This FAQ sheet will be best understood following a thorough reading of the LAMP Protocol.

Reagents

What do I do with the primers after receiving them? The primers will be shipped to you in powder form. You will then need to send them to a lab for resuspension. We recommend AL&L Crop Solutions as they are familiar with this procedure. Below is the protocol followed:

The LAMP primers from Integrated DNA Technologies need to be re-suspended in a buffer ('TE', 10mM Tris pH 8.0, 1.0 mM EDTA) to a concentration of 1mM. The 1mM oligonucleotides then need to be diluted in water to their respective concentrations to make up the LAMP primer stock. Aliquots of 12.7 µL (micro liter) will be needed.

LAMP Set I				10X LAMP primer mix			
			Final Concentration (µM)	Volume (µl) of 1 mM stock	Volume (µl) of 1 mM stock	Volume (µl) of 1 mM stock	Volume (µl) of 1 mM stock
				To make 200 µl	To make 200 µl	To make 1000 µl	To make 5000 µl
p1825	LAMP.168-F3	GAATCGTTTGAATCGTAAGAGA	0.2	0.4	1	2	10
p1826	LAMP.168-B3	CAGACAAATAAATACGATTCCTTTC	0.2	0.4	1	2	10
p1827	LAMP.168-FIP	AATGACTCCTGCGGCTTCTTTCGTATTTTGGGTTCGAAGA	1.6	3.2	8	16	80
p1828	LAMP.168-BIP	TCAAAGACGTCGTCTGGTTGTCATCATTACGTCCTCCACC	1.6	3.2	8	16	80
p1842	LAMP.168-LB	GCTTTTAAAAACGACGTGT	0.4	0.8	2	4	20
p1857	LAMP.168-LF	TTCACGCCAACAACAAGT	0.4	0.8	2	4	20
			Total	8.8	22	44	220
			Water to 200 µl	191.2			
			Water to 500 µl		478		
			Water to 1000 µl			956	
			Water to 5000 µl				4780

Should I dilute the DNA controls? Yes, the DNA controls should be diluted down and aliquoted into smaller volumes. You can dilute the controls down to 1:25 with 1 part DNA and 25 parts distilled water.

Should reagents be aliquoted? Yes. Volumes larger than what is needed for one reaction should be aliquoted into smaller amounts. This reduces the chance of contamination and limits the number of times the Master Mix reagent, the primers, and the DNA controls are thawed and re-frozen. Below are recommended aliquot measurements for each component of the LAMP reaction mix, however these can vary depending on preferences:

- Master Mix: One tube (1250 µL) from New England Biolabs is enough for 20 aliquots of 62.5 µL each. Each aliquot can be put into a 1.5ml centrifuge tube.
- DNA Controls: DNA dilutions are best aliquoted into PCR tubes.
- LAMP Primers: request 12.7 µL aliquots.
- Distilled water: Prepare several strips of PCR tubes, each tube with a 200uL aliquot. Distilled water does not need to be frozen.

Freezing Materials

What reagents need to be frozen? All DNA controls, primers, and Master Mix must be kept frozen at all times. You do not need to freeze the distilled water.

Can I freeze canes or petiole tissue to sample from later? Freezing petiole tissue to sample from later is not recommended as it can dry out, making it difficult to extract from. However, they can be placed in the fridge for up to a week prior to sampling from. Canes can be frozen for up to a month. After that, tissue acquisition may become increasingly difficult due to the material drying out.

Can I freeze the templates and test them later? Templates are a term used to describe the Eppendorf tube containing the pipette tip with tissue from the sampled vine. These can be stored in the freezer for up to 3-4 months before being tested on. Any longer and you risk the 10µL of water at the bottom of the tube evaporating.

Can I freeze the Eppendorf tubes containing the sample templates and test them later? Templates are a term used to describe the combination of the distilled water plus the tissue extract in the pipette tip (within the Eppendorf tube). The entire Eppendorf tube + pipette tip combination can be stored in the freezer for up to 3-4 months before being tested on. Any longer and you risk the 10µL of water at the bottom of the tube evaporating.

Lab Material Ordering Quantity

The quantity of materials used per vine can vary greatly depending on the number of controls used and how many samples are run at one time. You will also use various tips for aliquoting and preparing reagents. Based on the LAMP layout in Figure 1 below, you will be able to test at least 24 samples using 4 PCR strips and 4 reagent mixtures (Master mix, primers and water). There will usually be enough reagent mix for a few extra samples. Below is information to help guide decision making for each material.

How many pipette tips to order? In general, purchasing one box (960 tips) of each tip size is enough to get started using LAMP.

- **0.5 µL – 10 µL tips:** These will be used the most. Roughly 2 tips are used per vine. One to collect tissue and another to transfer 0.5 µL of water from the template into the reagent mixture during testing.
- **10 µL -20 µL tips:** These are used primarily for mixing reagents and will be used less than the 0.5 µL tips. One tip can be used for preparing Eppendorf tubes for sampling, one for adding primers to master mix solution and one for filling PCR tubes with the reagent solution.
- **20 µL -200 µL tips:** The primary use for these tips is adding 45 µL of distilled water to the reagent mixture and aliquoting larger amounts of reagents such as water and Master Mix. Of all three tip sizes, these will be used the least.

How many of each tube type to order?

- **Eppendorf tubes:** You will need one Eppendorf tube per vine so order according to the quantity of vines you plan on testing.
- **1.5ml Centrifuge tubes:** Each individual tube will hold 62.5 µL of master mix reagent which is enough 1 strip of PCR tubes.
- **PCR tubes:** One strip has 8 tubes. See Figure 1 for information on how many samples 1 strip can hold.

How many aliquots of the primers should I request from the lab? The primers are typically sent in boxes of 96 aliquots. Each aliquot is enough for 1 strip of PCR tubes. See Figure 1 for information on how many samples 1 strip can hold.

How much Master Mix should I order? One tube of master mix has 1250µL which is enough for 20 aliquots of 62.5µL each. Each aliquot is enough for 4-8 vine samples depending on how many controls are included in each testing strip.

Other Questions

How far in advance can I prepare the Eppendorf tubes with 10µL of water? At room temperature, the 10µL of water can evaporate from the Eppendorf tubes within a few weeks so preparing them any longer in advance is not recommended. However, storing the prepped tubes in the freezer can allow the water to last longer.

Can I reuse razor blades? Yes, with thorough cleaning razor blades are safe to reuse. They can be sterilized by baking them in an oven at 350° for 45 minutes.

What should I use to sterilize materials and equipment? It is best practice to routinely clean equipment and surfaces used for the LAMP Assay. Bleach (wipes work best) is more effective than alcohol at removing DNA viruses and should be used for routine cleaning.

Can I test more than one strip of samples at a time? Yes, the general limit for the number of PCR strips you can test at once depends on the heat block capacity. Most heat blocks have enough space for 5 strips. We recommend preparing enough LAMP reaction mix to fill four PCR strips. One LAMP reaction mix (62.5 µL mastermix+12.5 µL primers+45 µL of water) is required for each strip of 8 PCR tubes. Therefore, four LAMP reaction mixes will need to be prepared for 4 PCR strips. There will be enough LAMP reaction mix leftover to fill an extra 4 to 6 PCR tubes (see Figure 1)

Below is a general sample and control layout for testing 4.5 PCR strips at once. Please note that running numerous strips at once increases the risk of mistakes and should only be done once proficient LAMP testing skills are demonstrated.

Figure 1. Example LAMP assay layout for additional samples. There are 28 plant samples (blue) and 8 controls (orange). Each strip has 6 plant samples, one positive control and one negative (except the last strip that has extra samples). Typically, 4 controls are used per strip, this should still be done when first starting with LAMP or if only testing one strip at a time. When testing more than one strip, controls can be spaced out to allow for testing more samples. Negative controls can vary but it is recommended to have at least one of each type: no addition, water, and GRBV negative plant tissue. For more information of the type of controls, please see the LAMP Protocol.

	PCR Tube							
PCR Strip	1	2	3	4	5	6	7	8
1	1	2	3	4	5	6	(+) DNA	(-) No Addition
2	7	8	9	10	11	12	(+) DNA	(-) Water
3	13	14	15	16	17	18	(+) DNA	(-) Plant
4	19	20	21	22	23	24	(+) DNA	(-) Water
5	25	26	27	28				

Contamination LAMP for GRBV is a highly sensitive diagnostic assay, making contamination a very possible occurrence. You will know you have contamination if your negative controls begin turning yellow or all the reaction tubes turn yellow. Pay close attention to which controls they are, this will help you find the source. If it is the “no addition” the contamination likely occurred when mixing the reagents or it is

coming from one of the reagents. If it is controls like the "water" or "GRBV negative plant tissue", it could be carried over from positive samples or the positive control by your hands or other equipment when adding the 0.5 µL additions. Water controls can also indicate if your water is contaminated. Below are tips for identifying and removing the source of contamination.

1. Clean all surfaces and equipment with bleach
2. Perform a run with only controls. You should design your strip to try to identify the suspected source so the exact controls may vary depending on where you first saw contamination. For example, if you think it is water, try using a different source of water. If you think it was your hands carrying over the positive DNA, use gloves for each sample addition or add the DNA as the very last step. You will likely want a mix of negative controls with one positive.
3. You may need to run more than one strip but continue to try different combinations until you have found the source or have no more contamination.
4. Do not begin testing samples until you have no more contamination.

Additional note: While uncommon, it is possible to get contamination from equipment such as PCR tubes, microcentrifuge tubes or Eppendorf tubes that occur when the product was made. This is likely not contamination from GRBV but rather from chemicals on the products causing a pH change. This should be kept in mind when ordering products and choosing laboratory suppliers.

What tissue to use for testing There are three types of tissue that can be tested with LAMP: trunk, cane or petiole. Choosing which will depend on the time of year and your primary goal for testing samples. Below are two main goals that LAMP is typically used for.

Confirming visual symptoms: All three tissue types can be used for this purpose, choosing which depends on the time of year.

- Petiole: Use only after veraison and before leaf senescence.
- Cane: Use after harvest and up to pruning. You can test cane tissue as early as veraison but it isn't recommended as the canes are typically removed for testing.
- Trunk Tissue: Can be used year-round.

Identifying asymptomatic infected vines: Trunks are most effective for this purpose as canes and petioles will only be able to identify asymptomatic infected vines in the current growing season, right before they show symptoms. However, trunks can detect asymptomatic infected vines one year prior to when they will show symptoms.

Please see our recent publication "Trunk Cambium Facilitates Pre-Symptomatic and Year-Round Detection of Grapevine Red Blotch Virus Using the LAMP Assay" to learn more about testing various tissue types throughout the year.

Can I sample trunk tissue in the rain? While trunk samples can be used to detect GRBV in grapevines year-round, it is best practice to not sample in the rain nor immediately after heavy rains. When trunks are heavily saturated with water, there can be an increased risk of contamination due to sap droplets from vines easily landing on materials during sampling. Additionally, after the vines have taken up lots of water, it is more difficult to collect tissue samples as large amounts of sap can fill the pipette tip as you are scraping tissue, possibly diluting the sample.