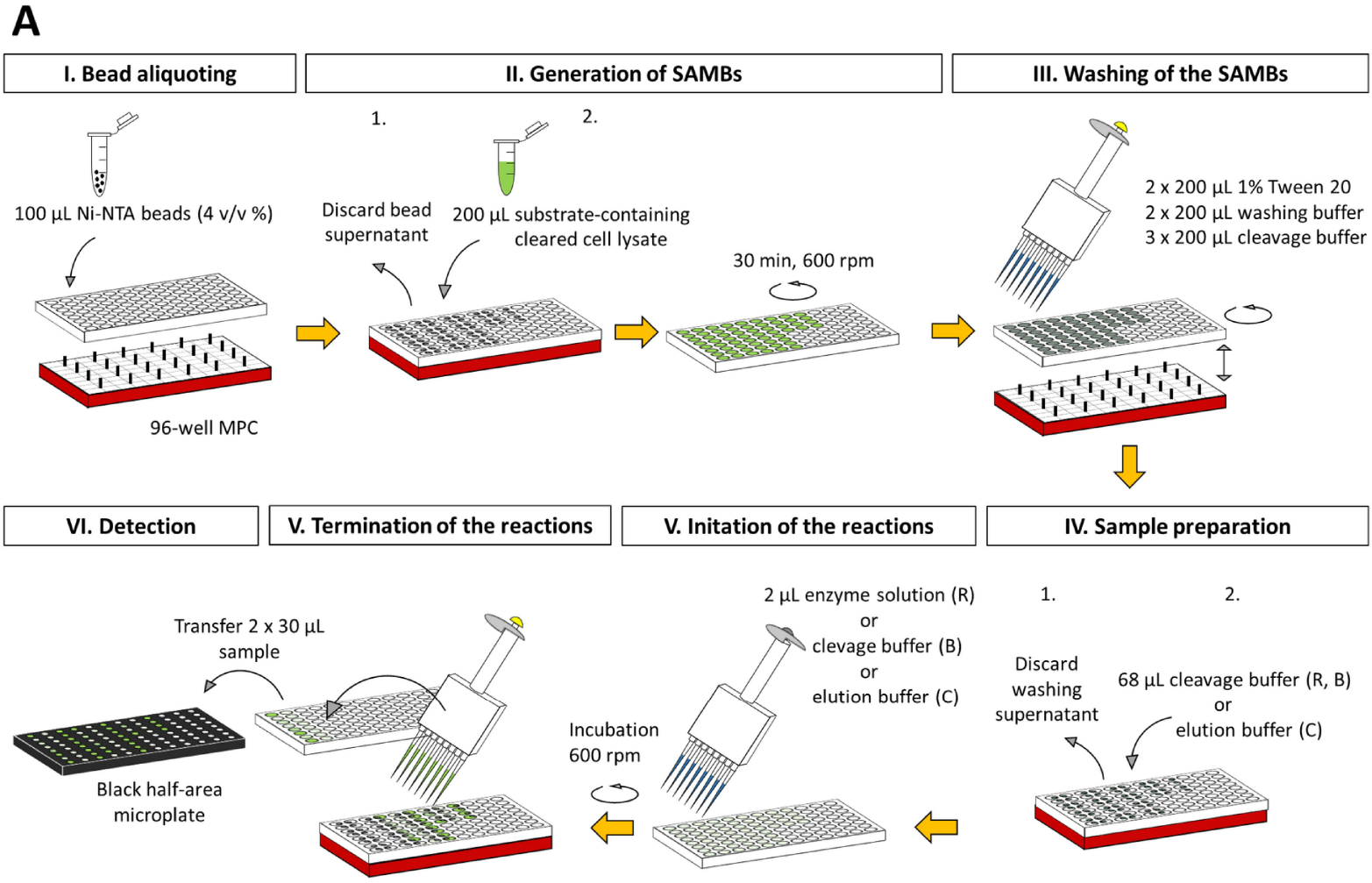
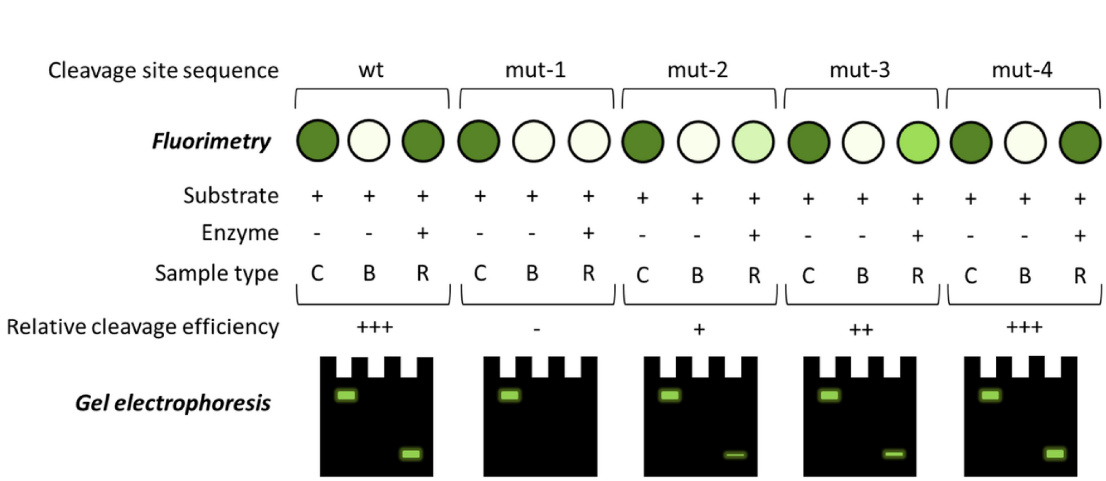

A
I. Bead aliquoting
II. Generation of SAMBs
III. Washing of the SAMBs
100 µL Ni-NTA beads (4 v/v %)
96-well MPC
1.
Discard bead supernatant
2.
200 µL substrate-containing cleared cell lysate
30 min, 600 rpm
2 x 200 µL 1% Tween 20
2 x 200 µL washing buffer
3 x 200 µL cleavage buffer
VI. Detection
V. Termination of the reactions
V. Initation of the reactions
IV. Sample preparation
Transfer 2 x 30 µL sample
Black half-area microplate
Incubation 600 rpm
2 µL enzyme solution (R) or clevage buffer (B) or elution buffer (C)
1.
Discard washing supernatant
2.
68 µL cleavage buffer (R, B) or elution buffer (C)


B
Cleavage site sequence
wt
mut-1
mut-2
mut-3
mut-4
Fluorimetry
Substrate
Enzyme
Sample type
C B R C B R C B R C B R C B R
Relative cleavage efficiency
+++
-
+
++
+++
Gel electrophoresis