# GMO Detective

## Wetware Guide:

The wetware guide will help you to gather and buy all the biological (wet) materials you need to set up the experiments. This consists of reagents and DNA (controls and primers). These companies usually do not sell to private individuals and the prices go down drastically when buying in bulk, so better to contact us :)



### 1. Enzyme mix

This is a premade mix consisting of an improved Isothermal Strand Displacing DNA polymerase enzyme along with Nucleotides and a buffer that allow the reaction to happen, all you need to add to this is a primer set and sample.

For simplicity we currently use NEB WarmStart® LAMP Kit (DNA & RNA) E1700:

https://www.neb.com/products/e1700-warmstart-lamp-kit-dna-rna

Available worldwide through their Subsidiaries / Distributors.

The small package is enough for 250 reactions which is 30 Experiments. (a regular LAMP reaction is 25ul, we do 8x10ul reactions per experiment)

## 2. Primers

The experiment uses 2 sets of  QUASR LAMP primers sets specific to are certain region. Each set has 7 Primers :
2 long internal primers
2 short external primers
2 loop primers to make the reaction faster
1 quenching primer to quench the fluorescence when the reaction is negative

Primers can be ordered from you Local Supplier, but are cheaper in bulk so contact us for purchasing together :)
Standard desalination is fine, except for quencher probes which need to be HPLC purified

Idtdna.com
Eurofinsgenomics.com
Thermofisher.com
Sigmaaldrich.com

### a. COX1 : Plant Gene primers

COX1 gene common to all plants

Based on: Rapid Detection of Phytophthora ramorum and P. kernoviae by Two-Minute DNA Extraction Followed by Isothermal Amplification and Amplicon Detection by Generic Lateral Flow Device J. A. Tomlinson, M. J. Dickinson, and N. Boonham
doi:10.1094/ PHYTO-100-2-0143

| Primer | Sequence 5'to3' | Modification |
|---|---|---|
| COX F3 | tatgggagccgtttttgc | |
| COX B3 | aactgctaagrgcattcc | 5' FAM |
| COX FIP | atggatttgrcctaaagtttcagggcaggatttcactattgggt | |
| COX BIP | tgcatttcttagggctttcggatccrgcgtaagcatctg | |
| COX F-Loop | atgtccgaccaaagattttacc | |
| COX B-Loop | gtatgccacgtcgcattcc | |
| COX FIPQ | YCAAATCCAT | 3' Black Hole Quencher®-1<br>or<br>Iowa Black® FQ |

## b. GMO Gene primers

## CaMV-35S promoter common to most GMO plants:

Based on: Real-time loop-mediated isothermal amplification for the CaMV-35S promoter as a screening method for genetically modified organisms; Fukuta, S., Mizukami, Y., Ishida, A. et al. Eur Food Res Technol (2004) 218: 496.
https://doi.org/10.1007/s00217-003-0862-5

| Primer | Sequence 5'to3' | Modification |
|---|---|---|
| 35S_FIP | aggcatcttcaacgatggccttaaaggaaggtggctcctac<br>a | |
| 35S_BIP | tgccgacagtggtcccaaagttgaagacgtggttggaacg | 5' FAM |
| 35S_F3 | tgcccagctatctgtcactt | |
| 35S_B3 | tcccttacgtcagtggagat | |
| 35S_FLoop | tcctttatcgcaatgatg | |
| 35S_BLoop | agcatcgtggaaaaagaag | |
| 35S_BIPQ | ACTGTCGGCA | 3' Black Hole Quencher®-1<br>Or Iowa Black® FQ |

In each 10ul reaction in a PCR tube we have in the end:

5ul LAMP reaction mix

3ul Primer mix

2ul sample

=10ul

Guide to 3x Primer mix dilutions (starting from 50/100 uM) for different numbers of reactions:

| Primer | Primer working stock conc. | Primer conc. in 10µl LAMP rxn | Vol. of primer (1 rxn) µL | Vol. of primer (10 rxn) µL | Vol. of primer (50 rxn) µL | Vol. of primer (100 rxn) µL | Vol. of primer (200 rxn) µL | Vol. of primer (500 rxn) µL |
|---|---|---|---|---|---|---|---|---|
| FIP | 50µM | 1.6µM | 0.4 | 4 | 20 | 40 | 80 | 200 |
| BIP | 50µM | 1.6µM | 0.4 | 4 | 20 | 40 | 80 | 200 |
| F3 | 50µM | 0.2µM | 0.05 | 0.5 | 2.5 | 5 | 10 | 25 |
| B3 | 50µM | 0.2µM | 0.05 | 0.5 | 2.5 | 5 | 10 | 25 |
| FLP | 50µM | 0.8µM | 0.2 | 2 | 10 | 20 | 40 | 100 |
| BLP | 50µM | 0.8µM | 0.2 | 2 | 10 | 20 | 40 | 100 |
| Quencher | 50µM | 2.0µM | 0.6 | 6 | 30 | 60 | 120 | 300 |
| DNaseFree H2O | | | 1.1 | 11 | 55 | 110 | 220 | 550 |
| Final volume | | | 3 | 30 | 150 | 300 | 600 | 1500 |
| | | | | | | | | |
| | | | | | | | | |

| Primer | Primer working stock conc. | Primer conc. in 10µl LAMP rxn | Vol. of primer (1 rxn) µL | Vol. of primer (10 rxn) µL | Vol. of primer (50 rxn) µL | Vol. of primer (100 rxn) µL | Vol. of primer (200 rxn) µL | Vol. of primer (500 rxn) µL |
|---|---|---|---|---|---|---|---|---|
| FIP | 100µM | 1.6µM | 0.2 | 2 | 10 | 20 | 40 | 100 |
| BIP | 100µM | 1.6µM | 0.2 | 2 | 10 | 20 | 40 | 100 |
| F3 | 100µM | 0.2µM | 0.025 | 0.25 | 1.25 | 2.5 | 5 | 12.5 |
| B3 | 100µM | 0.2µM | 0.025 | 0.25 | 1.25 | 2.5 | 5 | 12.5 |
| FLP | 100µM | 0.8µM | 0.1 | 1 | 5 | 10 | 20 | 50 |
| BLP | 100µM | 0.8µM | 0.1 | 1 | 5 | 10 | 20 | 50 |
| Quencher | 100µM | 2.0µM | 0.3 | 3 | 15 | 30 | 60 | 150 |
| DNaseFree H2O | | | 2.05 | 20.5 | 102.5 | 205 | 410 | 1025 |
| Final volume | | | 3 | 30 | 150 | 300 | 600 | 1500 |

## 3. Making the mastermix

We want to make enough mastermix for **X** experiments

Each experiment is 8 tubes, 4 for plant gene and 4 for the GMO gene.

We mix 5µl*4***X** LAMP mix with 3µl*4***X** Primer mix for each primer set.

It is better to usually make about 10% extra just in case of mistakes and spilling

Enjoy!