

Table 1: List of primers and probe used in this experiment

| probe Name                  | Primer    | modification            | Primer sequence 5'-3'                                                                                                                 |
|-----------------------------|-----------|-------------------------|---------------------------------------------------------------------------------------------------------------------------------------|
| Salmonella<br>InvA gene     | PLP probe | Phosphate at the 5' end | <u>TAAAGCTGGCTATGCCGAAATTATCGCCACGTTCGGG</u> <u>AAAGGTTCAGAAC</u><br><u>GTGTCCG</u> <u>ACTCATCTGTTTACCGGGCAATTGATGCCGTCAAAGGAACCG</u> |
|                             | F3        |                         | AAATTATCGCCACGTTCTGGG                                                                                                                 |
|                             | B3        |                         | ACTCATCTGTTTACCGGGCA                                                                                                                  |
|                             | Target    |                         | ACTGGAAAGGGAAAGC CAGCTTTA/CGGTTCTTTG ACGGTGCGA                                                                                        |
| InV MBO                     | MBO       | 5'VIC – 3'Dabcyl        | <u>AAAGGTTCAGAACGTGTCCG</u> CCTTT                                                                                                     |
| Staphylococcus<br>GlnA gene | PLP-glnA  | Phosphate at the 5' end | CAATATAACATGGTGGTCACCATGAAGTTGCCCCTGGTCAA <u>CGGTGTG</u><br><u>AATGGTAGCGGT</u> AGCAGTTTCCGTTAAGCCCATATATCATTACCACTCC<br>ATG          |
|                             | F3        |                         | ATGAAGTTGCCCCTGGTCAA                                                                                                                  |
|                             | B3        |                         | GCAGTTTCCGTTAAGCCCAT                                                                                                                  |
|                             | Target    |                         | ATGAAGCACC ATGTTATATTGCATGGAGT GGTAAAAACC                                                                                             |
| GlnA MBT                    | MBO       | 5'Atto425 – 3'Dabcyl    | <u>CGGTGTGAATGGTAGCGGT</u> ACCCG                                                                                                      |

Table 2: multiplex Hyper-branched RCA Ligation and amplification buffer used in this experiment.

| <b>Buffer stock</b>                             | <b>Concentration</b> | <b>2x ligation buffer (in 100µl stock)</b> | <b>2X amplification buffer (in 100µl Stock)</b> |
|-------------------------------------------------|----------------------|--------------------------------------------|-------------------------------------------------|
| MgCl <sub>2</sub>                               | 20mM                 | 20µl                                       | 20µl                                            |
| (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> | 20mM                 | /                                          | 20µl                                            |
| ATP                                             | 2mM                  | 40µl                                       | /                                               |
| DTT                                             | 20mM                 | 20µl                                       | 8µl                                             |
| dNTP                                            | 10mM                 | /                                          | 27.99µl                                         |
| NaOH                                            | 20mM                 | 17.5µl                                     | 20µl                                            |
| Water                                           |                      | 2.5µl                                      | 4.1µl                                           |

All the buffer were prepared with ultrapure water.

Table 3: statistical analyses of the effect of the Padlock probe concentration the fluorescence signal intensity

| Designation | Atto 425                       | VIC                            |
|-------------|--------------------------------|--------------------------------|
| control     | 2351,33 ± 58,01 <sup>a</sup>   | 1845,66 ± 156,12 <sup>a</sup>  |
| 0.1 µM      | 12578,33 ± 330,07 <sup>b</sup> | 4401 ± 65,60 <sup>b</sup>      |
| 0.2 µM      | 16588,66 ± 390,04 <sup>c</sup> | 7874 ± 169,34 <sup>c</sup>     |
| 0.3 µM      | 18150,33 ± 262,50 <sup>d</sup> | 9943,66 ± 85,15 <sup>d</sup>   |
| 0.4 µM      | 19597,33 ± 265,66 <sup>e</sup> | 11253,33 ± 253,06 <sup>e</sup> |
| 0.5 µM      | 23122,66 ± 126,02 <sup>f</sup> | 14110,33 ± 150,69 <sup>f</sup> |
| 0.6 µM      | 25603 ± 338,14 <sup>g</sup>    | 16404 ± 446,77 <sup>g</sup>    |

Mean ± SD,  $P \leq 0.05$ , within the same column, same letter indicate that the mean difference at 0.05 is not significant, while different letters indicate that the mean difference is significant. (\*) indicates that the mean difference at 0.01 is significant among the same letter.

Table 4: Statistical analysis of the effect of the Molecular beacon concentration on the fluorescent signal

| Designation | Atto 425                       | VIC                            |
|-------------|--------------------------------|--------------------------------|
| control     | 2648,66 ± 104,51 <sup>a</sup>  | 2172,67 ± 327,61 <sup>a</sup>  |
| 0,1 µM      | 6627,67 ± 531,59 <sup>b</sup>  | 4288,67 ± 267,33 <sup>b</sup>  |
| 0.2 µM      | 8913,33 ± 236,11 <sup>c</sup>  | 7123 ± 230,51 <sup>c</sup>     |
| 0.4 µM      | 11299,66 ± 285,16 <sup>d</sup> | 9244,33 ± 288,0 <sup>d</sup>   |
| 0.6 µM      | 16800,33 ± 374,02 <sup>e</sup> | 11075,66 ± 134,33 <sup>e</sup> |
| 0.8 µM      | 22372 ± 502,07 <sup>f</sup>    | 14223,67 ± 299,45 <sup>f</sup> |

Mean ± SD, P≤0.05, within the same column, same letter indicate that the mean difference at 0.05 is not significant, while different letters indicate that the mean difference is significant. (\*) indicates that the mean difference at 0.01 is significant among the same letter.

Table 5: statistical analysis of the reaction duration on the fluorescence signal

| Time (in minutes) | Atto 425                       | VIC                           |
|-------------------|--------------------------------|-------------------------------|
| control           | 1817,66 ± 88,74 <sup>a</sup>   | 1627 ± 256,74 <sup>a</sup>    |
| 30                | 7968,33 ± 288 <sup>b</sup>     | 3496,33 ± 543,48 <sup>b</sup> |
| 60                | 9013 ± 88,50 <sup>c</sup>      | 5281,33 ± 203,53 <sup>c</sup> |
| 90                | 10080,33 ± 185,39 <sup>d</sup> | 6979 ± 124,01 <sup>d</sup>    |
| 120               | 11556,33 ± 356,68 <sup>e</sup> | 8390,6 ± 162,05 <sup>e</sup>  |
| 150               | 12432,33 ± 121,69 <sup>f</sup> | 9193,3 ± 291,92 <sup>f</sup>  |
| 180               | 14745,6 ± 339,77 <sup>g</sup>  | 9990 ± 190,78 <sup>g</sup>    |

Mean ± SD, P≤0.05, within the same column, same letter indicate that the mean difference at 0.05 is not significant, while different letters indicate that the mean difference is significant. (\*) indicates that the mean difference at 0.01 is significant among the same letter.

Table 6: Statistical analysis of the effect of different concentration of the synthetic target on the signal.

| <b>Concentration of<br/>the synthetic target<br/>stock (in <math>\mu\text{M}</math>)</b> | <b>Atto 425</b>                    | <b>VIC</b>                          |
|------------------------------------------------------------------------------------------|------------------------------------|-------------------------------------|
| Control                                                                                  | 2056,33 $\pm$ 135,04 <sup>h</sup>  | 2018 $\pm$ 1088,47 <sup>f</sup>     |
| 0.002 $\mu\text{M}$                                                                      | 2013,33 $\pm$ 128,97 <sup>h</sup>  | 2010 $\pm$ 83,21 <sup>f</sup>       |
| 0.004 $\mu\text{M}$                                                                      | 2013 $\pm$ 149,88 <sup>h</sup>     | 2057 $\pm$ 100,36 <sup>f</sup>      |
| 0.009 $\mu\text{M}$                                                                      | 2041,66 $\pm$ 96,08 <sup>h</sup>   | 2154 $\pm$ 100,23 <sup>f</sup>      |
| 0.019 $\mu\text{M}$                                                                      | 2158,66 $\pm$ 96,00 <sup>h</sup>   | 2546,66 $\pm$ 128,58 <sup>ef*</sup> |
| 0.039 $\mu\text{M}$                                                                      | 4114,66 $\pm$ 98,10 <sup>g</sup>   | 2659,33 $\pm$ 136,23 <sup>ef*</sup> |
| 0.078 $\mu\text{M}$                                                                      | 5254 $\pm$ 86,53 <sup>f</sup>      | 3136,66 $\pm$ 107,97 <sup>ef*</sup> |
| 0.156 $\mu\text{M}$                                                                      | 10102 $\pm$ 257,47 <sup>e</sup>    | 3482,33 $\pm$ 105,29 <sup>de</sup>  |
| 0.312 $\mu\text{M}$                                                                      | 10677,33 $\pm$ 108,93 <sup>d</sup> | 4040 $\pm$ 259,42 <sup>cd</sup>     |
| 0.625 $\mu\text{M}$                                                                      | 12850 $\pm$ 114,56 <sup>c</sup>    | 4934,66 $\pm$ 173,50 <sup>c</sup>   |
| 1.25 $\mu\text{M}$                                                                       | 16643,6 $\pm$ 299,61 <sup>ab</sup> | 5998,66 $\pm$ 153,21 <sup>b</sup>   |
| 2.5 $\mu\text{M}$                                                                        | 16844,33 $\pm$ 274,21 <sup>a</sup> | 8491,66 $\pm$ 182,83 <sup>a</sup>   |

Mean  $\pm$  SD,  $P \leq 0.05$ , within the same column, same letter indicate that the mean difference at 0.05 is not significant, while different letters indicate that the mean difference is significant. (\*) indicates that the mean difference at 0.01 is significant among the same letter.