Fig. S1: Lifetime of valid and invalid published PCR primers used to amplify *V. cholerae* pathogenicity genes. For each primer, total number of primers is plotted against the number of years it was still cited in the literature after its first publication. Dotted curve: invalid primers, full curve: valid primers (124 invalid primers and 57 valid primers with a 1-year lifetime are not shown). Note the large number of primers used one or two years only (5 valid and 4 invalid primers).
Fig. S2: Efficiency of PCR primers used to amplify genes of *V. cholerae* in Senderovich et al. 2010. A phylogenetic analysis was performed with every genetic variant of *ctxA* (A), *hapA* (B), *hlyA* (C), *ompU* (D), *ompW* (E), *sto* (F), *tcpA* (G), *tcpI* (H), *toxR* (I), *zot* (J) genes. Tms were computed for each variant gene sequence and each primer using OHM. Accession number, number of other sequences identical to or contained in the sequence used in this tree, species names, and Tms are indicated (forward and reverse primers in columns from left to right). For strain, biovar and serotype information, see Table S9. Tms are symbolized by a color code described in the box on the right. A predicted Tm lower than the lowest temperature mentioned in the box is symbolized by a grey square. White boxes when a partial sequence does not encompass a primer. Please note that OHM tends to underestimate the Tm of primers (as compared to other tools).

*ctxA*: the forward and reverse primers amplify every allele of the gene and are specific of *V. cholerae*.

*hapA*: the forward and reverse primers amplify every allele of the gene and are specific of *V. cholerae*. However, a slight variation of Tm is observed for the reverse primer and the sequence CP001236, and a lower Tm (50.8°C) should be used for the detection.

*hlyA*: the forward and reverse primers detect every known sequence of *V. harveyi* but cannot amplify every allele of the gene in *V. cholerae* (AF194418, D58374, AY427780).

*ompU*: the forward and reverse primers amplify every allele of the gene in *V. cholerae*, but also detect some sequences of *V. mimicus* (e.g. DQ356331).

*ompW*: the high variation of Tms (from 46 to 54°C) for the second couple is probably too large for a proper detection. Only the first couple is probably useful to amplify *ompW* in *V. cholerae*.

*sto*: the couple of primer amplifies only one allele (M85198) of the gene in *V. cholerae*.
tcpA: the reverse primer is the primer targeting the classical strain of *V. cholerae*. The one targeting the El tor strain was not studied because of its localization outside the CDS. As shown in this figure, the forward and reverse primers cannot amplify every allele of the gene in *V. cholerae*, but will detect two sequences of *V. mimicus* (AF315787, DQ356010). The Tms of the forward primer vary from 43.8 to 51.4°C, according to the method of computation of Tm, with a high Tm (51.4°C) for the sequences of *V. mimicus*. For the reverse primers, the Tms vary from 52.1 to 52.5°C. The efficiency of this primer cannot be appreciated for sequences of *V. mimicus* because only partial sequences are available for the target region.

tcpI: the forward and reverse primers amplify every allele of the gene.

toxR: the primers of the first (Nandi et al. 2000) and the second (Halpern et al. 2005) published couples amplify every allele of the gene in *V. cholerae*. However, a variation of Tm is observed for the forward primer of the second couple for sequence CP001485. The lowest Tm (45.8°C) should be used for the detection, but this condition can favor the amplification of sequences of *V. harveyi*. Indeed, because of the partial sequences, it is not possible to estimate the efficiency of couples for the outgroup sequences, and therefore their specificity toward *V. cholerae*. As a result, toxR is not a validated genetic marker for the detection of *V. cholerae*.

zot: the forward and the reverse primers amplify every allele of the gene in *V. cholerae*. A variation of Tm is observed for the forward primer and sequence AF220606. A low Tm (47.7°C) should be used for the detection. The specificity of this couple cannot be estimated because of the partial sequence of *V. mimicus*. The zot gene is therefore not validated for detecting *V. cholerae*. 
Table S1: Summary of results obtained from the analysis of the complete list of sequences retrieved for *V. cholerae* pathogenicity genes.

Columns 1: pathogenicity island, 2: most frequently used gene name, 3: total number of sequences retrieved, 4: total number of unique sequences, 5: number of alternate gene and protein names found, 6: published and 7: valid primers retrieved from the literature.

| Gene Sequences | Unique Sequences | Keywords | Primers | Valid Primers |
|----------------|------------------|----------|---------|---------------|
| **CTX Prophage** |                  |          |         |               |
| ace            | 9                | 3        | 2       | 8             | 4          |
| cep            | 7                | 3        | 2       | 3             | 2          |
| ctxA           | 65               | 10       | 12      | 41            | 18         |
| ctxB           | 205              | 24       | 17      | 23            | 0          |
| orfU           | 9                | 3        | 1       | 9             | 7          |
| zot            | 21               | 4        | 4       | 24            | 8          |
| rstA           | 29               | 16       | 8       | 8             | 0          |
| rstB           | 12               | 4        | 5       | 3             | 0          |
| rstR           | 12               | 2        | 4       | 3             | 2          |
| **TCP Prophage** |                 |          |         |               |
| acfA           | 19               | 7        | 3       | 0             | 0          |
| acfB           | 10               | 4        | 3       | 6             | 4          |
| acfC           | 9                | 1        | 2       | 1             | 1          |
| tagE           | 18               | 7        | 4       | 0             | 0          |
| aldA           | 30               | 14       | 7       | 6             | 2          |
| int            | 13               | 4        | 6       | 5             | 4          |
| tcpA           | 98               | 46       | 19      | 27            | 0          |
| tcpB           | 40               | 11       | 3       | 3             | 0          |
| tcpC           | 9                | 3        | 4       | 1             | 0          |
| tcpD           | 7                | 1        | 3       | 1             | 1          |
| tcpE           | 8                | 1        | 3       | 1             | 1          |
| tcpF           | 10               | 5        | 4       | 5             | 5          |
| tcpH           | 17               | 10       | 3       | 6             | 0          |
| tcpI           | 10               | 3        | 5       | 7             | 4          |
| tcpJ           | 10               | 4        | 5       | 4             | 0          |
| tcpP           | 21               | 9        | 3       | 12            | 2          |
| tcpQ           | 17               | 8        | 3       | 5             | 3          |
| tcpR           | 8                | 1        | 3       | 0             | 0          |
| tcpS           | 8                | 1        | 3       | 0             | 0          |
| tcpT           | 8                | 2        | 3       | 0             | 0          |
| toxT           | 15               | 7        | 7       | 13            | 4          |
| **Sum**        | 780              | 230      | 159     | 230           | 73         |
Table S2: list of valid published primers.

Tms were calculated as described in the methods.

See also http://protein.bio.puc.cl/cardex/servers/dnaMATE/index.html for more details.

| Gene | Valid Primer | Tm (°C) | Bas | Sal | Bre | San | Sug |
|------|--------------|---------|-----|-----|-----|-----|-----|
| ace  | CCGCTTATCCACACGGCTATC | 54.4 | 61.3 | 59.9 | 49.5 | 54.6 |
|      | CCGCTTACCCACAGCTAT | 51.8 | 58.4 | 58.1 | 48.6 | 53.3 |
|      | TTTAAGCGTCCAGGCG | 49.5 | 54.8 | 59.8 | 49.2 | 52.8 |
|      | CGCCCTGAGGCGTTAAACCT | 55.9 | 62.5 | 64.8 | 54.5 | 57.8 |
| cep  | TTTAGCCTTACGAATTAAGCC | 48.5 | 55.5 | 54.7 | 44.8 | 50.4 |
|      | AACACAGCAAGAAACCGAGT | 53.0 | 60.3 | 62.3 | 52.1 | 55.0 |
| ctxa | CTCAGACGGGATTTGTTAGGCACG | 59.1 | 66.9 | 66.9 | 54.3 | 59.4 |
|      | ATGATCATGCAAGAGGAACTC | 50.4 | 57.4 | 55.6 | 46.7 | 51.5 |
|      | AGACGGGATTTGTTAGGCACG | 54.4 | 61.3 | 61.6 | 51.6 | 55.4 |
|      | CGTTAATGATGTATTAGGGGCATA | 52.3 | 60.1 | 58.3 | 47.2 | 52.8 |
|      | TTTGTTAGGCGAGGATGTAGGAT | 51.1 | 58.4 | 60.5 | 49.1 | 53.2 |
|      | ACCTGCTACGTTATTTTCA | 47.7 | 54.3 | 52.8 | 44.5 | 49.2 |
|      | TCTATTCGTCGAGGCCCTATCG | 55.7 | 63.5 | 57.2 | 49.4 | 55.9 |
|      | TCAGACGGGATTTGTTAGGCC | 51.8 | 58.4 | 58.9 | 48.7 | 52.2 |
|      | ATCTACTGCTGTAAGGCCCTATTAC | 54.0 | 61.8 | 53.4 | 47.3 | 53.3 |
|      | GCCGAGTTTACAGCTCCCTGAGAAATAAAA | 58.9 | 66.8 | 65.6 | 53.3 | 59.7 |
|      | GCAAAGGAAACTCAGACGGG | 55.9 | 62.5 | 61.1 | 51.4 | 55.3 |
|      | ACTCAGACGGGATTTGTTAGGCG | 54.8 | 62.1 | 60.7 | 51.3 | 55.0 |
|      | ACGGGATTTGTTAGGCACG | 51.1 | 57.3 | 59.8 | 49.2 | 52.3 |
|      | TGTGTTGACCTCAATTAGTTGAGAAGTGCCC | 61.8 | 71.2 | 71.3 | 57.6 | 62.0 |
|      | TGATCATGCAAGAGGCAACTCA | 50.5 | 57.4 | 58.2 | 48.2 | 52.7 |
|      | AGTCAGGTGGTCTTATGCC | 51.1 | 57.3 | 53.8 | 47.8 | 50.3 |
|      | AACTCAAGCCCGATTGTTAGG | 53.0 | 60.3 | 58.5 | 49.0 | 53.2 |
|      | TGAATAAAAGACTCAGGTTG | 47.7 | 54.3 | 52.8 | 44.5 | 49.2 |
|      | TCTATTCGTCGAGGCCCTATT | 50.5 | 57.4 | 51.5 | 45.8 | 51.0 |
| orfu | GTCACACCCAGTTACTTTTCC | 52.4 | 59.4 | 57.2 | 48.0 | 52.8 |
|      | CCTAAACAAATGAGCATGGC | 50.5 | 57.4 | 58.5 | 46.9 | 51.5 |
|      | ATGCGCTATTTCCTGCTTTTGTG | 50.6 | 58.4 | 58.0 | 47.3 | 53.8 |
|      | GGTGTATTTTGATGCTGATG | 53.0 | 60.3 | 61.4 | 49.0 | 53.5 |
|      | AGCTCAATACGAGGTTTATACGC | 53.0 | 60.3 | 59.8 | 50.2 | 55.7 |
|      | CAGAGCCCTGCTATTACTTATATTG | 56.0 | 64.1 | 64.1 | 51.3 | 59.1 |
|      | AGACCCTGCTATTACCTTTTATG | 54.0 | 61.8 | 61.9 | 50.5 | 57.6 |
| zot  | GCCCATAGACCACGATAA | 48.0 | 53.8 | 53.1 | 44.6 | 47.9 |
|      | GCCACTTATACCGCGCCAC | 55.4 | 61.6 | 64.9 | 53.5 | 55.9 |
|      | CTGCTTTTCCACAGGCACCG | 55.4 | 61.6 | 62.4 | 52.5 | 57.1 |
|      | CGCTTTAAGATGCGCCTTTT | 54.8 | 62.1 | 68.6 | 54.8 | 59.8 |
|      | CACGTGTGGGTGATGAGCCTATGC | 57.4 | 65.2 | 64.9 | 52.7 | 58.3 |
|      | CAAAGGCCAACATACAAAAACCAA | 54.4 | 62.5 | 65.8 | 51.8 | 55.9 |
|      | GATATCCCTGCTAACCGCGCTAAC | 57.4 | 65.2 | 61.3 | 51.8 | 56.7 |
|      | AGAGTAAGCAACAGTGATTGAAATCTCCGTAAC | 64.5 | 74.7 | 74.7 | 59.6 | 64.5 |
| rstr | CTCTCTACGACAGCAAAGCTCTCCTAC | 57.4 | 65.2 | 64.6 | 52.5 | 58.6 |
|      | AGCCCTTATCAAAATGATA | 45.6 | 52.3 | 53.7 | 43.4 | 47.8 |
| acfb | GATGAAAGACAGGAGAG | 46.8 | 53.0 | 48.5 | 42.3 | 47.0 |
|      | TTTGCTGAGCGCTATGTCG | 51.8 | 58.4 | 58.7 | 48.8 | 53.7 |
|      | GAGCGTGCTTTATCTAGTGGCTG | 55.3 | 62.9 | 63.6 | 52.0 | 57.5 |
|      | CAGCAACACAGCGACACAC | 51.1 | 57.3 | 59.1 | 49.0 | 51.6 |
| aefc | CACTATTGTCGGCGAAAGC | 49.7 | 56.4 | 59.3 | 46.6 | 50.6 |
| alda | GTCAATGGAAGTGCGACACAGTG | 57.4 | 65.2 | 65.0 | 53.1 | 56.9 |
|      | AACCAGGTTAGGTTTGTACC | 52.4 | 59.4 | 56.9 | 49.2 | 50.8 |
| int  | GATAAGAGATCAAAGCC | 43.5 | 49.3 | 45.6 | 38.3 | 43.1 |
|      | GAAGTGAAAGGAACGGATAGTG | 51.7 | 59.3 | 55.9 | 46.0 | 51.2 |
|      | TGCTTGTACACGATCAGATAG | 53.5 | 61.1 | 56.9 | 49.0 | 54.1 |
|      | CCAACCTTTGTGACGTT | 48.9 | 55.2 | 55.2 | 45.2 | 48.5 |
| Gene | Sequence                                      | Tagg    | Tcpd    | Tcep   | Tcpf    | Tcpi   |
|------|----------------------------------------------|---------|---------|--------|---------|--------|
|      | ACCTACTTTAGGAAAAGAGCC                      | 50.5    | 57.4    | 52.7   | 46.0    | 50.5   |
| tcpd | GCCTCTGTAATCAAGAC                         | 48.0    | 53.8    | 53.0   | 44.7    | 48.8   |
| tcep | GCTCCTGACAATGGCGCTTTATTCA                  | 56.0    | 64.1    | 64.6   | 52.6    | 57.4   |
|      | ACTGTATAGCAAAAGCGATATTCAGAGA               | 53.2    | 61.6    | 57.0   | 49.0    | 56.0   |
|      | GAGTTCACATTGCAGAAGACAGA                    | 55.3    | 62.9    | 62.6   | 52.0    | 55.7   |
|      | GACGCTACCCACTCGACAGA                       | 53.8    | 60.5    | 60.8   | 50.6    | 54.3   |
|      | GGAGTTATCTATGACCCCTGTT                     | 50.4    | 57.4    | 51.3   | 45.3    | 49.1   |
|      | AGGAGATGGAAGTTGGTG                         | 51.1    | 57.3    | 54.6   | 47.7    | 50.3   |
|      | GCAATAGTGCTCGAGCTCGTA                     | 54.8    | 62.1    | 60.6   | 51.2    | 56.6   |
|      | TAGCCTTAGTTCCTCAGCAGGCA                    | 54.8    | 62.1    | 60.5   | 52.4    | 57.7   |
|      | CGACTGCTTTATGCGGAAGT                      | 51.8    | 58.4    | 59.4   | 49.4    | 55.7   |
|      | GATGGTCAGATAAAAGACGCAGG                    | 55.7    | 63.5    | 62.2   | 50.5    | 56.1   |
|      | TAGCAAGGTTACCGGGATAACAA                    | 56.0    | 64.1    | 64.6   | 53.0    | 56.5   |
|      | TGGATTGTATCCCCGGA                         | 49.7    | 56.4    | 58.9   | 47.3    | 49.7   |
| tcpp | ACCGTTAAATCAGCCCAAG                       | 51.8    | 58.4    | 58.9   | 49.3    | 52.5   |
|      | AGCCAATCTAGTAAACTTTGTTC                   | 52.3    | 60.1    | 57.2   | 48.8    | 53.3   |
|      | GCACAGGAGAGATGCAAA                       | 51.8    | 58.4    | 58.8   | 49.5    | 53.3   |
|      | TACCGTAATTGGCGTGGGAG                      | 58.8    | 66.4    | 70.5   | 56.9    | 61.0   |
|      | ACAAAATATCTGCCAACG                        | 45.8    | 51.6    | 53.2   | 43.8    | 47.4   |
|      | CTGGTGCTACATTGAGG                        | 48.9    | 55.2    | 53.7   | 44.7    | 48.9   |
|      | TCACCAAATATCTGCCA                         | 49.7    | 56.4    | 58.7   | 48.2    | 50.8   |
Table S3: Proportion of valid primers according to a threshold set for the computed melting temperature. Three conditions were used to determine valid primers from the theoretical Tms: no threshold (Tm>0°C), Tm above 50°C (Tm>50°C) and Tm above 55°C (Tm>55°C). The published primers, which are not valid with no threshold of Tms (Tm>0), are primers that are not specific to *V. cholerae* and/or cannot detect every allele of their target gene. In some cases (e.g. *tcpA* or *ctxB*), none of many published primers is valid because the sequence of the target gene is very variable into the species and primers were not designed in conserved regions. Generally, this observation corresponds to very well studied genes, which were resequenced many times, increasing the number of different sequences in public databases.

| Gene | Published Primers | Valid Primers |
|------|-------------------|---------------|
|      |                   | Tm>0°C | Tm>50°C | Tm>55°C |
| *ace* | 8                 | 4      | 4       | 4       |
| *acfb* | 6               | 6      | 5       | 4       |
| *acfc* | 1                | 1      | 1       | 1       |
| *alda* | 6                | 2      | 2       | 2       |
| *cep*  | 3                | 2      | 2       | 2       |
| *ctxa* | 41               | 18     | 18      | 18      |
| *ctxb* | 23               | 0      | 0       | 0       |
| *int*  | 5                | 4      | 4       | 4       |
| *orfu* | 9                | 7      | 7       | 7       |
| *rsta* | 8                | 0      | 0       | 0       |
| *rsth* | 3                | 0      | 0       | 0       |
| *rstr* | 3                | 2      | 2       | 2       |
| *taga* | 4                | 0      | 0       | 0       |
| *tagd* | 1                | 1      | 1       | 1       |
| *tcpa* | 27               | 0      | 0       | 0       |
| *tcpb* | 3                | 0      | 0       | 0       |
| *tcpc* | 1                | 0      | 0       | 0       |
| *tcpd* | 1                | 1      | 1       | 1       |
| *tcpv* | 1                | 1      | 1       | 1       |
| *tcpf* | 5                | 5      | 5       | 5       |
| *tcph* | 6                | 0      | 0       | 0       |
| *tcpi* | 7                | 4      | 4       | 4       |
| *tcpj* | 4                | 2      | 2       | 0       |
| *tcpk* | 12               | 2      | 2       | 2       |
| *tcpq* | 5                | 3      | 3       | 3       |
| *toxt* | 13               | 4      | 4       | 4       |
| *zot*  | 24               | 8      | 8       | 8       |
| **Total** | **230**             | **77** | **76** | **73** |
Table S4: Alignments of published primers and sequences of the ctxB gene.

Local alignments of 23 ctxB primers with their target sequences, perfect matches are shown as dots. Hyphens correspond to INDELs or missing part of the sequence (partial sequences).

Columns: 1: strand, 2: Tm computed with OHM that is often slightly underrated, 3: alignment, 4: number of such target sequences. F is for forward primers, R for reverse ones.

| Tm | Alignment | Nbr. | Tm | Alignment | Nbr. |
|----|-----------|------|----|-----------|------|
| 49.8 | ACTATCTTCAGCATATGCACATGG | 22 | 4 | AGATATTTTCGTATACAGAATCTCAG | 20 |
| 44.2 | .........................A.. | 1 | 38.6 | ................G.. | 4 |
| 42.9 | .........................T.. | 1 | 50.9 | TGATAGCCATCCTCCTTTTTCAG | 22 |
| 47.4 | TATCTTCAGCATATGCACATGCACAT | 22 | 47.5 | .....................G.. | 2 |
| 39.9 | .........................A.. | 1 | 48.4 | GATAGCCATCCTCCTTTTTC | 22 |
| 38.2 | .........................T.. | 1 | 43.4 | ..................G.. | 2 |
| 56.0 | GAGGAGCTCCATGTGCATATGCTG | 1 | 51.0 | AAAGCGATGAAAGGATGAAAG | 22 |
| 44.2 | .........................T.. | 1 | 49.1 | ..................A.. | 1 |
| 38.5 | .........................T.. | 1 | 48.8 | ..................C.. | 1 |
| 30.8 | .........................T.. | 1 | 50.7 | GCCATGGAAGGATGAAAGGATAAC | 22 |
| 0.0 | .........................T.. | 1 | 48.7 | ..................C.. | 1 |
| 53.2 | AGGTGTTCCATGTGCATATGC | 21 | 45.6 | ..................ACCGA | 1 |
| 48.6 | .........................T.. | 1 | 52.4 | GCCATGGAAGGATGAAAGG | 22 |
| 44.8 | .........................T.. | 1 | 50.0 | ..................A.. | 1 |
| 43.9 | .........................A.. | 1 | 49.5 | ..................C.. | 1 |
| 51.4 | TATGCACATGGAACACCTCAAA | 20 | 52.2 | TGAAGGATGAAAGGATAACCC | 22 |
| 49.2 | .........................C.. | 1 | 49.4 | ..................C.. | 1 |
| 45.4 | .........................A.. | 1 | 32.7 | ..................ACCGA | 1 |
| 40.3 | .........................G.. | 1 | 54.8 | TCCTCAGGGTATCCTCCTC | 23 |
| 37.5 | .........................A.. | 1 | 36.1 | ..................ATCGGT | 1 |
| 44.7 | ACGCTCAAATATTACTGA | 19 | 52.4 | GATATGCAATCCTCAGGATACCC | 22 |
| 43.3 | .........................C.. | 1 | 47.4 | ..................A.. | 1 |
| 43.1 | .........................C.. | 1 | 38.4 | ..................ACCGA | 1 |
| 38.9 | .........................C.. | 1 | 49.1 | GCTTCAGTGAGTATGCAATCCTC | 21 |
| 38.3 | .........................A.. | 1 | 46.4 | ..................T.. | 1 |
| 34.2 | .........................G.. | 1 | 44.9 | ..................A.. | 1 |
| 50.1 | ACTGATGTTGTCAGAATTACAC | 15 | 42.9 | ..................TT.. | 1 |
| 48.9 | .........................C.. | 1 | 48.8 | CGATAGGGGCTTTTATTATCC | 23 |
| 48.8 | .........................C.. | 1 | 37.8 | ..................G.. | 1 |
| 46.4 | .........................G.. | 1 | 56.2 | TAAATGCGGCAATCGCAGTGACGGT | 23 |
| 44.7 | .........................CA.. | 1 | 45.6 | ..................T.. | 1 |
| 36.8 | .........................G.. | 1 | 59.1 | ATGCGCCGAATCGCAGTGACGGT | 23 |
| 32.9 | .........................A.. | 1 | 47.5 | ..................T.. | 1 |
| 49.7 | GGTATTTTCGCAACAAATCAC | 18 | 62.2 | TGCGCACAATCGCAGTGACGG | 23 |
| 48.8 | .........................C.. | 1 | 48.4 | ..................T.. | 1 |
| 44.3 | .........................G.. | 1 | 52.7 | GCCATACTAATGCGCAGTACCGTG | 23 |
| 44.3 | .........................G.. | 1 | 51.0 | ..................T.. | 1 |
| 43.6 | .........................C.. | 1 | 34.3 | ..................A.. | 1 |
| 34.3 | .........................C.. | 1 | 34.3 | ..................T.. | 1 |
Table S5: primers sets for *ctxA*, *ctxB* and *tcpA* genes.

Columns: 1: gene name, 2: forward primer, 3: reverse primer, 4: minimal melting temperature of the pair of primers (°C), 5: method used for the design. “Manual” corresponds to a design using the alignment of every genetic variant of the gene and a manual selection of conserved regions. Since Primaclade provided a large number of forward primers (and not 4 couples as Prifi), only partial Primaclade results are shown. The third column is filled by a double hyphen (--) for Primaclade, because this software returns only forward primers and not a couple of primers. For *ctxA*, only primers corresponding to valid published primers (and thus experimentally tested) were selected from the output of Primaclade.

| Gene | Forward sequence | Reverse Sequence | Tm | Method |
|------|------------------|------------------|----|--------|
| **ctxB** | | | | |
| | TCGTATACAGAATCTGACCTGAA | GCCATACAAATTGCCCAATTGCGC | 55.0 | Manual |
| | TCGTATACAGAATCTGACCTG | ATTGCACTACTAATTTGCGC | 59.4 | Prifi |
| | GGTGTTTTTTTTAYAGTTTTACTATCTTCAGC | ATTTGCCATACTAATTTGCGC | 59.4 | Prifi |
| | GGTGTTTTTTTTAYAGTTTTACTATCTTCAGC | ATACGCTTTTTTTTGTTAACTAATTTGAC | 59.2 | Prifi |
| | TCGTATACAGAATCTGACCTG | ATACGCTTTTTTTTGTTAACTAATTTGAC | 59.2 | Prifi |
| | TACAGAATCTGACCTGAAAMAGAG | -- | 59.7 | Primaclade |
| | ATCTCTGAAAMAGAGAGATG | -- | 59.6 | Primaclade |
| | TAGCTGGAAAMAGAGAGATGCGCTAT | -- | 61.0 | Primaclade |
| | GGAAMAGAGAGATGCTATCATTAC | -- | 59.9 | Primaclade |
| | TTAAGAATGTTGMAAYTTTTCCAAGTA | -- | 61.4 | Primaclade |
| | AAATGTTGMAAYTTTTCCAAGTAAG | -- | 60.4 | Primaclade |
| | AAAAGCGATATGAGAGATGGAAG | -- | 60.9 | Primaclade |
| | AACGATGAGAGATGGAAG | -- | 59.3 | Primaclade |
| **tcpA** | | | | |
| | CRCGAATAAGAAAAMCGGTCAAGGG | CCGACRCTCATTGRTATTTTCTC | 55.6 | Manual |
| | CATCTCTATATATATATATTGATGACGCTTGAC | GGATGTTCTCTAAGGAAATTTTACACCTGC | 59.5 | Prifi |
| | AAAATGAAATCAACCCCTTATTGATCATGCAAG | CAAGGAATTTTACACTTAGGATTTGAC | 59.8 | Prifi |
| | AAAATGAAATCAACCCCTTATTGATCATGCAAG | ATGATCTGGAAGCATCCTC | 59.8 | Prifi |
| | TTTCACCTCTCATTGATGTAGTGAAG | ATGATCTGGAAGCATCCTC | 59.8 | Prifi |
| | ATGATCTGGAAGGAAACTC | -- | 57.4 | Primaclade |
| | CGTAAATGATGTAGGATGAGCACA | -- | 60.1 | Primaclade |
| | CACCGTACTGTCTTTATTTGCA | -- | 54.3 | Primaclade |
| | TCATCTCTGAGGCCCTATTACG | -- | 63.5 | Primaclade |
| | TCATCTCTGAGGCCCTATTACG | -- | 58.4 | Primaclade |
| | AGGGATTTTGTAGGACGGA | -- | 57.3 | Primaclade |
| | TGATCTGGAAGGAAACTC | -- | 57.4 | Primaclade |
| | TGAAATGAAATCAACCCCTTATTG | -- | 54.3 | Primaclade |
| | TCATCTCTGAGGCCCTATT | -- | 57.4 | Primaclade |
Table S6: Examples of possible improvements for *ctxB* PCR primers.

Local alignments of primers with their target sequences, perfect matches are shown dots. Shorter primers contained into these primers are not shown. Columns: 1: Tm computed with OHM that is often slightly underrated, 2: consensus Tm computed with dnaMATE, 3: alignments, 4: number of target sequences. Primers can be improved by adding ambiguities shown in bold. Note that for the last primer, the last position could be simply removed.

| Tm (°C) | Cons. Tm (°C) | Sequence alignment | Targets |
|---------|--------------|--------------------|--------|
| 49.8    | 52.7         | ACTATCTTCAGCATATGCACATGG \ ...........................A... \ ...........................T... \ ACTATCTTCAGCATATGYAMATGG | 21     |
| 44.2    | 50.1 – 53.7  |                     | 1      |
| 42.9    | 50.1 – 53.7  |                     | 1      |
| 44.9 – 49.8 | 50.3 – 53.7 |                     | 24     |
| 42.2    | 50.3         | AGATTTTCGTATACAGAATCTCTAG \ ........................... | 20     |
| 38.6    | 50.3 – 51.3  |                     | 4      |
| 42.2 – 43.9 | 52.4 – 53.8 |                     | 22     |
| 50.9    | 52.4         | TGATAGCCATCTCTCTTTTCCAG \ ...........................G... \ TGATAGCCATCTCTCTKTTTCAG | 24     |
| 47.5    | 52.4 – 53.8  |                     | 2      |
| 50.9 – 53.0 | 50.9         | AAAGCGATTGAAAGGATGAAGG \ ...........................A... \ ...........................C... \ AAAGCGATTGAMAGGATGAAGGR | 24     |
| 51.4    | 50.9 – 53.1  |                     | 1      |
| 49.1    | 51.1         |                     | 1      |
| 48.8    | 51.7 – 52.7  |                     | 24     |
| 50.0 – 53.1 | 59.0         | GCCATACATTGGCGCAATCGCATG | 23     |
| 56.2    | 59.0         | GCCATACATTGGCGCAATCGCATG | 23     |
| 45.6    | 59.0         | GCCATACATTGGCGCAATCGCATG | 23     |
| 51.9 – 56.2 | 58.4 – 59.0 |                     | 1      |
| 51.7 – 52.7 | 58.4 – 59.0 |                     | 1      |
Table S7: Examples of possible improvements for tcpA.

Local alignments of primers with their target sequences, perfect matches are shown by dots. Shorter primers contained into these primers are not shown. Columns: 1: Tm computed with OHM that is often slightly underrated, 2: consensus Tm computed with dnaMATE, 3: alignments, 4: number of target sequences. Primers can be improved by adding ambiguities shown in bold.

| Tm (°C) | Cons. Tm (°C) | Sequence alignment          | Targets |
|---------|---------------|-----------------------------|---------|
| 39.5    | 51.5          | ATGCAATTATTTAAACAGCTTTTTAAG | 43      |
| 37.9    | 51.5 – 52.4   | ........................... | 3       |
| 39.5 – 41.1 | 51.5 – 52.4 | ATGCAATTAYTTAAACAGCTTTTTAAG | 46      |
| 52.5    | 55.1          | CACGATAAGAAAAACGGTCAAGGG   | 44      |
| 47.9    | ........................... | 1       |
| 45.3    | ........................... | 1       |
| 50.5 – 52.5 | 55.1 – 57.5 | CRGATAAGAAAAACGGTCAAGGG   | 46      |
Table S8: Examples of possible improvements for other genes.

Local alignments of primers with their target sequences, perfect matches are shown dots. Columns: 1: gene name, 2: Tm computed with OHM that is often slightly underrated, 3: consensus Tm computed with dnaMATE, 4: alignments, 5: accession number of target sequences. Primers can be improved by adding ambiguities shown in bold.

| Gene | Tm (°C) | Cons. Tm (°C) | Sequence alignment | Targets |
|------|---------|---------------|--------------------|---------|
| cep  | 58.1    | 53.1          | AACCCGAGGTAAAGAGTG | CP001486 |
|      | 54.4    |               |                    | CP001236, AF220606 |
|      | 58.1 – 58.3 | 51.7 – 53.1 | AACCCGAGGTAAAGAGTG | CP001486, M83563 |
|      |         |               |                    | AF220606 |
| zot  | 54.1    | 51.6          | AACCCGTTTTCTCTCTACCA | X64098, FJ209009, X74730, FJ209005, FJ209007 |
|      | 47.7    |               |                    | FJ209006, FJ209008, FJ209010 |
|      | 54.1 – 59.5 | 51.6 – 55.5 | AACCCGTTTTCTCTCTACCA | AE003852, AF220606 |
|      |         |               |                    | CP001235.PE1548 |
| tcpQ | 54.1    | 56.6          | GAGGACTTGTCTGAATCTGCTCAT | FJ209011, X64098, FJ209009, X74730, FJ209005, FJ209007 |
|      | 48.2    |               |                    | FJ209006, FJ209008, FJ209010 |
|      | 51.5 – 54.1 | 53.9 – 56.6 | GAGGACTTGTCTGAATCTGCTCAT | AE003852, AF220606 |
|      |         |               |                    | CP001235.PE1548 |
| orfU | 58.8    | 58.7          | AACCTCGGTCTCGCCCTCTGCATC | FJ209005, FJ209007, CP001235, FJ209010, FJ209006, FJ209009, X64098, FJ209011, X74730 |
|      | 54.6    |               |                    | FJ209008 |
|      | 58.8 – 60.5 | 58.7 – 61.2 | AACCTCGGTCTCGCCCTCTGCATC | FJ209004 |
| tcpB | 56.5    | 51.7          | CGTTGCCGTCAGTCTTTG | FJ209005, FJ209007, CP001235, FJ209010, FJ209006, FJ209009, X64098, FJ209011, X74730 |
|      | 53.4    |               |                    | FJ209008 |
|      | 52.0    |               |                    | FJ209004 |
|      | 55.2 – 57.9 | 46.5 – 51.5 | YGTTGGCGTCAGTCTTTG | FJ209005, FJ209007, CP001235, FJ209010, FJ209006, FJ209009, X64098, FJ209011, X74730 |
Table S9: Strains, biovars and serotypes of nucleic sequences.

Columns: 1: gene name, 2: accession number, 3: number of other sequences identical to or contained in the sequence, 4: species names, 5: biovar, serotype and strain information.

| Accession number | Duplicates | Species                        | Biovar, Serotype and Strain                                                                 |
|------------------|------------|--------------------------------|-------------------------------------------------------------------------------------------|
| D30052.CTXA      | 30         | Vibrio cholerae                | O1 0402, O1 0403, O1 0404, O1 0405, O1 0406, O1 0407, O1 0408, O1 0409, O1 0410, O1 0502, O1 0503, O1 0504, O1 0505, O1 0506, O1 0507, O1 0508, O1 0601, O1 0602, O1 0603, O1 0604, O1 0605, O1 0606, O1 0702, O1 0706, O1 0709, O105 571-88, O139 0401, O141 203-95, O37 1322-69, O37 87 |
| FJ748608.CTXA    | 0          | Vibrio cholerae                | non O1/O139 J31W                                                                           |
| AJ375590.TOXA    | 0          | Vibrio cholerae                | Inaba                                                                                     |
| X00171.PE1       | 1          | Vibrio cholerae                | 2125                                                                                      |
| X58786.PE1       | 14         | Vibrio cholerae                | Classical 569B, El Tor 2125, El Tor O1 B33, El Tor O1 N16961, El Tor O1 VC44, Inaba O1 VC04, MJ-1236, MTCC 3906, O1 O395, O139 JS9803, O139-Bengal 1854 |
| AF175708.CTXA    | 0          | Vibrio cholerae                | KNIH002                                                                                   |
| AF390572.CTXA    | 10         | Vibrio cholerae                | Classical O1 GP8, Classical O1 H218, Classical O1 NIH35A3, Classical O1 NIH41, Classical O1 V154, Classical O1 VC44, El Tor O1 B33, El Tor O1 CO457, El Tor O1 VC106, El Tor O1 VC20, O27 65-96 |
| AY376268         | 0          | Vibrio cholerae                | non O1/O139 F                                                                             |
| AY376267         | 0          | Vibrio cholerae                | non O1/O139 B                                                                             |
| DQ132785         | 0          | Vibrio cholerae                |                                                                                            |
| CP001486.PE458   | 3          | Vibrio cholerae                | El Tor O1 N16961, MJ-1236, O1 M66-2, Ogawa O1                                              |
| M59466.PE1       | 0          | Vibrio cholerae                | DH5-alpha                                                                                 |
| CP001236.HAP     | 1          | Vibrio cholerae                | Ogawa O1 O395                                                                             |
| AB435238         | 0          | Vibrio mimicus                 | ES-39                                                                                     |
| AB071709.VFPA    | 0          | Vibrio fluvialis               | AQ0005                                                                                   |
| M64809.PE1       | 0          | Vibrio proteolyticus           |                                                                                            |
| EU675309         | 0          | Vibrio tubiashii               | RE22                                                                                      |
| FJ455120         | 0          | Vibrio tubiashii               | 00-90-6                                                                                   |
| FJ455119         | 0          | Vibrio tubiashii               | X00-12-1; RE98                                                                            |
| FJ455121         | 0          | Vibrio tubiashii               | ATCC 19105                                                                                 |
| US50548.PE1      | 0          | Vibrio vulnificus              |                                                                                            |
| CP000062.HLYA    | 0          | Vibrio cholerae                | Ogawa O1 O395                                                                             |
| A3757090.HLYA    | 0          | Vibrio cholerae                | Inaba                                                                                     |
| Y00557.HLYA      | 2          | Vibrio cholerae                | O17, El Tor O1 N86, O1 M66-2                                                             |
| CP001486.PE34    | 7          | Vibrio cholerae                | ATCC 14035, CIP104154, Clin2200, El Tor N16961, Env25, M793, MJ-1236, O139                |
| M36855.PE1       | 0          | Vibrio cholerae                |                                                                                            |
| GU809235         | 0          | Vibrio cholerae                | Env40                                                                                     |
| GU586279         | 0          | Vibrio cholerae                | Env8                                                                                      |
| GU809234         | 0          | Vibrio cholerae                | Env8Q                                                                                     |
| GU586281         | 0          | Vibrio cholerae                | Env34                                                                                     |
| AF194418.HLYA    | 0          | Vibrio cholerae                | 3509                                                                                      |
| GU593975         | 0          | Vibrio cholerae                | Env4Q                                                                                     |
| DS8374.PE1       | 1          | Vibrio cholerae                | N037                                                                                      |
| AY427780.PE1     | 0          | Vibrio cholerae                | El Tor non O1/O139                                                                       |
| GU586277         | 0          | Vibrio cholerae                | Env31Q                                                                                   |
| GU230682         | 0          | Vibrio harveyi                | STD-3-0945                                                                                 |
| GU230681         | 0          | Vibrio harveyi                | STD-3-0945                                                                                 |
| GU137288.VMHA    | 0          | Vibrio mimicus                 | ATCC 33653                                                                                 |
| U68271.VMHA      | 0          | Vibrio mimicus                 | ATCC 33653                                                                                 |
| GU137289.VMHA    | 0          | Vibrio mimicus                 | 03-4472g2                                                                                 |
| GU137291.VMHA    | 0          | Vibrio mimicus                 | 05-3478                                                                                   |
| GU137290.VMHA    | 0          | Vibrio mimicus                 | CDC08-2487                                                                                |
| EF187438         | 0          | Vibrio mimicus                 | HX4                                                                                       |
| FJ222406         | 0          | Vibrio mimicus                 |                                                                                            |
| AF348455         | 0          | Vibrio mimicus                 |                                                                                            |
| CP001485.PE2734  | 2          | Vibrio cholerae                | El Tor O1 N16961, MJ-1236, O1 M66-2                                                        |
| CP001235.OMP6    | 2          | Vibrio cholerae                | Ogawa O1 O395                                                                             |
| AF253529         | 0          | Vibrio cholerae                | El Tor                                                                                   |
| DQ356330         | 0          | Vibrio mimicus                 | 04-14                                                                                     |
| DQ356331         | 0          | Vibrio mimicus                 | HX4                                                                                       |
| DQ846741         | 0          | Vibrio mimicus                 | 04-5                                                                                      |
| Accession | Organism                          | Strain/Reference       |
|-----------|-----------------------------------|------------------------|
| EU285491  | Vibrio tubiashii                  | ATCC 19109             |
| DQ846743  | Vibrio mimicus                    | 04-13                  |
| EU285490  | Vibrio tubiashii                  | ATCC 19105             |
| AE016795.PE1557 | Vibrio vulnificus            | CMCP6                  |
| BA000357.VV2720 | Vibrio vulnificus             | YJ016                  |
| DQ090545  | Vibrio vulnificus                 | VV7-00                 |
| DQ090544  | Vibrio vulnificus                 | NCIMB 2136             |
| CP001805.PE989 | Vibrio sp.                     | EX25                   |
| CP000789.PE3292 | Vibrio harveyi               | ATCC BAA-1116; BB120   |
| FJ919231  | Vibrio harveyi                    | zj2008                 |
| CP001808.PE1228 | Vibrio sp.                   | EX25                   |
| CP000790.PE374 | Vibrio harveyi               | ATCC BAA-1116; BB120   |
| aompW     | Vibrio cholera                     | ompW                   |
| BA000332.VPA0096 | Vibrio paraohaemolyticus        | O3:K6, ATCC 17802      |
| DQ425109  | Vibrio paraohaemolyticus          | ZJ2003                 |
| AY944132  | Vibrio alginoleticus              | H4901                  |
| DQ075316  | Vibrio alginoleticus              | H4901                  |
| GG991116  | Vibrio alginoleticus              | H4901                  |
| CP001808.PE902 | Vibrio sp.                  | EX25                   |
| CP000790.PE2148 | Vibrio harveyi               | ATCC BAA-1116; BB120   |
| FJ9908286 | Vibrio harveyi                    | ZJ2008                 |
| FM954973.PE1234 | Vibrio splendidus            | LGP32                  |
| M85198.STN | Vibrio cholera                     | NG1-36                 |
| M97591.STO | Vibrio cholera                     | NG1-36                 |
| tcpA      | Vibrio cholera                     | tcpA                   |
| AF030546  | Vibrio cholera                     | non O1/O139 #151       |
| DQ132784.PE1 | Vibrio cholera                   | non O1/O139 #208       |
| FJ209006.TCPA | Vibrio cholera                  | O27 M1112              |
| AF414371  | Vibrio cholera                     | O36 VCE 22             |
| AF512421.TCPA | Vibrio cholera                  | EVC O1 ZI6061         |
| EU622531.TCPA | Vibrio cholera                  | EVC O1 LN9309, EVC O1 LN93097, EVC O1 ZI4042, HB84419, O1 ZI59 |
| AF512423.TCPA | Vibrio cholera                  | EVC O1 ZI4042         |
| AF209004.TCPA | Vibrio cholera                  | O80 M1098, O77 8-76    |
| AF452571  | Vibrio cholera                     | O26 63                 |
| FJ209009.TCPA | Vibrio cholera                  | O37 1322-69, O37 V52, non O1/O139 M1618 |
| X64098.TCPA | Vibrio cholera                     | Classical O1 Z17561   |
| M33514.TCPA | Vibrio cholera                     | Classical Z17561      |
| AF315787  | Vibrio cholera                     | 343CAS                 |
| AB012946  | Vibrio cholera                     | OXQ9065                |
| AY056618.TCPA | Vibrio cholera                  | O1 ZI24                |
| AF452580  | Vibrio cholera                     | O191 366-96            |
| AF452573  | Vibrio cholera                     | O48 AQ1875             |
| EU622527.TCPA | Vibrio cholera                  | O1 ZI22                |
| EU362122  | Vibrio cholera                     | OS6 A199               |
| GU797082  | Vibrio cholera                     | OS6 A217               |
| EU622526.TCPA | Vibrio cholera                  | O1 ZI20                |
| FJ209005.TCPA | Vibrio cholera                  | O105 577-88, O105 M1118, O4 VCE232 |
| AF452585  | Vibrio cholera                     | O44 SCE188             |
| AF390571  | Vibrio cholera                     | O27 365-96             |
| AF452574  | Vibrio cholera                     | O49 507-94             |
| EU622525.TCPA | Vibrio cholera                  | ZI109                  |
| AF139626  | Vibrio cholera                     | O59 10259              |
| FJ209008.TCPA | Vibrio cholera                  | O141 203-93, O141 M1593 |
| GU797083  | Vibrio cholera                     | OS6 K216               |
| GU797084  | Vibrio cholera                     | non O1/O139 A213       |
| FJ209007.TCPA | Vibrio cholera                  | O115 523-80, O115 M1567 |
| AY056619.TCPA | Vibrio cholera                  | XJ90006               |
| Accession | Strain | Type |
|-----------|--------|------|
| DQ356010 | Vibrio mimicus | HX4 |
| EU622529.TCPA | Vibrio cholerae El Tor O139 JS9803, O1 ZJ25, O139 HLJ9803, O139 JS9801, O139 JX98108, O139 LN1997 | 5 |
| AF512411.TCPA | Vibrio cholerae O139 GD9512, O139 NM1996 | 1 |
| EU649677.TCPA | Vibrio cholerae Y1 | 0 |
| EL622532.TCPA | Vibrio cholerae O1 ZJ65 | 0 |
| AF512408.TCPA | Vibrio cholerae O139 XJ93131 | 0 |
| X74730.TCPA | Vibrio cholerae EVC O1 FJ62168, EVC O1 GD1961, EVC O1 GD98224, EVC O1 GX9525, EVC O1 LN9337, EVC O1 SD76137, EVC O1 SD7783, EVC O1 SD7763, EVC O1 WUJANG2, EVC O1 XJ73329, El Tor E7946, El Tor O1 C6706, El Tor O1 H1, El Tor O1 N16961, El Tor O1 SC8511, El Tor O1 SC98107, El Tor O1 SM115, El Tor O139 FJ98352, El Tor O139 JX94484, MJ-1236, O1 M2140, O1 M66-2, O1 ZJ47, O139 63-93(MO45), O139 FJ9510, O139 MO10, O139 SD01001, O37 CO130, O44 S66-94, O53 S585 | 29 |
| EU649678.TCPA | Vibrio cholerae O139 XJ93006 | 0 |
| FJ209003.TCPA | Vibrio cholerae O1 M794 | 0 |
| AY052831.PE1 | Vibrio cholerae SD95001 | 0 |
| EL622533.TCPA | Vibrio cholerae O1 ZJ75, O8 V54 | 1 |
| AF452570 | Vibrio cholerae O8 153-94 | 0 |
| FJ209010.TCPA | Vibrio cholerae non O1/O139 M1619 | 0 |
| tcpI | Vibrio cholerae Ogawa O1 O395 | 3 |
| L25659.TCPJ | Vibrio cholerae Ogawa O1 O395 | 0 |
| X4098.TCPI | Vibrio cholerae Classical O1 Z17561 | 0 |
| CP001485.PE23461 | Vibrio cholerae El Tor O1 H1, El Tor O1 N16961, MJ-1236, O1 M66-2 | 4 |
| toxR | Vibrio cholerae CECT 514T | 2 |
| CP001235.TOXR | Vibrio cholerae Ogawa O1 O395 | 1 |
| CP001485.PE2314 | Vibrio cholerae MJ-1236, O1 M66-2, El Tor O1 N16961 | 2 |
| GU230678.TOXR | Vibrio harveyi STD 3-0953 | 0 |
| GU230677.TOXR | Vibrio harveyi STD 3-0949 | 0 |
| EF693743.TOXR | Vibrio mimicus 1.1969 | 0 |
| AF170881 | Vibrio mimicus ATCC 33655 | 0 |
| zot | Vibrio cholerae Classical O1 GP8, Classical O1 H218, Classical O1 NIH35AJ, Classical O1 NIH41, Classical O1 V154, Classical O1 VC44, El Tor O1 B33, El Tor O1 CO457, El Tor O1 VC106, El Tor O1 VC20, Ogawa O1 O395 | 14 |
| M83563.ZOT | Vibrio cholerae El Tor O1 N16961, KNIH002, MJ-1236, Ogawa O139 | 3 |
| CP001486.PE741 | Vibrio cholerae El Tor O1 N16961, KNIH002, MJ-1236, Ogawa O139 | 3 |
| AF207857 | Vibrio mimicus P15 | 0 |
| AF220606.ZOT | Vibrio cholerae El Tor O1 86015 | 0 |
Table S10: List of softwares available for the design of primers. “Speciality” describes if softwares can design primers for specific molecular methods and “Input” reports the several types of format compatible with each software. “Parameter settings” presents common parameters of softwares and the methods used to compute melting temperature (Tm). “Option” shows if a program supports degenerated positions (IUPAC code), checks specificity (BLAST of primers) and coverage (BLAST of input sequences) of primers, and allows using target sequences and non-target sequences. qPCR: quantitative PCR, RFLP: Random Fragment Length PCR, SNP: Single Nucleotide Polymorphism.

| Type                        | Primer properties determination | %GC | Amplicon size | Primer size | Secondary structure | Substrate concentrations | Basic method (Tm) | Salt-adjusted method (Tm) | Nearest neighbor method (Tm) | BLAST for input sequences | BLAST for primers | IUPAC code | Non-target sequences | Region selection |
|-----------------------------|--------------------------------|-----|---------------|-------------|--------------------|-------------------------|-------------------|--------------------------|-----------------------------|----------------------|----------------|------------|---------------------|-------------------|
| **De novo primer design**   |                                |     |               |             |                    |                         |                   |                          |                             |                      |               |            |                     |                   |
| **Primer properties determination** |                        | *   |               |             |                    |                         |                   |                          |                             |                      |               |            |                     |                   |
| Long-Range PCRs             |                                | *   |               |             |                    |                         |                   |                          |                             |                      |               |            |                     |                   |
| Multiplex PCRs              |                                | *   |               |             |                    |                         |                   |                          |                             |                      |               |            |                     |                   |
| qPCRs                       |                                |     |               |             |                    |                         |                   |                          |                             |                      |               |            |                     |                   |
| Regular PCRs                |                                | *   |               |             |                    |                         |                   |                          |                             |                      |               |            |                     |                   |
| RFLP                        |                                |     | *             |             |                    |                         |                   |                          |                             |                      |               |            |                     |                   |
| SNP Genotyping / Mutagenesis|                                | *   | *             |             |                    |                         |                   |                          |                             |                      |               |            |                     |                   |
| GenBank ID                  | *                              |     |               |             |                    |                         |                   |                          |                             |                      |               |            |                     |                   |
| Multiple amino acid sequences|                                 | *   |               |             |                    |                         |                   |                          |                             |                      |               |            |                     |                   |
| Multiple nucleic acid sequences|                               | *   |               |             |                    |                         |                   |                          |                             |                      |               |            |                     |                   |
| Reference SNP ID            |                                |     |               |             |                    |                         |                   |                          |                             |                      |               |            |                     |                   |
| Single amino acid sequence  | *                              |     |               |             |                    |                         |                   |                          |                             |                      |               |            |                     |                   |
| Single nucleic acid sequence| *                              | *   |               |             |                    |                         |                   |                          |                             |                      |               |            |                     |                   |

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