CO-EXTRA

GM and non-GM supply chains: their CO-EXistence and TRAceability

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Food Quality and Safety

Deliverable D5.5

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Dissemination Level	
PU Public	
PP Restricted to other programme participants (including the Commission Services)	
RE Restricted to a group specified by the consortium (including the Commission Services)	
CO Confidential, only for members of the consortium (including the Commission Services)	**CO**

1 Summary

Lumora, in conjunction with NIAB have been addressing the potential of an alternative method to PCR for the detection and quantification of GM as part of Task 5.10
Whilst this work is still ongoing, we believe that we have demonstrated that LAMP, in conjunction with Lumora's proprietary BART technology, offers an exciting alternative to real-time PCR, in particular for on-site testing. Specifically:

- hardware costs will be significantly less than for PCR
- hardware is significantly simpler than for PCR and hence more appropriate for on-site testing
- high throughput applications are significantly less costly as very small sample volumes can be used, without the need for more sophisticated hardware
- We have demonstrated sensitivity sufficient to detect 0.1% presence of GM event 40-3-2 using LAMP
- LAMP did not amplify from standards which were validated to be GM free..

Our assessment is that the performance of this alternative to PCR is extremely promising, however there is still work to be done before the method can proceed into inter-laboratory validation procedure.

We report here a protocol for the detection and quantification of GM event 40-3-2 as required by Milestone 5.8 and we also discuss issues associated with sensitivity, specificity and cost.

2 Introduction

Deliverable D5.5 is linked to Task 5.10 which addresses the potential benefits of non-PCR molecular methods for the detection and quantification of GM. One of the benefits can be the potential to bring molecular tests to on-site testing, something which has been difficult and expensive with PCR due to the complexity of the required hardware.

Two non-PCR methods are being addressed in Task 5.10, LAMP and NASBA, by Lumora and NIB respectively. Lumora is working closely with NIAB who are providing sequence data and material to evaluate the LAMP method.

Key to task 5.10, is to demonstrate whether LAMP or NASBA, bring benefits over that offered by PCR, like for on-site testing or for improved accuracy. This therefore requires investigation of the inherent performance of the methods (sensitivity, specificity, speed, etc.) but also the practical and commercial implications of bringing LAMP or NASBA to on-site testing.

Clear benefits of LAMP are that it is an isothermal technique, unlike PCR that requires samples to be thermocycled. This facet substantially simplifies hardware requirements: a particular issue for the development of inexpensive on-site devices. A further benefit of LAMP is that it can be integrated with novel detection technologies in a manner not possible with PCR. In particular, LAMP can be integrated with Lumora's proprietary BART technology that allows real-time, quantitative analysis with extraordinarily simple hardware.

Deliverable D5.5 takes the form of a report on the analytical specificity and sensitivity of GMO detection using LAMP, with special emphasis on the detection of specific events. This is to be accompanied by a cost analysis of the methodology.

This deliverable is associated with Milestone 5.8, a 'first protocol for GMO detection using LAMP', also enclosed.

This document aims to provide for Milestone 5.8, whilst explaining the background to methodologies, collaborations and planning issues associated with the work so far.

3 Background to LAMP and BART technologies

Loop-Mediated Amplification (LAMP) was first described in academic literature by Notomi *et al.* (2000)[1], and has since been associated with over 100 publications. Lumora were one of the first EU bodies to recognize the potential benefits of LAMP over PCR. LAMP isothermally amplifies DNA targets with high specificity even without confirmatory probes. This is a reflection of the amplification mechanism which requires four independent target sites to be recognized for amplification to occur unlike just two as in PCR (Mori *et al.* 2001[2]; Nagamine *et al.* 2002[3]). LAMP has now been demonstrated with a wide variety of different targets from different matrices, including the detection of GMO (Fukuta *et al.* 2004[4]).

LAMP has become a very well characterized method with high specificity and sensitivity and has been validated for diagnostic products.

LAMP is one of a number of isothermal amplification technologies that can be interfaced with Lumora's unique reporter system known as BART (bioluminescent assay for real-time).

The BART assay follows the accumulation of pyrophosphate (in a sample) that necessarily occurs as dNTPs are incorporated into amplicon. As such, the level of pyrophosphate indicates the extent of amplification and hence the presence or not (and quantity) of target nucleic acid in the sample.

The biochemistry of BART is an adaptation of the 'Enzymatic Luminometric Inorganic pyrophosphate Detection Assay, *or* "ELIDA" (figure 3.1).

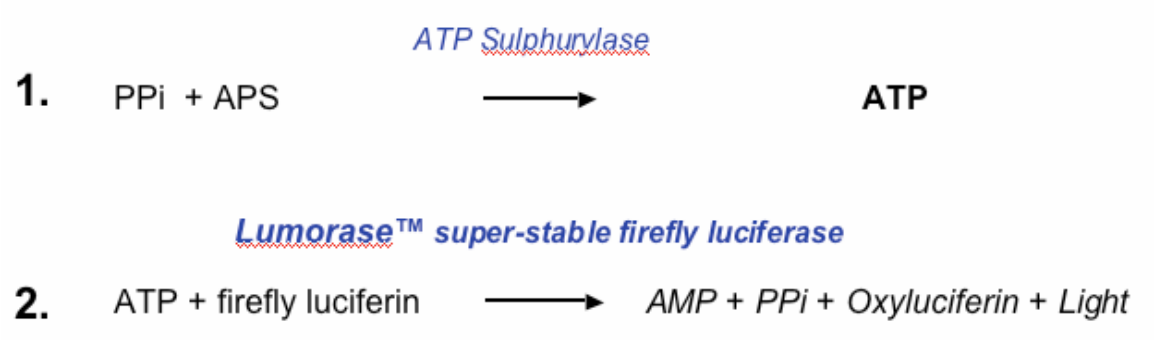
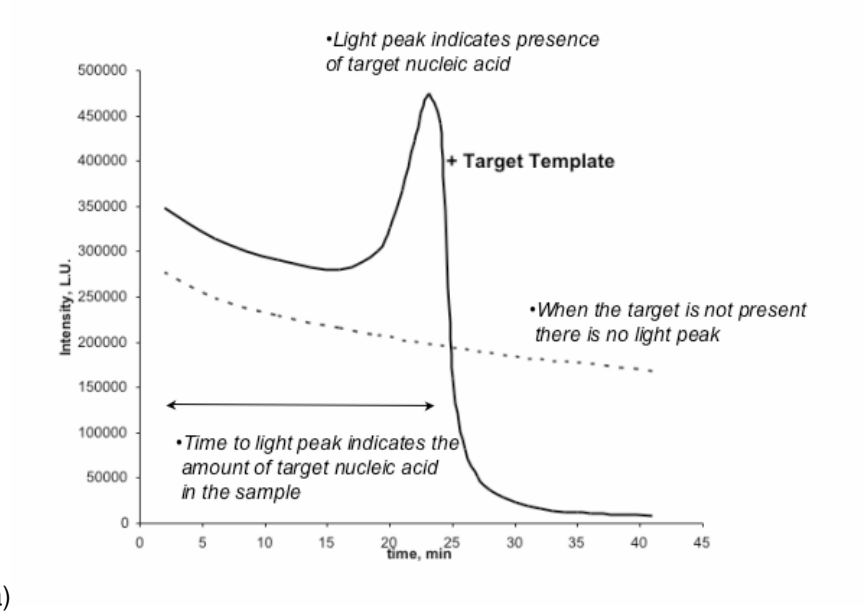


Figure 3.1 the two-enzyme coupled assay for pyrophosphate (PPi) detection: an adaptation of the 'ELIDA' assay (Nyren & Lundin, Anal Biochem1985; 151:504-509) that utilises highly thermostable firefly luciferases.

Unlike previous applications of the ELIDA assay (most notably Pyrosequencing), BART allows pyrophosphate levels to be assessed in real-time over long periods and harsh conditions (>55-60˚C for 2 hours or more). This allows real-time analysis of the extent of an amplification reaction.
A unique feature of BART outputs is that they are associated with both an increase in light intensity as amplicon is generated, and a rapid decrease in light. As such the outputs show a light peak in time; the time to reach this light peak is a function of the amount of target DNA in the sample at the beginning of the reaction (figure 3.2).
Therefore the key features/benefits of the BART outputs are:

- the key parameter: time to light peak, is not dependent on absolute light intensity, this greatly simplifies data interpretation and required hardware
- the time to light peak has been shown to correlate with the amount of specific target template at the beginning of the reaction.
- the measurements are made in real-time in a closed-tube format.

BART does not work with PCR or LCR as the luciferase enzymes are not stable enough to operate at 95˚C (further LCR does not release significant amounts of pyrophosphate). However, we have already proven BART to give excellent results with 5 of the 15 or more Isothermal methods now available. Hence we have data with LAMP (Loop-mediated AMPlification), Rolling Circle, LIMA (Linear Isothermal Multimerisation Amplification) and two further methods that we must keep confidential.



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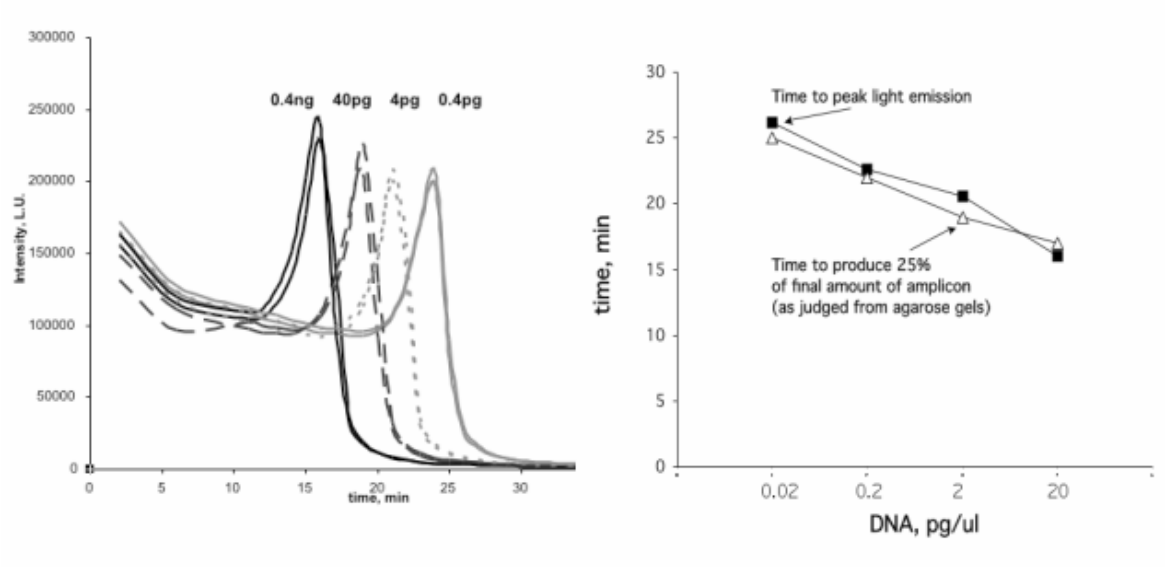


b)

Figure 3.2 a) typical light profile of a +ve and –ve amplification reaction. In the positive sample, as PPi is released by generation of amplicon, it is converted into ATP causing an increase in light (the background light intensity is caused by dATP reacting with luciferase), however, as amplification progresses the light intensity drops to below the level of the control. This drop in light intensity is caused by a number of factors including inhibition of luciferase by high [PPi]; b) the quantitative nature of BART is shown (using LAMP) using different template concentrations

Whilst figure 3.2 demonstrates BART working with LAMP, figure 3.3 shows similar results when BART is interfaced to an entirely different (mechanistically) type of Isothermal NAAT (which we cannot disclose). In fact, similar quantitative results have now been obtained with five Isothermal NAATs.

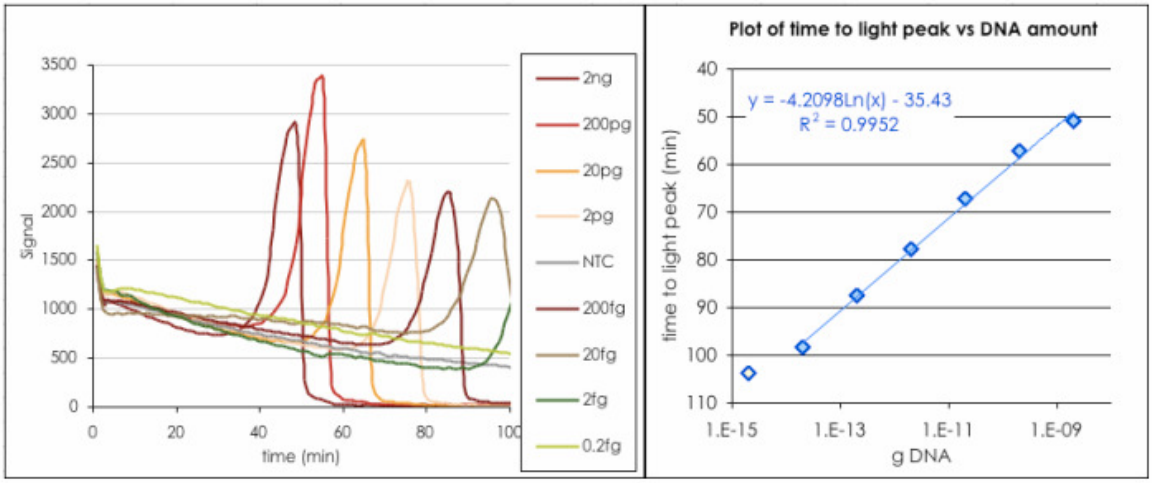
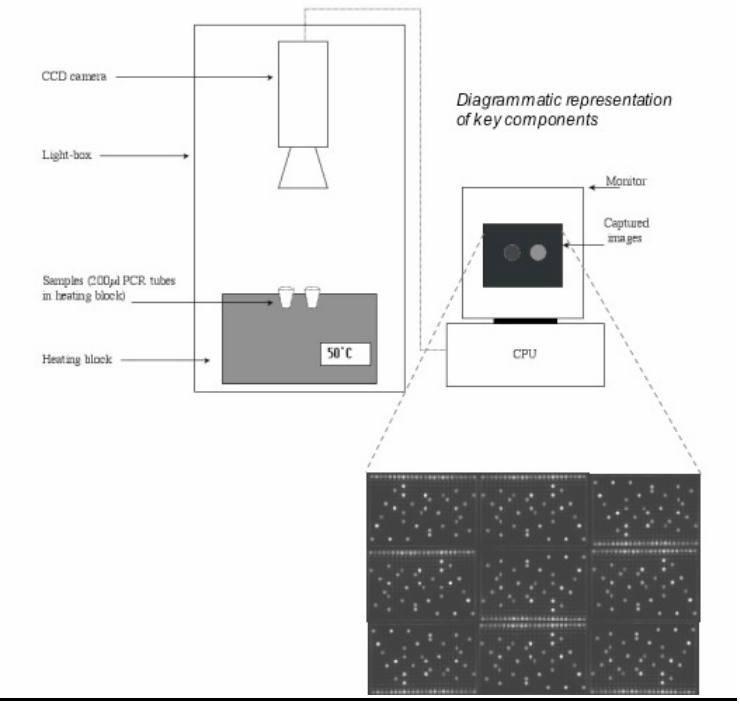


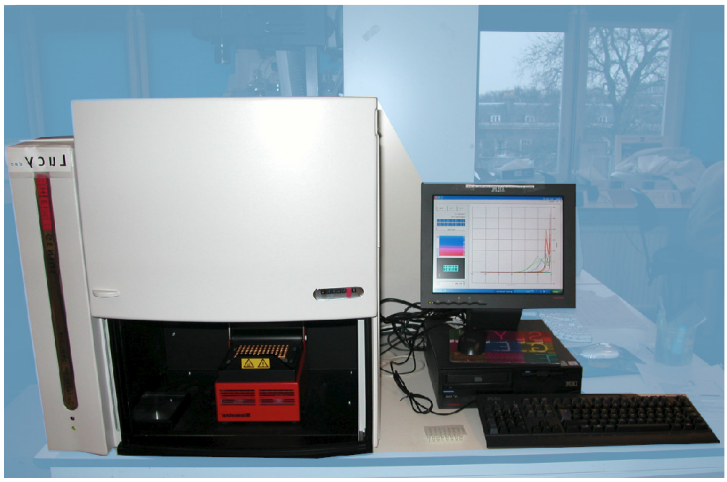
Figure 3.3 where BART is demonstrated with a novel isothermal NAAT

We have shown that interfacing the BART technology with an Isothermal NAAT, does not affect the inherent sensitivity of the NAAT in question. However, one significant difference is that when BART is the reporting system, amplification occurs more rapidly than with fluorescent reporting systems. This, presumably, is because fluorescent systems can slow the kinetics of amplification.

The hardware requirements for BART are extremely simple as neither thermocyling is required, nor irradiation of samples: only light detection alone is needed. We have designed our prototype systems based on CCD cameras rather than luminometers. As such there is little price difference between following 1 or several thousand samples in real time: such sample number expansion is far more costly in fluorescent-based systems (figure 3.4, overleaf).



a)



b)

Figure 3.4 illustrates a) the hardware concept for running BART reactions; b) one of the three working prototype systems that have been constructed with bespoke software. Each is capable of monitoring 96 samples in real-time, but this can be expanded to >3000 samples with little more than a minor software modification.

The prototype hardware we have developed, though presently crude, is still capable of measuring amplification reactions over a large variety of sample sizes from 0.2->50µl (figure 3.5). Using fluorescent methods, reducing the sample volume and density of samples greatly increases the cost of hardware; this is not so with BART since sample irradiation is not necessary.

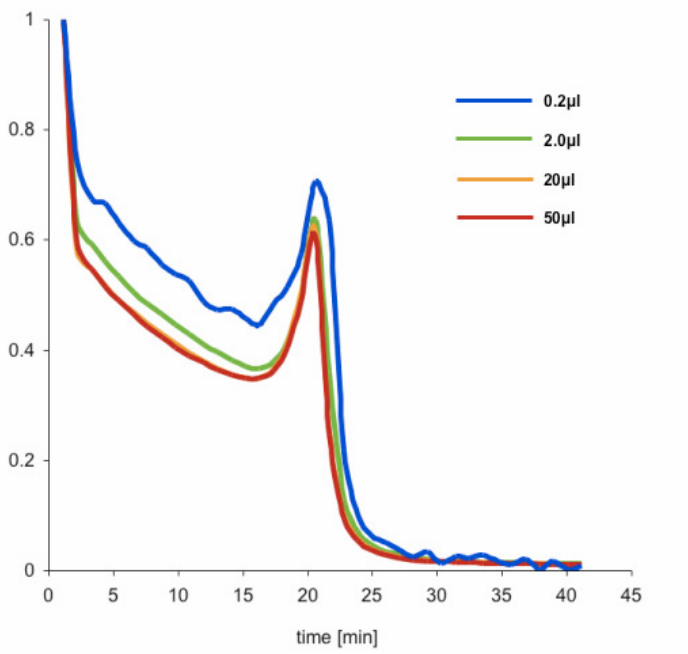


Figure 3.5 where BART reactions are shown for several samples, all containing the same concentration of target template, but with different reaction volumes. The Time to Peak, is identical for all samples. Note, the hardware was identical regardless of whether 50µl samples were used or just 0.2µl samples were used.

We anticipate, that the above format could provide significant competition to Genechips for many applications where multiple targets need to be quantified. In particular, where it is necessary to assess targets very urgently (e.g. as in sepsis tests, or pharmacogenetic profiling for an urgently required drug such as warfarin) the above format will allow multiple targets to be quantitatively assessed in a suitable timescale: at present it will take hours to run a Genechip-based IVD, this is far too slow for many applications.
Further, the BART-based approach will be many times less costly and will be a single-step, rather than multi-step operation as with Genechips.
The simplicity of the hardware requirements means low-cost hardware. Further, the ease with which very low sample volumes can be analysed, means that reagent costs can be greatly reduced.
Alternatively, it is clear that the hardware concept is particularly suitable for portable IVD hardware. Attempts to make portable PCR machines have been complicated by the need to thermo-cycle with real-time fluorescent detection. However, for BART the situation is far simpler as thermo-cycling is not required, nor irradiation of the sample.

To summarise, the BART technology is a unique technology that has only recently been made possible by the development of highly stable recombinant luciferases. The key features of the technology include:

- Quantitative Real time output
- Totally closed system
- Proven in clinical pilot study for Chlamydia (DTI funded)
- Can be used for Immuno-NAAT applications
- Proven with 5 isothermal NAATs to date
- Simplest ever hardware for real-time
- Uniquely suited for portable devices

The BART technology is covered by:

PCT/GB04/000127
PCT/GB05/002998

4 Project plan & role of collaborators

Lumora have been collaborating with the National Institute of Agricultural Botany, NIAB on Task 5.10. A combined project plan was submitted to the Co-Extra administrators in mid-2005 describing the activities of both partners in addressing the deliverables of the project.

In the initial phase of the project, Lumora evaluated plant sample-preparation methods on a model system to identify any adverse effects on LAMP or BART from plant contaminants. This was achieved by testing for a plant pathogen using BART-LAMP in barley grain. We were able to show that BART-LAMP could detect small amounts of pathogen DNA in a large background of barley DNA prepared from grain. This was an important milestone in our internal project plan as it suggested that detection of small amounts of GM presence should be possible.
The next step was to test the BART-LAMP system on a model plasmid system that would mimic GM events. The aim was to show that BART-LAMP could differentiate between the same transgene in different sequence contexts. This work was undertaken whilst awaiting sequence data and plant material from NIAB from their transgene junction identification work.
The work on the plasmid systems, ultimately, proved fruitless. In short, our approach had been to test BART-LAMP on two plasmids with the same transgene inserted in different orientations. The aim was to see if BART-LAMP could distinguish between the plasmids. This did not work because plasmid recombination events could occasionally flip inserted transgenes.
Rather than generate improved plasmid test systems, Lumora decided to move straight to testing GM certified reference material (CRMs) as obtained from JRC-IRMM. This also allowed Lumora to continue developments whilst waiting on sequence information from NIAB. This had become an issue since NIAB had encountered problems releasing sequence information they had obtained. Much of the work presented below is based on Lumora's work with Round-up Ready Soya CRMs. Lumora generated test plasmids to assess analytical sensitivity and demonstrated BART-LAMP working on the standards supplied.
Whilst Lumora has not yet demonstrated BART-LAMP on multiple independent events, just Round-up Ready Soya, we think the essence of Milestone 5.8 has been addressed in that the BART-LAMP system clearly has the desired properties for on-site devices while retaining the quantification abilities of real-time PCR.
During the course of this work, Lumora has also undertaken considerable commercial development to understand the cost implications of implementing BART on an on-site device either with LAMP or other methods. This work, in part presented below, also addresses key issues associated with Deliverable 5.5 and Milestone 5.8.

5 BART-LAMP as applied to GM event 40-3-2

PCR primers were designed to amplify the sequence of the Monsanto Roundup Ready insert and the flanking *Glycine max* genomic DNA (GM event 40-3-2). Sequence information was obtained from accession numbers AJ308514 and AJ308515. This fragment was cloned and the sequence confirmed by DNA sequencing. The subsequent, plasmid (pRoSI), was used as an artificial template for assessment of analytical sensitivity.

DNA was extracted from Roundup Ready reference samples (0, 0.1, 0.5, 1, 2 and 5 % Roundup Ready in WT background) obtained from JRC-IRMM. A series of BART-LAMP experiments were undertaken on both the pRoSI artificial template and DNA extracted from the reference material.

5.1. Methods and materials:

Primer design
Primers were designed to the 3' junction of the Round-up Ready Soya insert. The final sequences used in the detection protocol are as follows:

B1cB2	cat gct gtt gcc tat tag ggt c tttt gat taa gct tca aca tgt gaa gg
B3	ctc ctg cta tta cat gag
LoopB	gta gta cac tca cca gt
F1cF2	ggg atc gga gaa gaa ctg ttt g tttt ctt ctt cac gaa ctt ctc gac
F3	gag aag gta gtt ctc ttc
LoopF	gaa tgg cct ggt cgt cg

These primers are spatially associated with the target site as follows:

```
5' ---[B3][B2]-[LoopB]-[B1]-----------------------------------------
                          junction: |
3' -----------------------------------------[F1]-[LoopF]-[F2][F3]----
```

Specifically:

```
CAGCAATTTG AATATTAAGT AACTGCTTCT CCCAGAATGA TCGGAGTTTC
  [       B3        ]                               [
TCCTCCTGCT ATTACATGAG CAAAAATAAA AAATAAATAA AAGATAAGAT
       B2           ]  [     Loop B     ][        B1
TAAGCTTCAA CATGTGAAGG AGTAGTACAC TCACCAGTGA CCCTAATAGG
         ]
CAACAGCATG AAAAAAAATA AAAAAGAATA AAAATAGCAT CTACATATAG
                   Junction:|   [      F1
CTTCTCGTTG TTAGAAAAAC AAAACTATTT GGGATCGGAG AAGAACTGTT
 ]    [     Loop F      ]          [          F2
TGAGGCGAAT GGCCTGGTCG TCGCGGCCAT CGTCGAGAAG TTCGTGAAGA
 ]             [      F3          ]
AGCTCGAATG CGGTGAGAAG GTAGTTCTCT TCCAACAGAA AGTTCACCAC
```

Preparation of genomic DNA from certified reference material (CRM)
DNA was extracted from 50mg flour of Roundup Ready Soya from Sigma (Fluka # 94162, Lot # 1129490) using a Promega Wizard genomic DNA purification kit (A79).
The following materials were extracted:
0%, 0.1%, 0.5%, 1%, 2% & 5%.
The resulting genomic DNA was quantified using UV spectrophotometry and stocks of 1ng/ul genomic DNA were prepared for all. DNA was stored at -20˚C.

pRosi cloning

Using genomic DNA extracted from the 5% CRM, a junction fragment was cloned by PCR. The primers B3 and F3 above, were used to amplify the required region of DNA. The resulting PCR product was gel-purified and cloned using an Invitrogen TOPO-cloning kit as per the manufacturers instructions.

Preparation of pRoSI dilutions series

Plasmid DNA was extracted purified using a Qiagen mini-prep kit as per the manufacturers instructions. The resulting DNA was quantified using a 'Nanodrop' Spectrophotometer (which also confirmed plasmid purity) and alliquoted into 100ng/µl samples and frozen at -20˚C.
Dilution series of known concentration of pRoSI were freshly prepared from a 100ng/µl stock (as assessed by UV spectroscopy) in the range 10ng/µl to 10fg/µl to be used in experiments to assess sensitivity and quantitative properties.

Reaction setup

BART-LAMP reactions were performed in 20µl volumes. The following table indicates how to make up 1ml of BART-LAMP assay reagent, equating to 50 reactions of 19µl to which 1µl of sample DNA is added:

Reagent	**Volume (µl)**
Thermopol buffer (x10; NEB)	100
10mM dNTPs	80
*x10 primer mix	100
8u/µl Bst Polymerase (NEB)	20
**BART reagent (Lumora)	215
UHP water	435

* The final concentration of each of the LAMP primers is as follows:
B1cB2 & F1cF2 0.8µM
B3 & F3 0.2µM
LoopB & LoopF 0.4µM

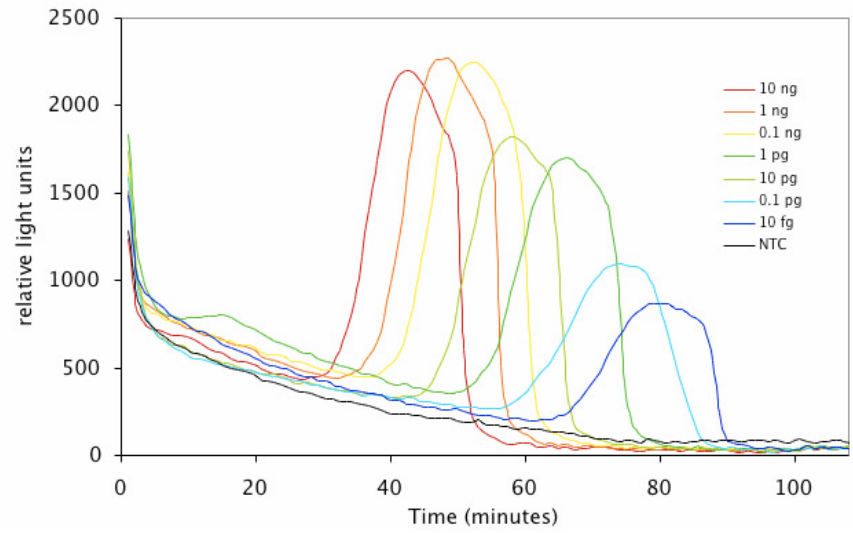
**available under MTA.

Where necessary, the above reagent composition was altered such that, per 20µl reaction volume, 5µl of sample DNA could be added rather than just one. 5µl was preferred where the pipetting of 1µl samples was considered to introduce unacceptable user errors.
The assay reagent can be snapped frozen in liquid nitrogen and stored at -20˚C for several months.
In a 200µl thin-walled PCR tube, either 1µl or 5µl of sample DNA was added to either 19µl or 15µl respectively of BART-LAMP assay reagent on ice. PCR tubes were selected to have transparent lids such that light emission from the sample could be detected from above.
Samples were covered with 10µl of oil and the lids closed.

Samples were immediately loaded into one of Lumora's in-house hardware units and incubated at 55˚C for anything up to 150 minutes. The in-house hardware captures light emission from the samples and displays the output in real-time using our proprietary ReactIVD software.
The resulting data was transferred to Microsoft Excel for further analysis.
Where appropriate, samples were subsequently run on 1% agarose gels stained with ethidium bromide to confirm amplification had occurred.

5.2 Experimental results

Preliminary results indicate that the primers are event-specific – that is, peaks in luminescence occur when the template is 0.1, 0.5, 1, 2 or 5 % Roundup Ready but not when the template is 0 % Roundup Ready DNA. Figure 5.1 shows an example dilution series with the RoSI template.



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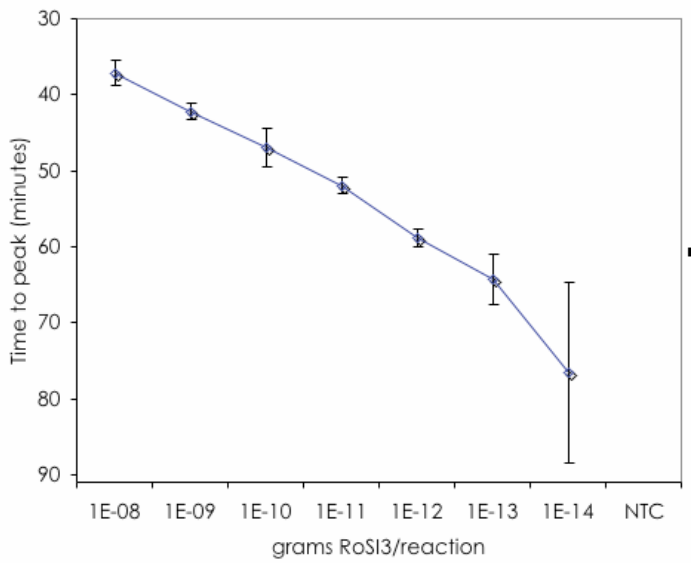

b)

Figure 5.1 a) raw BART data from a dilution series of the RoSI template and b) a calibration curve derived from a).

LAMP gave reasonable results with the artificial template though the rate of amplification was slower than expected. Sensitivity of reaction was determined to be caa. 10 fg, corresponding to few thousands copies of tamplate in reaction.

Using the reference material a series of experiments were performed to test the BART-LAMP system. A typical output using 5% contaminated Soya is shown in figure 5.2.

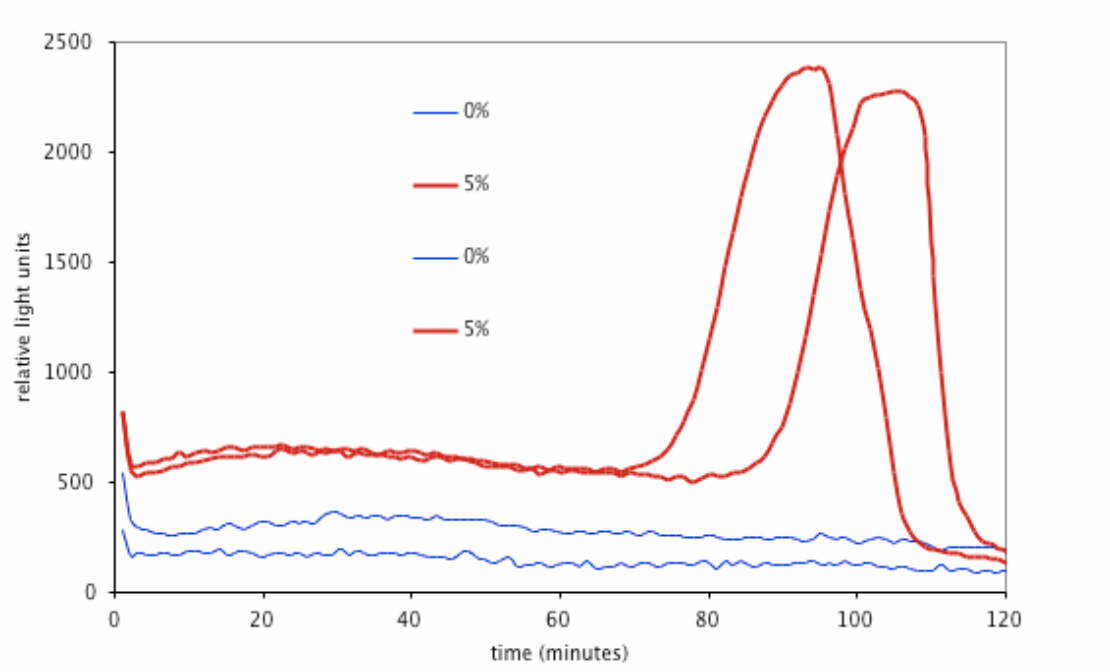

Figure 5.2 event specific detection of the GM 40-3-2 event, in duplicate.

In the course of further evaluation of the specific primer set being used, it became clear that there was room for improvement in the primer optimisation. For example, in figure 5.3, it is clear that lower percentages of GM 40-3-2 are not being reliably detected and that the amplification is very slow.

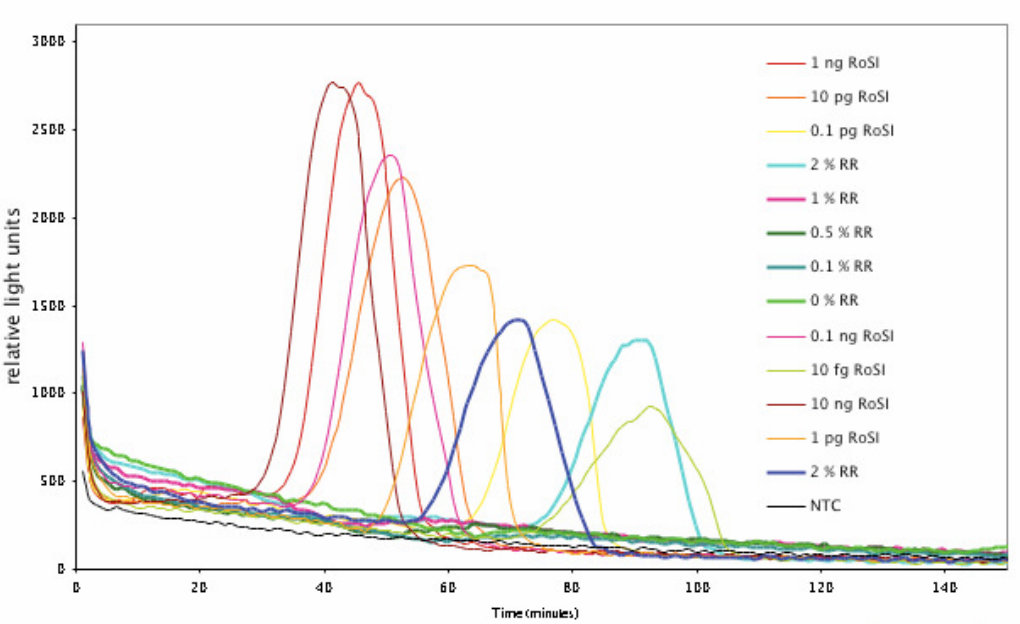

Figure 5.3 results of an experiment where the limit of detection of the transgene was 2%; other experiments have detected down to 0.1% however.

With Isothermal methods, other parameters other than primer design *per se* can affect the performance of the assay. Therefore, considerable time was spent investigating means to improve the reliability of the amplification reactions with this primer set. In Lumora's experience, the faster the DNA amplification, the better the sensitivity and reliability hence maximising the kinetics is crucial. Having followed Lumora's proprietary buffer optimisation process, we were able to significantly increase the rate of amplification (figure 5.4).

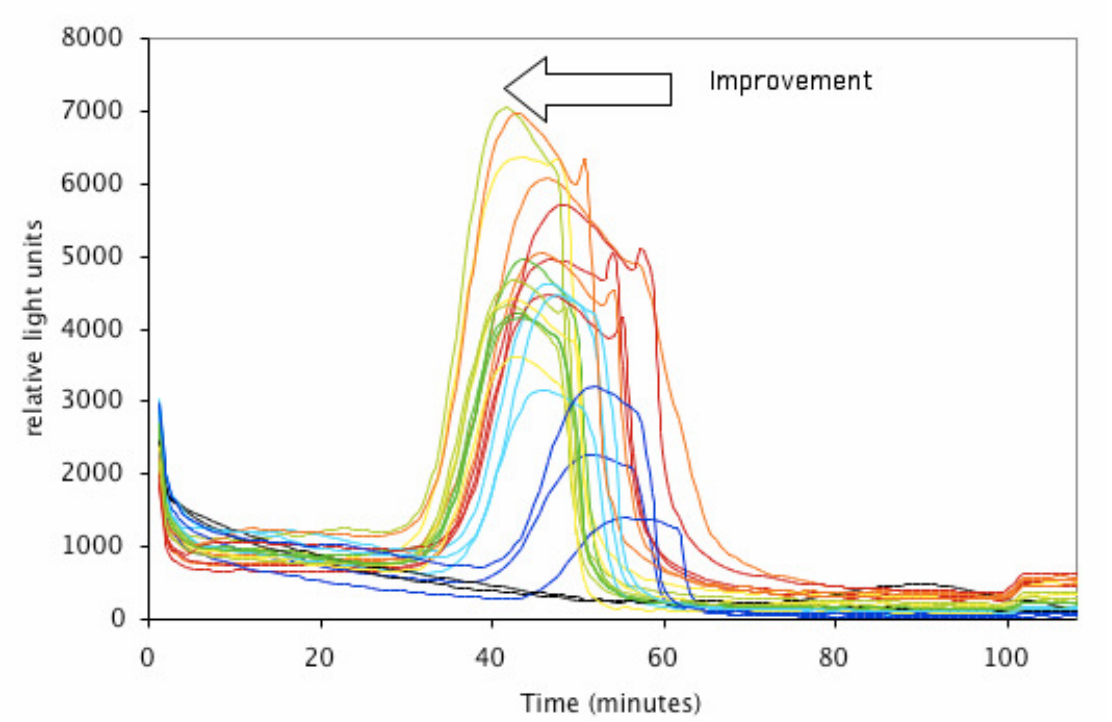

Figure 5.4 where several BART-LAMP reactions were performed with identical amounts of target template but with differing buffer compositions. An improvement of almost 20 minutes in time-to-peak was obtained for our most optimal formulation.

The results obtained in experiments such as those displayed in figure 5.4 are very encouraging. We anticipate that with modification to the primers we can reach the reproducible sensitivities (we have proven single copy detection on other targets).

The next stage of our project is to build on the afore mentioned improvements and better characterise the results with a view to providing SOPs for future validation.

The results show that the GM event 40-3-2 is detected in all the standards containing the event, but not in uncontaminated material. As such, the specificity of the method appears high. However, more persuasive analytical specificity can only be assessed when Lumora is given access to a broad range of material from *different* events containing the same transgene. This is an important next step. Unfortunately, progress along these lines has been affected by IP issues associated with novel junctions that NIAB have identified from 3rd party material. However, presently there are more than 10 GM CRMs available to purchase, as well as material from other parties in the Co-Extra consortium, that will allow Lumora to assess the specificity of the method during the next phase of the project.

With respect to the sensitivity of the BART-LAMP system, we have shown that we can detect down to 0.1% GMO presence using the LAMP system. However, as previously reported, our analytical sensitivity, as based on standard curves with pRoSI have given lower sensitivities than other targets Lumora has addressed. For example, Lumora has demonstrated single copy detection of a gene from the cryptic plasmid of *Chlamydia trachomatis*. Further, the time taken to detect clinically relevant levels of *Chlamydia*, was around 40 minutes, whereas our detection of GM event 40-3-2 was extremely slow.

LAMP has been demonstrated to be as sensitive as PCR in a large number of publications. As such we are confident that there is no inherent problem with obtaining higher sensitivities

for detection of the above event, it is simply a matter of improving on the primer design: an issue shared with PCR. Further, we have found that more sensitive detection is associated with faster amplification; we therefore anticipate improving both the speed and sensitivity of detection for GM event 40-3-2 to be as good or better as our *Chlamydia* detection reagents.

6 Practical and economic benefits of BART-LAMP

Deliverable 5.5 requires that Lumora address not just performance issues associated with non-PCR methods, but other added value the methods may bring.

Reagent cost

The LAMP method employs six primers, whereas PCR employs just two. However, primers are not a major cost-component of amplification reactions. Further, whilst the BART reagents add to the cost of the BART-LAMP system, real-time PCR must use expensive confirmatory probes. As such, we do not believe there is a significant cost difference between PCR and BART-LAMP reagents on a per volume basis.

However, as disclosed in our technical summary (see www.lumora.co.uk), a major feature of BART is that extremely low reaction volumes can be analysed on very low-cost hardware. In fact, we have demonstrated that we can measure BART reactions over the range of 50µl to 0.2µl on the same in-house hardware we used in this project.

This reagent volume issue has significant cost implications for high throughput applications. We refer the reviewers of this document to Task 5.5 of CoExtra where the method being employed requires large numbers of PCR reactions to be performed at significant reagent cost: the costs of this method would be dramatically reduced if each reaction could be performed with 0.2µl of reagent (or perhaps lower) as is possible using BART.

Hardware: robustness and cost

The BART technology requires just a single temperature heating block and a simple light detection system. These are far less complex requirements than for real-time PCR, as reflected in the price of present systems.

In the past 6 months, outside of its work on Co-extra, Lumora has been actively engaging with design companies and consultants to obtain parameters for a point-of-use, portable testing system employing the BART technology. Lumora is convinced that hardware based around the BART technology will be substantially less costly to manufacture than PCR equivalents. The low complexity of the hardware will confer physical robustness on devices (e.g. no moving parts are anticipated). Further, technical difficulties with PCR machines are often associated with temperature modulation, which is particularly challenging when the temperature is constantly changing. Since BART works at a single temperature, the temperature control is far simpler and hence more robust.

Consumable cost

As well as simple hardware requirements, the BART technology also offers low consumable cost. The only requirement of BART-based consumables is that some part of them is translucent to allow light to be detected. This is far simpler than consumables that are used in fluorescent-based methods where excitation and emission from samples must be measured and simpler still than methods requiring semi-conductors integrated into the consumable.

The simplicity of BART also allows for the use of simple liquid circuit-based consumables to be manufactured at a viable cost-point. In this regard, Lumora has been working on developing an integrated consumable-hardware platform that can be used for on-site testing.

We are presently raising capital to execute a development programme in this regard.

Licence costs

Real-time PCR is associated with costly licence fees which represent a significant part of the cost of assays based on the technology. In particular, diagnostic services relying on real-time PCR pay significant amounts of their income to use the method.

With respect to LAMP, Lumora is under confidentially with the Eiken Chemical Company, Japan, who are marketing the LAMP technology. Whilst we would not disclose the details of any of our discussions regarding licensing terms for the LAMP technology, Eiken's business model for their LAMP technology is to license it non-exclusively to multiple parties. Therefore Lumora would encourage partners to make their own independent enquiries.

With respect to the BART technology itself, Lumora is open to discuss access to this with a view to generating competitively costed products.

Finally, through a recently negotiated deal with the French diagnostic company bioMérieux, Lumora has access to another isothermal amplification method as an alternative to LAMP.

In conclusion, combining BART with isothermal methods such as LAMP, significantly reduces the complexity of hardware and consumables. This represents a crucial factor in making on-site testing an economically viable reality. Further, the use of BART can significantly reduce costs in high throughput applications by virtue of the ease with which reagent volumes can be reduced.

7 Conclusions

The data Lumora has collected on the LAMP method, in particularly when combined with the BART technology, indicate that it is a promising method to further develop. Whilst the technical performance data is not yet as extensive or impressive as Lumora has obtained for other targets, we believe that it is simply a matter of time and recourses before this is demonstrated.

One of the objectives to Task 5.10 is to assess whether PCR itself is going to be the most appropriate method for on-site testing. The results that Lumora have obtained strongly suggest that alternatives to PCR should continue to be explored. The simplicity of the hardware requirements for the BART system (whether combined with LAMP or another isothermal system) has major implications for the cost of highly distributed devices used for on-site testing. Simple, robust, low-cost hardware and consumables are the key to taking molecular approaches out of the laboratory: it is clear to us that the method we have disclosed promises these characterises.

Further work is required before the described methods can proceed to inter-laboratory validated; in particular, improved primer sets need to be evaluated and more material obtained to assess analytical specificity more rigorously.

Abreviations list:

CRM - certified reference material, , member of reference material familiy as defined by Emons 2006

QCM -. . quality control material,, synonnim for in house reference material, member of reference material familiy as defined by Emons, 2006
BART – Bioluminescent Assay in Real Time
LAMP – Loop mediated Amplification

References:

1. Notomi T. Okayama H. Masubuchi H. Yonekawa T. Watanabe K. Amino N. Hase T. Loop-Mediated isothermal amplification of DNA. Nucleic Acids Research 2000: Vol.28 No.12 e63

2. Mori Y. Nagamine K. Tomita N. Notomi T. Detection of Loop-Mediated Isothermal Amplification Reaction by Turbidity Derived from Magnesium Pyrophosphate Formation. Biochemical and Biophysical Research Communications 2001: Vol 289, 150-154.

3. Nagamine K, Hase T, Notomi T. Accelerated reaction by loop-mediated isothermal amplification using loop primers. Mol Cell Probes 2002;16:223-9.

4. Fukuta S. Mizukami Y. Ishida A. Ueda J. Hasegawa M. Hayashi I. Hashimoto M. Kanbe M. Real-time loop-mediated Isothermal amplification for the CaMV-35S promoter as a screening method for genetically modified organisms. European food research and technology A 2004: 218, 496-500.

5. Emons H. The 'RM family'—Identification of all of its members, Accred Qual Assur 2006 10: 690–691