

RT-PCR NS5A (2)

| (5'-3') (Forward) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|-------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Subtypes | 6227 | 6228 | 6229 | 6230 | 6231 | 6232 | 6233 | 6234 | 6235 | 6236 | 6237 | 6238 | 6239 | 6240 | 6241 | 6242 | 6243 | 6244 | 6245 | 6246 | 6247 | 6248 | 6249 | 6250 | 6251 | 6252 | 6253 | 6254 | 6255 |
| 1a | | | | | | | | A | G | C | T | C | G | G | A | G | T | G | Y | A | C | C | A | C | T | C | C | R | T |
| 1b | | | | | | | | A | A | Y | G | A | G | G | A | C | T | G | C | T | C | C | A | C | G | C | C | A | T |
| 2a | | | | | | | | A | C | T | G | A | G | G | A | C | T | G | C | C | C | C | A | T | C | C | C | A | T |
| 2b | | | | G | A | T | C | A | C | T | G | A | A | G | A | T | T | G | C | C | C | A | G | T | C | C | C | A | T |
| 2c | | | | | | | | | | | G | A | R | G | A | Y | T | G | C | C | C | Y | G | T | C | C | C | T | |
| 2j | | | G | G | A | T | C | A | C | T | G | A | G | G | A | C | T | G | Y | C | C | C | G | T | | | | | |
| 3a | | | G | A | T | C | A | A | T | G | A | A | G | A | C | T | A | C | C | C | A | A | G | Y | C | C | T | T | |
| 4a | | | G | A | T | C | A | A | T | G | A | A | G | A | T | G | Y | T | C | C | A | A | G | Y | C | C | A | T | |
| 4d | | | | | | | | A | A | C | G | A | G | G | A | C | T | G | C | T | C | T | A | C | T | C | C | A | T |
| 4f | G | T | G | G | A | T | C | A | A | T | G | A | A | G | A | C | T | G | Y | C | C | Y | A | C | T | | | | |

| (5'-3') (Reverse) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|-------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|---|---|
| Subtypes | 6882 | 6881 | 6880 | 6879 | 6878 | 6877 | 6876 | 6875 | 6874 | 6873 | 6872 | 6871 | 6870 | 6869 | 6868 | 6867 | 6866 | 6865 | 6864 | 6863 | 6862 | 6861 | 6860 | 6859 | 6858 | 6857 | 6856 | 6855 | 6854 | 6853 | | |
| 1a | | | | G | G | G | A | G | G | G | A | T | C | G | G | T | G | A | G | C | A | T | G | G | A | | | | | | | |
| 1b | | | | G | G | G | A | G | G | G | A | T | C | G | G | T | G | A | G | C | A | T | G | G | A | | | | | | | |
| 2a | | | T | G | G | G | A | Y | G | G | A | T | C | T | G | T | T | A | G | C | A | T | G | G | A | | | | | | | |
| 2b | | | | G | G | G | A | C | G | G | A | T | C | T | G | T | C | A | A | C | A | T | G | G | A | | | | | | | |
| 2c | T | G | T | G | G | G | A | Y | G | G | G | T | C | Y | G | T | T | A | G | C | A | T | G | G | A | | | | | | | |
| 2j | | | | G | G | G | A | Y | G | G | G | T | C | C | G | T | C | A | G | C | A | T | G | G | A | | | | | | | |
| 3a | | | | | | | | | G | G | G | T | C | T | C | T | C | A | A | C | A | T | C | G | A | G | G | A | G | T | C | A |
| 4a | | | | G | T | G | A | T | G | G | G | T | C | T | G | T | C | A | R | C | A | T | G | G | A | | G | G | T | C | A | |
| 4d | | | | G | A | G | A | T | G | G | A | T | C | T | G | T | C | A | G | C | A | T | A | G | A | | | | | | | |
| 4f | | | | G | G | G | A | T | G | G | G | T | C | T | G | T | A | A | G | C | A | T | G | G | A | | | | | | | |

PCR NS5A (2.1)

| (5'-3') (Forward) | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|-------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Subtypes | 6299 | 6300 | 6301 | 6302 | 6303 | 6304 | 6305 | 6306 | 6307 | 6308 | 6309 | 6310 | 6311 | 6312 | 6313 | 6314 | 6315 | 6316 | 6317 | 6318 | 6319 | 6320 | 6321 | 6322 | 6323 | 6324 | 6325 |
| 1a | G | G | T | G | C | T | G | A | G | C | G | A | Y | T | T | T | A | A | G | A | C | C | T | G | G | C | T |
| 1b | G | G | T | G | C | T | G | A | C | T | G | A | Y | T | T | C | A | A | G | A | C | C | T | G | G | C | T |
| 2a | C | A | T | C | C | T | R | A | C | A | G | A | C | T | T | Y | A | A | A | A | A | Y | T | G | G | C | T |
| 2b | C | A | T | Y | C | T | C | A | C | A | G | A | C | T | T | T | A | A | G | A | A | C | T | G | G | C | T |
| 2c | R | A | T | C | C | T | G | A | C | A | G | A | C | T | T | Y | A | A | G | A | R | T | T | G | G | C | T |
| 2j | C | A | T | C | T | T | G | A | C | T | G | A | C | T | T | Y | A | A | R | A | A | Y | T | G | G | C | T |
| 3a | G | G | T | G | Y | T | G | T | C | Y | G | A | C | T | T | C | A | A | G | A | C | A | T | G | G | C | T |
| 4a | C | G | T | G | C | T | G | A | G | T | G | A | C | T | T | C | A | A | G | A | C | G | T | G | G | C | T |
| 4d | C | G | T | A | C | T | G | A | G | T | G | A | C | T | T | T | A | A | A | A | C | G | T | G | G | C | T |
| 4f | C | G | T | A | Y | T | G | T | C | T | G | A | C | T | T | Y | A | A | G | A | C | Y | T | G | G | C | T |

| (5'-3') (Reverse) | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|-------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|--|--|--|--|--|--|
| Subtypes | 6735 | 6734 | 6733 | 6732 | 6731 | 6730 | 6729 | 6728 | 6727 | 6726 | 6725 | 6724 | 6723 | 6722 | 6721 | 6720 | 6719 | 6718 | 6717 | 6716 | 6715 | | | | | | |
| 1a | T | A | T | G | Y | A | R | G | C | G | C | A | C | C | C | C | G | T | C | C | A | | | | | | |
| 1b | T | G | T | G | C | A | R | C | C | G | Y | A | C | C | C | C | R | T | C | C | A | | | | | | |
| 2a | T | R | T | G | G | A | T | Y | T | G | C | A | C | T | C | C | G | T | C | C | A | | | | | | |
| 2b | G | G | T | G | T | A | T | Y | T | G | C | A | C | C | C | C | G | T | C | C | A | | | | | | |
| 2c | G | A | T | G | G | A | T | Y | T | G | R | A | C | Y | C | C | G | T | C | C | A | | | | | | |
| 2j | G | R | T | G | G | A | T | C | T | G | Y | A | C | C | C | C | R | T | C | C | A | | | | | | |
| 3a | G | G | T | G | G | A | G | T | C | T | C | A | C | C | C | C | R | T | C | C | A | | | | | | |
| 4a | G | G | T | G | T | A | G | Y | C | T | G | A | Y | G | C | C | G | T | C | Y | A | | | | | | |
| 4d | G | A | T | G | G | A | G | C | C | T | G | A | C | G | C | C | A | T | C | C | A | | | | | | |
| 4f | G | G | T | G | A | A | K | T | C | T | R | A | C | R | C | C | A | T | C | C | A | | | | | | |

PCR NS5A (2.2)

| (5'-3') (Forward) | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|-------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|--|
| Subtypes | 6256 | 6257 | 6258 | 6259 | 6260 | 6261 | 6262 | 6263 | 6264 | 6265 | 6266 | 6267 | 6268 | 6269 | 6270 | 6271 | 6272 | 6273 | 6274 | 6275 | 6276 | 6277 | 6278 | 6279 | 6280 | 6281 | 6282 | |
| 1a | | | | | C | G | G | T | T | C | C | T | G | G | C | T | A | A | G | G | G | A | C | A | T | | | |
| 1b | | | | C | C | G | G | C | T | C | G | T | G | G | C | T | A | A | G | G | G | A | C | A | T | | | |
| 2a | | | | | C | G | G | M | T | C | V | T | G | G | C | T | C | C | G | C | G | A | Y | G | T | | | |
| 2b | | | | | G | G | G | G | T | C | T | T | G | G | C | T | C | C | R | G | G | A | | | | | | |
| 2c | G | C | T | C | Y | R | G | C | T | C | A | T | G | G | C | T | Y | C | G | A | G | A | | | | | | |
| 2j | | | | | G | G | C | T | C | T | T | G | G | C | T | T | C | G | C | G | A | C | A | T | A | T | | |
| 3a | | | | | C | G | G | Y | G | A | T | T | G | G | C | T | G | C | G | T | A | C | C | A | T | | | |
| 4a | | | | | C | G | A | A | T | C | T | T | G | G | C | T | R | T | G | G | A | G | G | T | | | | |
| 4d | G | T | G | A | T | C | G | C | T | C | T | T | G | G | T | A | T | G | G | A | G | A | T | | | | | |
| 4f | G | T | G | A | C | T | C | A | T | C | Y | T | G | G | C | C | T | R | T | G | G | A | T | | | | | |

| (5'-3') (Reverse) | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
|-------------------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Subtypes | 6799 | 6798 | 6797 | 6796 | 6795 | 6794 | 6793 | 6792 | 6791 | 6790 | 6789 | 6788 | 6787 | 6786 | 6785 | 6784 | 6783 | 6782 | 6781 | 6780 | 6779 | 6778 | 6777 | 6776 | 6775 | 6774 | 6773 | 6772 | 6771 |
| 1a | T | C | G | T | G | K | A | G | T | C | C | Y | A | C | T | C | T | G | A | A | Y | G | A | | | | | | |
| 1b | T | G | G | T | T | G | A | G | C | C | C | G | A | C | C | C | T | G | A | A | T | G | T | | | | | | |
| 2a | | | | | | | A | G | C | C | C | A | A | C | G | C | W | R | A | A | C | G | A | G | A | C | C | T | C |
| 2b | G | A | A | T | T | G | A | G | C | C | C | Y | A | C | R | G | T | G | A | A | C | G | T | | | | | | |
| 2c | G | A | A | T | T | G | A | G | M | C | C | A | A | C | G | C | W | G | A | A | C | G | A | | | | | | |
| 2j | G | A | A | T | T | G | A | G | C | C | C | A | C | A | C | G | C | T | A | A | A | C | G | A | | | | | |
| 3a | G | A | A | T | T | C | A | W | C | C | C | T | A | C | C | R | T | G | A | A | A | G | T | G | A | T | | | |
| 4a | G | A | A | T | T | G | A | G | T | C | C | T | A | C | Y | G | A | G | A | A | C | G | A | | | | | | |
| 4d | G | T | G | T | T | G | A | G | G | C | C | A | C | T | G | T | A | T | G | G | A | G | A | T | | | | | |
| 4f | A | A | G | T | T | R | A | G | G | C | C | C | A | C | G | G | A | G | A | A | C | G | A | | | | | | |

Figure S2. Subtype-specific oligonucleotides designed to sequence the NS5A-coding region. Residue numbering is according to the reference strain AF009606. Positions in red are conserved among the different subtypes, and positions with different colors are discriminatory of a specific subtype (color codes given in the left column at each panel). Discriminatory positions for genotype are highlighted in pink.