**Colorimetric LAMP Protocol for the detection of *BmHha* I, *OvGST1a* and *WbLDT***

|  |  |  |  |
| --- | --- | --- | --- |
| Components | Volume (l) | 2X concentration | 1X concentration |
| 10 mM dNTP solution mix (NEB# N0447) | 1400  | 2.8 mM | 1.4 mM |
| 3M (NH4)2SO4 | 33.4  | 20 mM | 10 mM |
| 100 mM MgSO4 | 800 | 16 mM | 8 mM |
| a2M KCl | 50 (if wt*Bst,* LF) | 20 mM | 10 mM |
|  | 250 (if *Bst* 2.0 or *Bst* 2.0 WS) | 100 mM | 50 mM |
| Tween 20 | 10  | 0.2 % v/v | 0.1 % v/v |
| bH2O | 2165.3 (if wt*Bst*, LF) | --- | --- |
|  | 2005.3 (if *Bst* 2.0 or *Bst* 2.0 WS) | --- | --- |
| cTotal Volume | 5000 | --- | --- |

**1.** **Prepare** **2X colorimetric solution mix:**

**2. Prepare 25X Dye Stocks of neutral red or phenol red:**

|  |  |  |
| --- | --- | --- |
| Components | Volume (l) | 25X concentration |
| d50 mM Dye solution | 50  | 2.5 mM |
| H2O | 950 | --- |
| eTotal Volume | 1000 | --- |

**3. cMaster Mix:**

|  |  |  |
| --- | --- | --- |
| Components | Volume (l)/rxn | Volume (l)/100 rxn |
| 2X colorimetric solution mix pH 8.6-8.8 | 12.5  | 1250  |
| 25X Dye solution pH 8.6 | 1 | 100  |
| f*Bst* polymerase (120,000 U/ml) | 0.067  | 6.7  |
| bH2O | 1.433  | 143.3  |

**4. 25X Primer Mixes:**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| gStandard Primers | Volume (l) | 25X concentration | 1X concenration |  |
| 100 M FIP | 40 | 40 M | 1.6 M |  |
| 100 M F3 | 5  | 5 M | 0.2 M |  |
| 100 M BIP | 40  | 40 M | 1.6 M |  |
| 100 M B3 | 5  | 5 M | 0.2 M |  |
| H2O | 10  | ---- | ---- |  |
| Total Volume | 100 | ---- | ---- |  |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| gLoop Primers | Volume (l) | 25X concentration | 1X concentration |  |
| 100 M LF | 10  | 10 M | 0.4 M |  |
| 100 M LB | 10  | 10 M | 0.4 M |  |
| H2O | 80  | ---- | ---- |  |
| Total Volume | 100 | ---- | ---- |  |

**5. hColorimetric LAMP reactions:**

|  |  |
| --- | --- |
| Components | Volume (l) |
| Master mix pH 8.6-8.8 | 15  |
| 25X Standard Primer mix | 1 |
| 25X Loop primer mix | 1 |
| iSubstrate DNA | 2 |
| H2O | 6 |
| Total Volume | 25 |

a. Separate solution mixes must be prepared for *Bst* DNA polymerase, Large fragment (wt*Bst,* LF) and *Bst* DNA polymerase 2.0 (*Bst* 2.0) as well as its WarmStart version (*Bst* 2.0 WS) as these enzymes require different KCl concentrations.

b. Adjust the pH of the solution to between 8.6-8.8 with 1M KOH before bringing the solution up to its final volume with H2O.

c. Store aliquots frozen in screw top tubes.

d. Neutral red and phenol red dyes are available from Sigma-Aldrich.

e. Adjust the pH of the dye solution to 8.6 or greater with 1M KOH before bringing the solution up to its final volume with H2O.

f. Highly concentrated polymerase is used to minimize carry over of Tris into the master mix. NEB catalog numbers for concentrated polymerase are as follows: wt*Bst* LF, M0275M; *Bst* 2.0, M0537M and *Bst* 2.0 WS, M0538M.

g. 100 M primer stocks are prepared in H2O to minimize carry over of Tris.

h. LAMP reactions are incubated in a NINA heater @ 63oC for 40 to 70 min depending on the primer set as described in the Materials and Methods.

i. Substrate DNA can be dissolved in either T10E 0.1 orH2O.