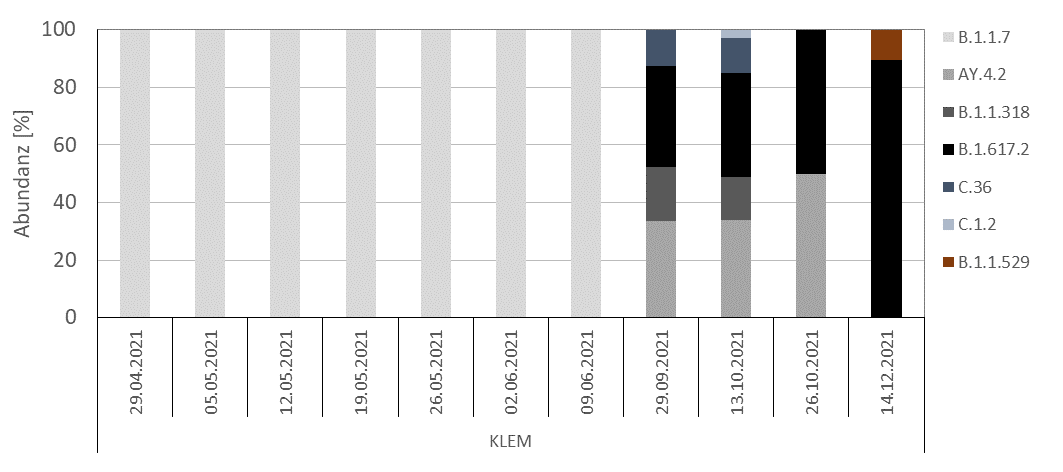
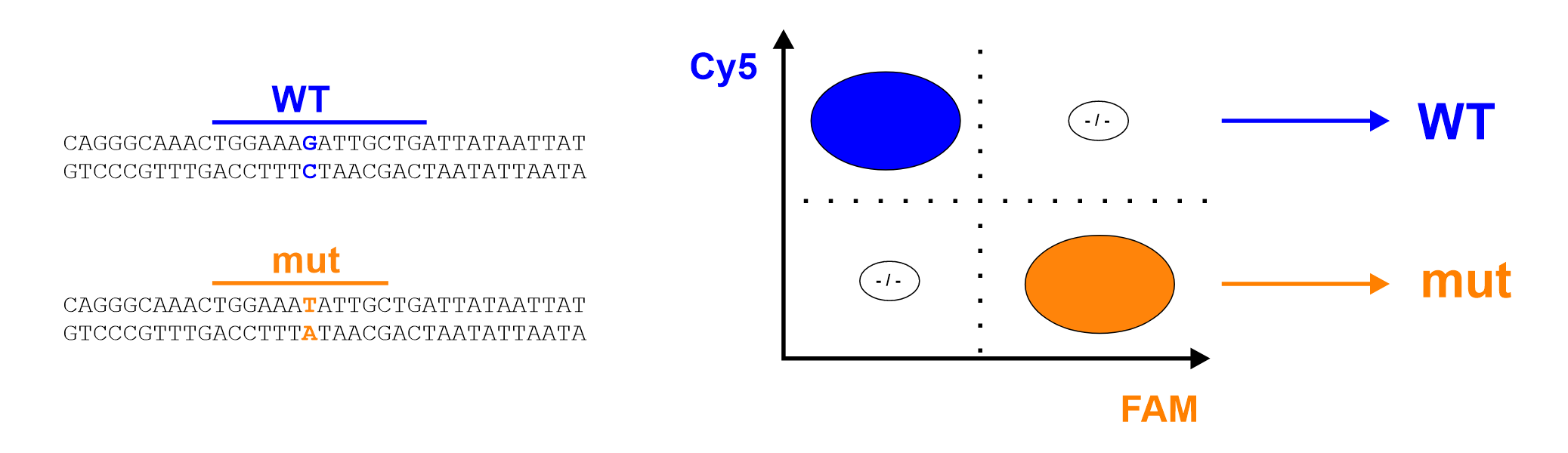
**Supplementary Material:**

**A)**

**B)**

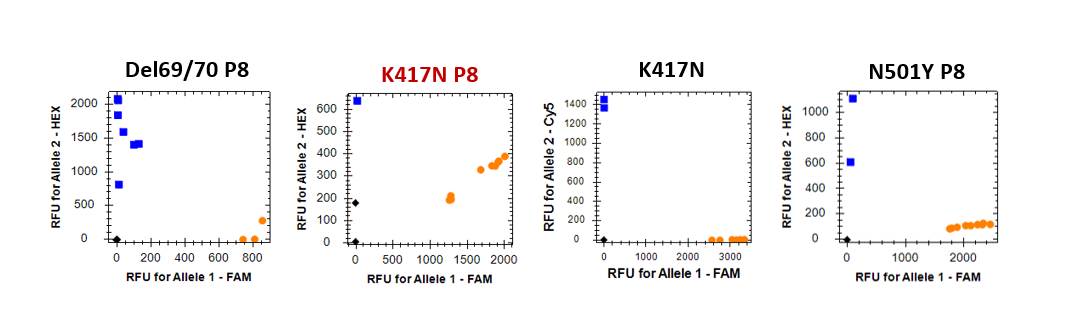


**Supplementary Figure 1: Relative SARS-CoV-2 variant distribution. A)** Epidemiological data on SARS-CoV-2 cases including the variant specific portions in NRW were obtained from the official data repository of the Federal Robert Koch Institute (RKI) in charge of public health surveillance. **B)** Relative proportion of SARS-CoV-2 variants as determined by NGS-sequencing of wastewater samples derived from KLEM at the indicated sampling dates.

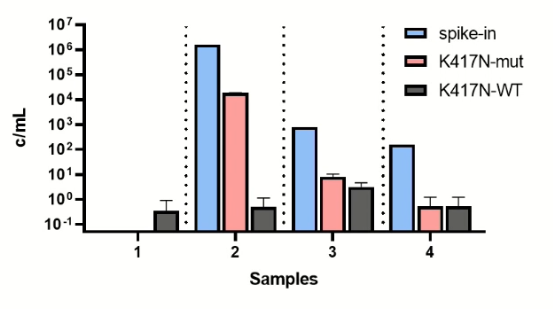


**B)**

**A)**



**C)**



**Supplementary Figure 2: Specificity and sensitivity of different SARS-CoV-2 target genes.**

**A)** To evaluate the proportion of SARS-CoV-2 RNA carrying a specific substitution, we used a multiplex approach based on the simultaneous use of two differently labeled LNA probes. Together with a single forward- and reverse-primer, a FAM-labelled locked-nucleic acid (LNA) probe for the detection of a specific mutation and a Cy5-labelled LNA probe for the detection of SARS-CoV-2 RNA lacking the respective substitution were combined a single PCR reaction. **B)** Samples spiked with inactivated SARS-CoV-2 were used for assay validation and VoCs carrying the respective mutation were successfully detected in the FAM-channel (orange dots). The allelic discrimination plot revealed a crosstalk between the Cy5- and FAM-channel in the primer-probe pair “K417N P8” (highlighted in red), while *Del69/70 P8, K417N and N501Y P8* exhibited high specificity in both fluorescence channels. P8: SARS-CoV-2 Variant Panel-8 Targets Kit (Promega) **C)** The primer-probe pair *K417N* (purchased from IDT) was evaluated in wastewater spiked with different amounts of K417N-carrying SARS-CoV-2 variants (blue bars). The samples 1 to 4 originate from a single wastewater sample.

**Supplementary Table 1:** Sequences of primers and probes used for SARS-CoV-2 detection. All assays were verified for specific detection of SARS-CoV-2 by RT-qPCR using samples spiked with authentic SARS-CoV-2 as described above. “+” indicates LNA positions. FAM, 5' 6-FAM (Fluorescein) modification; ZEN, internal quencher for fluorescence-quenched probes (IDT). 3IABkFQ, 3' Iowa Black FQ quencher; 3IAbRQSp, 3' Iowa Black RQ quencher; Cy5, 5' Cy5 fluorescence dye.

|  |  |  |
| --- | --- | --- |
| **primer/probe** | **SARS-CoV-2 gene** | **Sequence (5′–3′)** |
| N1 probe | N | FAM/ACCCCGCAT/ZEN/TACGTTTGGTGGACC/3IABkFQ/ |
| N2 probe | N | FAM/ACAATTTGC/ZEN/CCCCAGCGCTTCAG/3IABkFQ/ |
| N1 fwd | N | GACCCCAAAATCAGCGAAAT |
| N1 rev | N | TCTGGTTACTGCCAGTTGAATCTG |
| N2 fwd | N | TTACAAACATTGGCCGCAAA |
| N2 rev | N | GCGCGACATTCCGAAGAA |
| K417N fwd | S | GAGGTGATGAAGTCAGACAAATC |
| K417N rev | S | AGCTATAACGCAGCCTGTAA |
| K417 wt probe | S | Cy5/TGG+AA+A+G+ATT+G+CT/3IAbRQSp/ |
| K417 mt probe | S | FAM/TG+GAA+A+T+ATTG+CT+GA/3IABkFQ/ |