|
| EGFR exon 12 | TGGTCAGTTTTCTCTTGCAGTCGTCAGCCTGAACATAACAT CCTTGGGATTACGCTCCCTCAAGGAGATAAGTGATGGAGA TGTGATAATTTTCAGGAAACAAAAATTTGTGCTATGCAAATAC AATAAACTGGAAAAAACTGTTTGGGACCTCCGGTCAGAAAA CCAAAATTATAAGCAACAGAGGTGAAAACAGCTGCA |
| KRAS exon 2 | GCCTGCTGAAAATGACTGAATATAAACTTGTGGTAGTTGGA GCTGGTGGCGTAGGCAAGAGTGCCTTGACGATACAGCTAA TTCAGAATCATTGTGGACGAATATGATCCAACAATAGAG |
| KRAS exon 3 | GATTCCTACAGGAAGCAAGTAGTAATTGATGGAGAAACCT GTCTCTTGGATATTCTCGACACAGCAGGTCAAGAGGAGTA CAGTGCAATGAGGGACCAGTACATGAGGACTGGGGAGGG CTTTCTTTGTGATTTGCCATAAATAACTAAATCATTGGA AGATATTCACCATTATAG |
| KRAS exon 4 | GGACTCTGAAGATGTACCTATGGTCCTAGTAGGAAATAAAT GTGATTTGCCTTCTAGAACAGTAGACACAAAACAGGCTCAG GACTTAGCAAGAAGTTATGGAATTCCTTTTATTGAAACATCA GCAAAGACAAGACAG |
| NRAS exon 2 | GTTCTTGCTGGTGTGAAATGACTGAGTACAACTGGTGGT GGTTGGAGCAGGTGGTGTGGGAAAAGCGCACTGACAATC CAGCTAATCCAGAACCACTT |
| NRAS exon 3 | GATTCTTACAGAAAACAAGTGGTTATAGATGGTGAAACCTG TTTGTGGACATACTGGATACAGCTGGACAAGAAGAGTACA GTGCCATGAGAGACCAA |
| NRAS exon 4 | GGAGCAGATTAAGCGAGTAAAAGACTCGGATGATGTACCT ATGGTGCTAGTGGGAAACAAGTGTGATTTGCCAACAAGGA CAGTTGATACAAAACAAGCCCACGAACTGGCCAAGAGTTA CGGGATTCCATTGAAACCTCAGCCAAGACCAGACAG |
| HRAS exon 2 | GGCAGGAGACCCTGTAGGAGGACCCCGGGCCGCAGGCC CCTGAGGAGCGATGACGGAATATAAGCTGGTGGTGGTGG GCGCCGGCGGTGTGGGCAAGAGTGCGCTGACCATCCAGC TGATCCAGAACCATTTTGTGGACGAATACGACCCCACTATA GAG |
| HRAS exon 3 | GATTCCTACCGGAAGCAGGTGGTCATTGATGGGGAGACGT GCCTGTTGGACATCCTGGATACCGCCGGCCAGGAGGAGT ACAGCGCCATGCGGGACCAGTACATGCGCACCGGGGAGG GCTTCCTGTGTGTGTTTG |