

## SOP\_ variantPCRV5.0\_SSI

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### Variant PCR L452R RT-qPCR

For more information about the variant PCR and detailed protocols please see also:

[Rapid surveillance platforms for key SARS-CoV-2 mutations in Denmark | medRxiv](#)

#### Primer and probes

| Primer/probe name                          | Primer sequence                              | Fluorophore       | Quencher |
|--|--|-------------------|----------|
| SARS-CoV-2_L452R_F                         | 5'-CAGGCTGCGTTATAGCTTGA -3'                  |                   |          |
| SARS-CoV-2_L452R_R                         | 5'-CCGGCCTGATAGATTTTCAGT -3'                 |                   |          |
| SARSCoV2_WT_L452 (omicron variant)_BHQplus | 5-TATAATTACCTGTATAGATTGTTA-3'                | Cal Fluor Red 610 | BHQ2     |
| SARSCoV2_Mut_452R (delta variant)_BHQplus  | 5'-TATAATTACCGGTATAGATTGTT-3'                | HEX               | BHQ1     |
| SARSCoV2_WT_L452 (omicron variant)_LNA*    | 5'-TAT AAT TAC +C+TG TA+T A+GA TTG TTT AG-3' | Cal Fluor Red 610 | BHQ2     |
| SARSCoV2_Mut_452R (delta variant)_LNA*     | 5'-TAT AAT TAC +C+GG TA+T AGA TTG TTT AG-3'  | HEX               | BHQ1     |

\* LNA probes can be used if BHQplus probes cannot be ordered

#### Mastermix 96 well plate

| Luna® Universal Probe One-Step RT-qPCR Kit         | <i>per reaction</i> |
|--|---------------------|
| Luna® Universal Probe One-Step RT-qPCR Kit 2x      | 12.5 µl             |
| Luna WarmStart® RT Enzyme Mix (20X)                | 1.25 µl             |
| SARS-CoV-2_L452R_F (100µM)                         | 0,1 µl              |
| SARS-CoV-2_L452R_R (100µM)                         | 0,1µl               |
| SARSCoV2_WT_L452 (omicron variant)_BHQplus (100µM) | 0,05 µl             |
| SARSCoV2_Mut_452R (delta variant)_BHQplus (100µM)  | 0,05 µl             |
| H2O  | 5.95 µl             |
| Total  | 20 µl               |
| Sample   | 5µl                 |
| Total  | 25µl                |

### 384 well plate (15µL total volume)

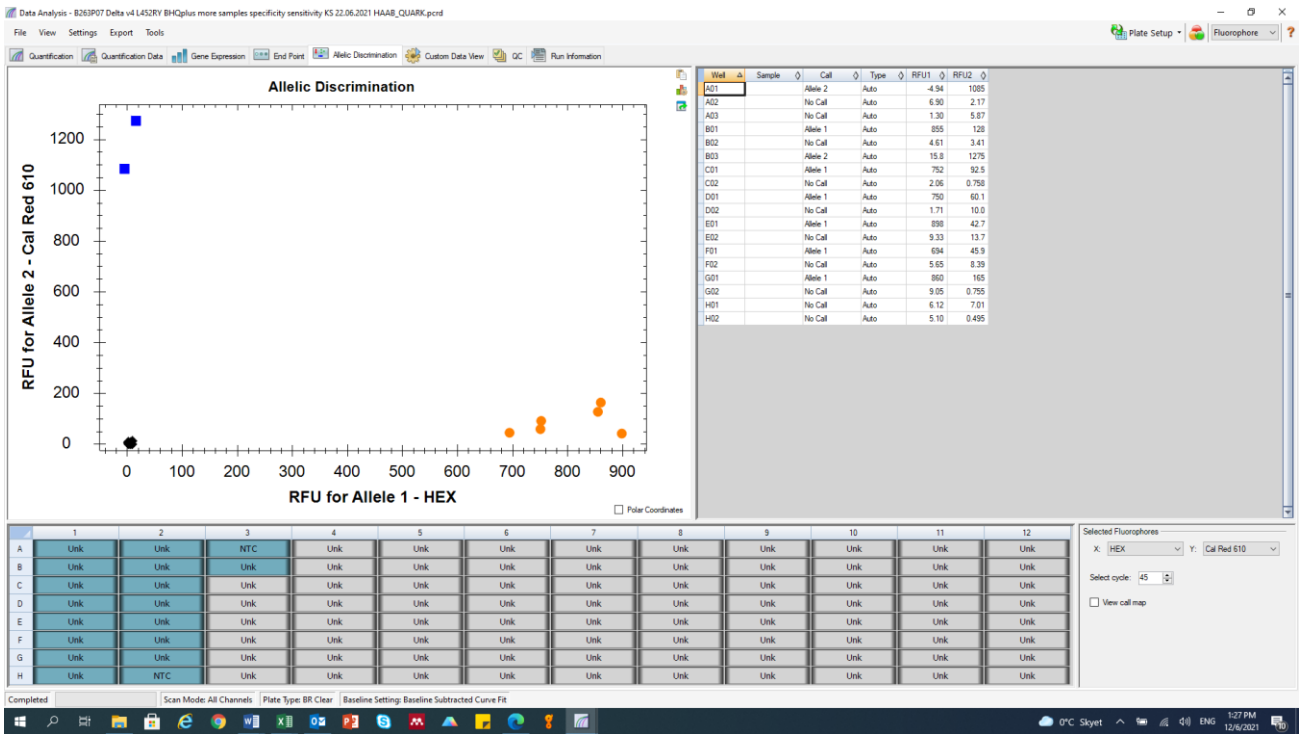
| Luna® Universal Probe One-Step RT-qPCR Kit         | <i>per reaction</i> |
|--|---------------------|
| Luna® Universal Probe One-Step RT-qPCR Kit 2x      | 7,5µl               |
| Luna WarmStart® RT Enzyme Mix (20X)                | 0,75 µl             |
| SARS-CoV-2_L452R_F (100µM)                         | 0,1 µl              |
| SARS-CoV-2_L452R_R (100µM)                         | 0,1 µl              |
| SARSCoV2_WT_L452 (omicron variant)_BHQplus (100µM) | 0,05 µl             |
| SARSCoV2_Mut_452R (delta variant)_BHQplus (100µM)  | 0,05 µl             |
| H2O  | 1,45 µl             |
| Total  | 10 µl               |
| Sample   | 5µl                 |
| Total  | 15µl                |

### Cycler protocol

| Cycle     | Time  | Temperature |
|-----------|-------|-------------|
| 1 cycle   | 10min | 55°C        |
| 1 cycle   | 3min  | 95°C        |
| 45 cycles | 15s   | 95°C        |
|           | 30s   | <b>58°C</b> |

### Data evaluation Biorad CFX 96

1. Mark NTC as NTC in plate set-up. If this is not done the wildtype and mutant will not be grouped correctly.
2. Correct undershooting curves (often for weak samples) by adjusting "Cycles to analyze". This also helps the software to group wildtype and mutant correctly.
3. In the tab Allelic discrimination: Set Selected Fluorophores (right low corner) to X: HEX, Y: Cal Fluor Red 610, see example below.
4. Allele 2 – Cal Fluor Red 610 is the mutant (delta variant). Allele 1 – HEX is the wildtype (omicron variant), see example below.



### Interpreting results

| Result                      | L452 WT (Cal Fluor Red 610)                                    | 452R MUT (HEX)   |
|-----------------------------|--|--|
| Potentially Omicron variant | Grouped as allele 1 (RFU Cal Fluor Red 610 > RFU HEX at Ct 45) |  |
| Potentially Delta variant   |  | Grouped as allele 2 (RFU HEX > RFU Cal Fluor Red 610 at Ct 45) |