Lab Notebook

Protocols from papers:

- Protocol for DNA extraction from PCR diagnostics paper (Toda et al, 2014):

Adult females were homogenized individually in 10 µl of lysis buffer including 9 µl of STE buffer [100 mM NaCl, 1 mM EDTA (pH 8.0), 10 mM Tris–HCl (pH 8.0)], and 1 µl of proteinase K (10 mg/ml). The homogenate was incubated for 15 min at 65 °C and then heated for 5 min at 95 °C to inactivate the proteinase K . PCR was per-formed in 20 ul reaction volume, containing 20 pmol of each primer, 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 2.5 mM MgCl2, 0.25 mM of each dNTP, and 0.5 units of Taq-polymerase (AmpliTaq Gold, Applied Biosystems). The PCR program in the DNA thermal cycler (DNA thermal cycler 2720, PE Applied Biosystems) was 94°C for 9 min as a preheat step, followed by 35 cycles at 94°C for 30 s, 52°C for 30 s, and 72°C for 1 min, and then 72 °C for 7 min as a final extension.

- Protocol for DNA extraction from LAMP diagnostics paper (Toda et al, 2015):

Place one individual *Scirtothrips dorsalis* insect into a 0.2 ml PCR tube (using a fine-tipped brush). Add 10 µl of 100 mM Tris-HCl (pH 8.0) to the tube. Grind the insect thoroughly in this solution (using a fine-tipped brush or a heat-modified pipette tip as mentioned previously). Heat the homogenate at 99.9°C for 10 minutes. The resulting supernatant/lysate containing the DNA is then used directly as the template for the LAMP reaction (0.5 µl of this extract was used per 20 µl LAMP reaction).

2025/05/04. Field sampling

- Visit to the Mango farm.
- Collected 6 eppendorf tubes of thrips (approximately >10 thrips in each tube). They are stored in 99% ethanol.

2025/06/11 DNA extraction of collected samples.

Quick Protocol: DNeasy Blood & Tissue Kit (for insects)

Materials Needed:

- DNeasy Blood & Tissue Kit (Qiagen)
- 1.5 mL microcentrifuge tubes
- Ethanol (96–100%)
- Proteinase K
- Buffer ATL, AL, AW1, AW2, AE

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1. Sample Prep

- 5 samples collected from farms were used (4 samples collected by Baha and 1 sample collected by Wahei)
- Samples were centrifuged to spin down the insects and ethanol was discarded
- Incubated in 56 °C until excess ethanol dried out
- Add 180 µL Buffer ATL.
- Add 20 µL Proteinase K.
- Vortex briefly or flick.
- Incubate at 56 °C for 1–3 hours (or overnight) until fully lysed.

2. Lysis

- Add 200 µL Buffer AL, mix.
- Add 200 µL ethanol (96–100%), mix well.

3. Binding

- Transfer the mixture to a DNeasy spin column in a collection tube.
- Centrifuge 1 min at ≥6000 x g (8000 rpm).
- Discard flow-through.

4. Wash

- Add 500 µL Buffer AW1, spin 1 min, discard flow-through.
- x

5. Elution

- Place column in a clean 1.5 mL tube.
- Add 100 µL Buffer AE (or nuclease-free water).

- Incubate 1 min, then centrifuge 1 min at ≥6000 x g.

Store:

- Store eluted DNA at –20 °C.

2025/06/12 PCR tests of extracted DNA samples.

PCR reaction mix prep:

- 12.5 ul of Phusion Plus Master Mix
- 1.25 ul of 10uM of each ThripsITSF
- 1.25 ul of 10uM of each ThripsITSR
- 1.25 ul of 10uM of each SdITSF3
- 1.25 ul of 10uM of each SdITSF7
- 3 ul of extracted DNA
- 4.5 ul of H_2O

PCR conditions:

Initial denaturation	98°C	30 sec	
Denaturation	98°C	10 sec	} 25 cycles
Annealing	60°C	10 sec	
Extension	72°C	30 sec	
Final extension	72°C	5 min	

Run on 1% agarose gel (0.5 g of agarose and 50 ml of TAE buffer) with 180 V for 5 min

The result was unclear.

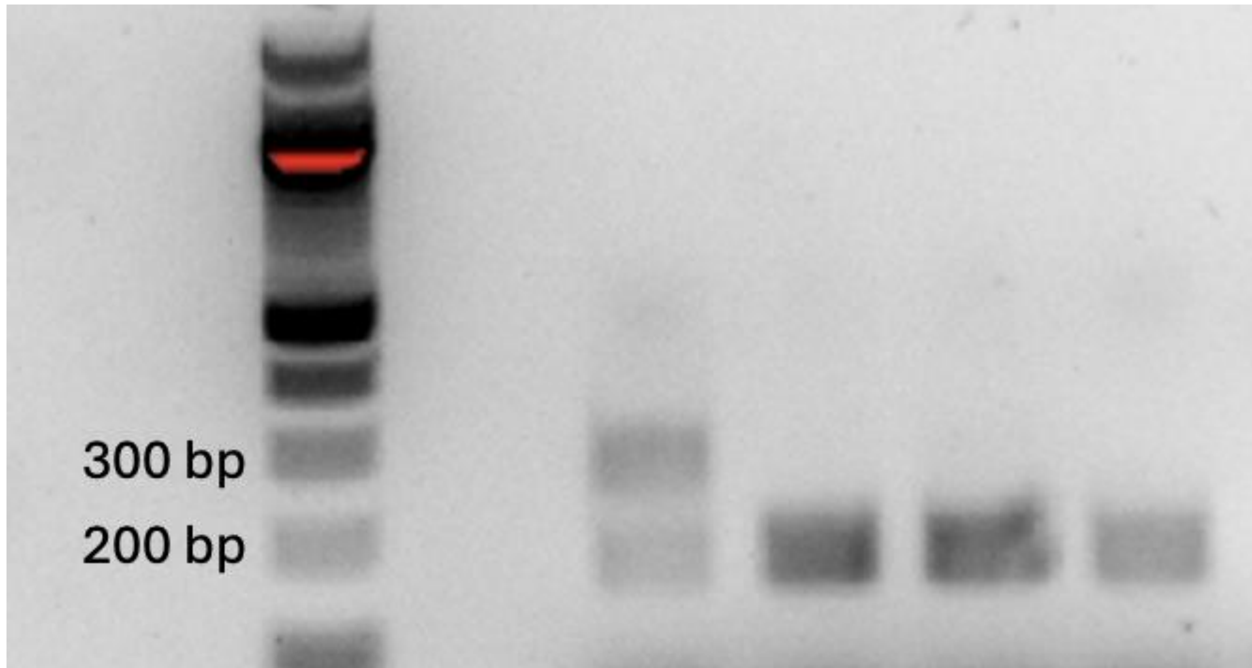
26/06/2025 PCR test extracted DNA samples (optimize)
This time, we used PCR protocol from paper:
PCR reaction mix prep:

- 12.5 ul of Phusion Plus Master Mix
- 1 ul of 20uM of each ThripsITSF (20 pmol)
- 1 ul of 20uM of each ThripsITSR (20 pmol)
- 1 ul of 20uM of each SdITSF3 (20 pmol)
- 1 ul of 20uM of each SdITSF7 (20 pmol)
- 5 ul of extracted DNA
- 3.5 of H_2O

PCR conditions:

Initial denaturation	94°C	9 min	
Denaturation	94°C	30 sec	} 35 cycles
Annealing	52°C	30 sec	
Extension	72°C	1 min	
Final extension	72°C	7 min	

Mix PCR samples with loading dye (5:1) and use the appropriate ladder (with bands on 200-500 bp). Run on gel 2% agarose gel (1 g of agarose dissolved in 50 ml of TAE) with 130 V for 15 min.
Clear bands near 200 bp (characteristic to C strain), one sample has band on 300 bp (characteristic to 300 bp).
Samples were loaded in this order: diluted ladder, miss, 7ng/ul extracted dna (trap water), 3.3 ng/ul, 2.6 ng/ul, 0.8 ng/ul, 0 ng/ul (Figure 1).



13/07/2025- Sticky sheet DNA extraction optimization
Aim: To extract DNA from the water sample where the sticky sheet was soaked

A. Material

250713
Sticky Sheet in the RO water (Kept in my room)
Sticky Sheet in the 80% ethanol (Kept in 2 tube in -20 freezer)
Leaf and thrips in the 80% ethanol (Kept in 4 tube in -20 freezer)
Thrips in the 80% ethanol (Kept in 4 tube in -20 freezer)
3 water bottles

B. Procedure

1. Get sticky sheet from farmer (250713)
2. Soak it in the bottle filled with 500ml water (250713)
3. Take 5 ml water from the bottle and put them in the tube everyday (from day0-day7)
   Day0: 250713
4. Freeze the tube
5. Extract DNA from these tubes in same procedure in previous experiment
6. Check PCR amplification

2025/07/29 DNA extraction from Stick sheet sample (SS1)
Wahei and Youjung

Aim
To extract DNA from the samples for 7 days where the sticky sheet was soaked by the protocol (**2025/06/11**)

Procedure
Water sample: 200 µl
See protocol 2025/06/11 DNeasy Blood & Tissue Kit (for insects)
Conduct from “Add 180 µL Buffer ATL.”

Result
Get extraction from 7 samples

Next
They will be checked by PCR following methods on **26/06/2025** in 08/01

Rest of work
1. Extraction dna from water sample
Sticky Sheet (SS) (n=3)
7 sample for 7 days (or 14 days) each replicate
Total reaction 63 reactions for PCR
SS1

- PCR on extracted DNA on 250729
- PCR on water
- PCR on heating water

SS2 and SS3

- Extract DNA and PCR
- PCR on water
- PCR on heating water

Thrips water (T) (n=2)
7 sample for 7 days each replicate (Not yet)
T1 and T2

- PCR on water and heating water
- Extract DNA and PCR

After above, Conduct qPCR on positive samples

2025/08/01 PCR

Sticky Sheet (SS) (n=3)
8 sample for 8 days (from day0)
Total reaction 24 reactions for PCR
SS1

- PCR on extracted DNA on 250729
- PCR on water
- PCR on heating water

DNA Concentration

- Water sample
  Day0 2.1ng/ul Day1 2.7ng/ul Day2 3.0ng/ul Day3 3.6ng/ul Day4 4.3ng/ul Day5 4.8ng/ul
  Day6 5.3ng/ul Day7 5.8ng/ul

- Extracted sample
  Day0 1.8ng/ul Day1 1.3ng/ul Day2 3.3ng/ul Day3 0.0ng/ul Day4 0.1ng/ul Day5 1.2ng/ul
  Day6 1.1ng/ul Day7 0.4ng/ul

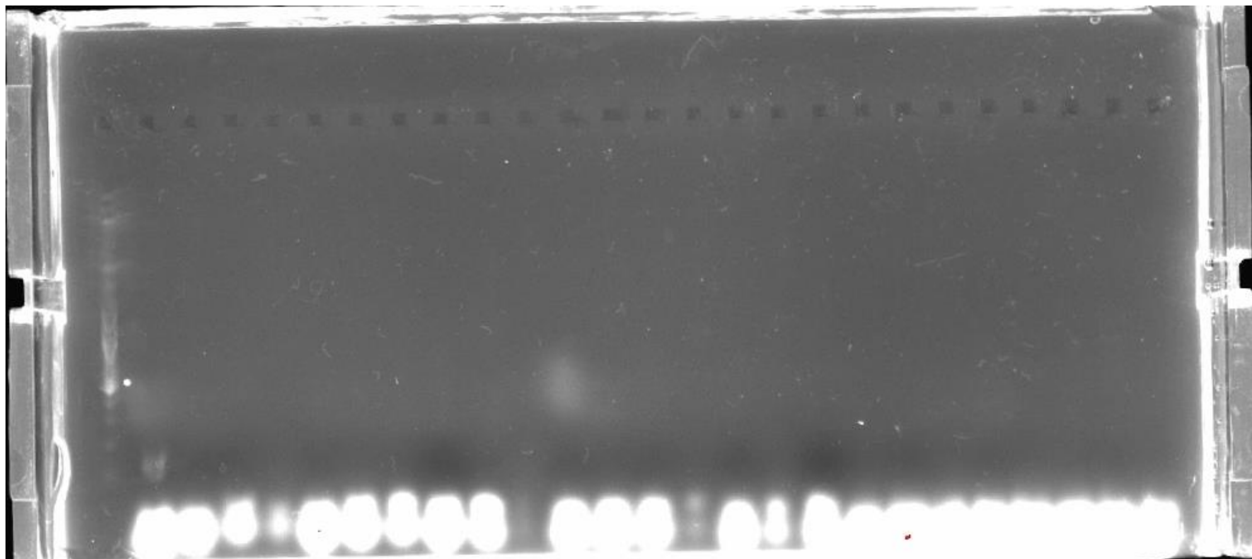
PCR reaction mix prep (250625)

- 12.5 ul of Phusion Plus Master Mix
- 1 ul of 20uM of each ThripsITSF (20 pmol)
- 1 ul of 20uM of each ThripsITSR (20 pmol)
- 1 ul of 20uM of each SdITSF3 (20 pmol)
- 1 ul of 20uM of each SdITSF7 (20 pmol)
- 5 ul of extracted DNA
- 3.5 of H_2O

PCR conditions:

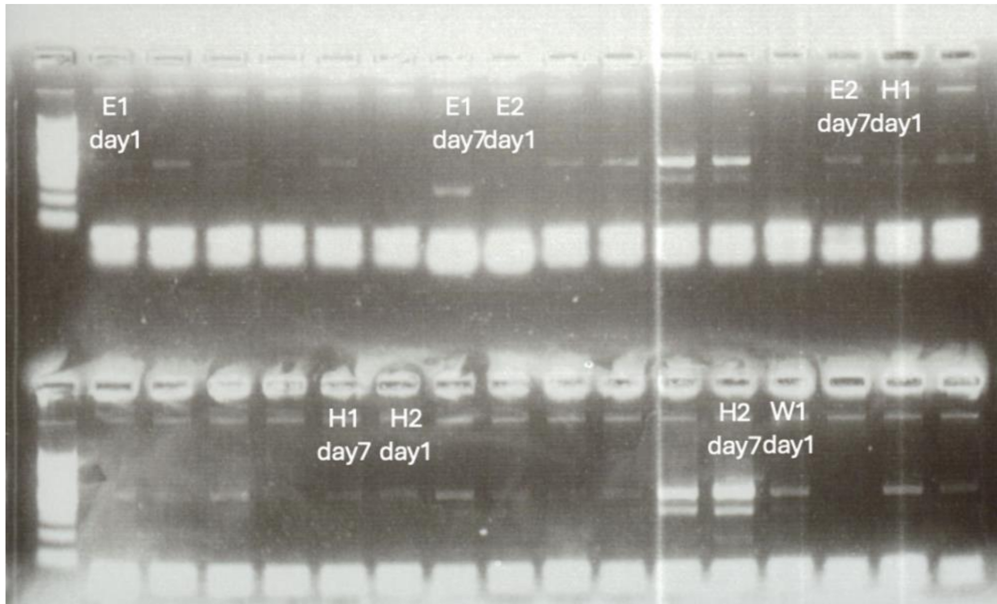
Initial denaturation	94°C	9 min	
Denaturation	94°C	30 sec	} 35 cycles
Annealing	52°C	30 sec	
Extension	72°C	1 min	
Final extension	72°C	7 min	

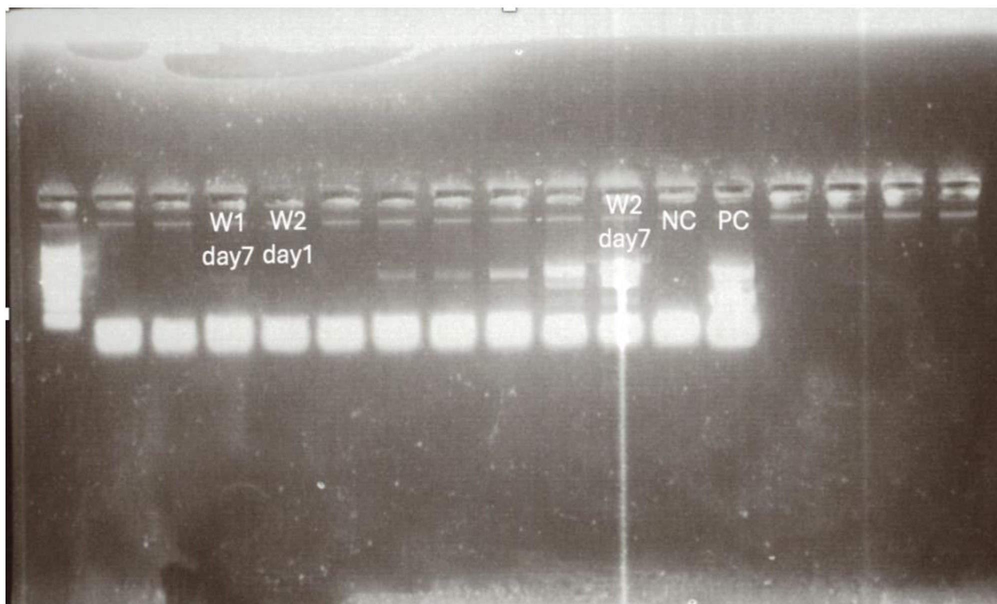
Mix PCR samples with loading dye (5:1) and use the appropriate ladder (with bands on 200-500 bp). Run on gel 2% agarose gel (2 g of agarose dissolved in 100 ml of TAE) with 130V for 15 min.
Samples were loaded in this order: ladder, positive control, extracted DNA (day1-7, day0), heated samples (day1-7, day0), water samples(day1-7, day0). No bands.



PCR of water samples.

E1 - **extracted samples from water with thrips replicate #1**, E2 - extracted samples from water with thrips replicate #2. H1 - heated samples from water with thrips replicate #1, **H2** - **heated samples from water with thrips replicate #2**, W1 - crude water samples with thrips replicate #1, W2 - crude water samples with thrips replicate #2





2025/08/06 eDNA Extraction from Ethanol-Preserved Environmental Samples

Method: Qiagen DNeasy Blood & Tissue Kit
Sample types: Ethanol-preserved leaf wash & soil samples
Batch size: 4 samples
Total time: ~1 hr 45 min

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heat block/incubator (56°C)

Pre-Preparation (Ethanol Removal & Pellet Concentration)

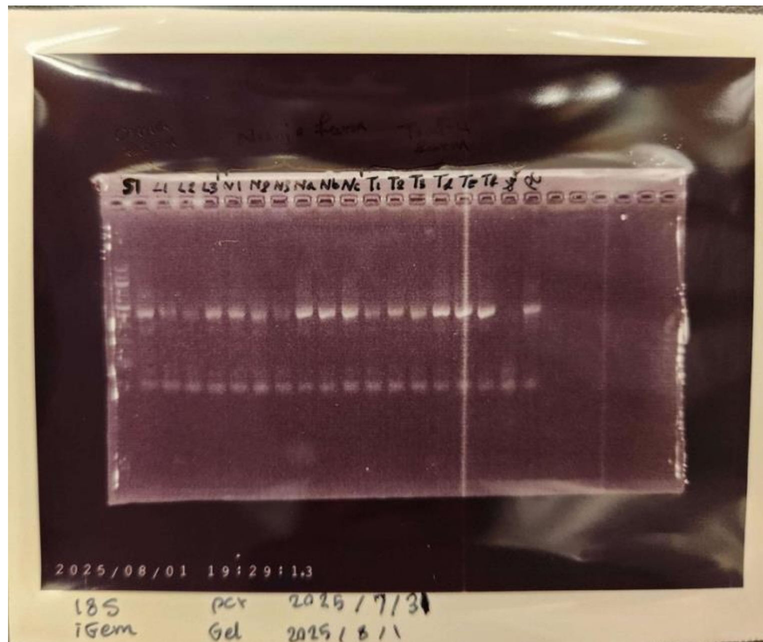
1. **Centrifuge 50 mL Falcon tubes** at **4,000 × g for 20 minutes at room temp** to pellet debris.
2. **Carefully decant ethanol** without disturbing pellet.
3. **Air-dry pellet** with lids open in clean area (or 37°C incubator) for **5–10 minutes**.

DNA Extraction Protocol

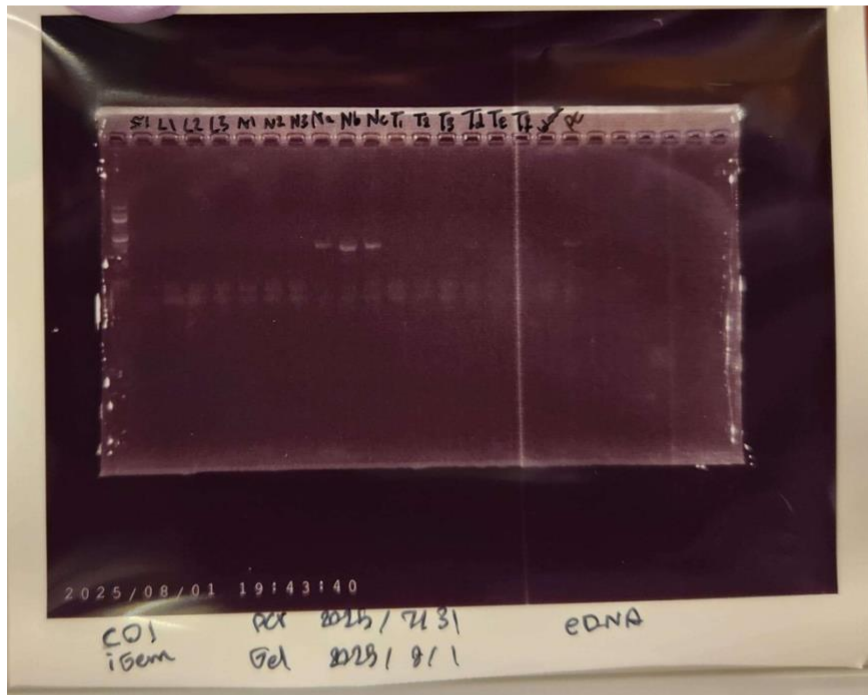
5. **Add lysis solution to each pellet:**
    - 180 µL Buffer ATL
    - 20 µL Proteinase K
      (double volumes if pellet is large)
6. **Vortex briefly** or pipette up/down to mix.
7. **Incubate at 56°C for 1 hour.**
8. After incubation, add:
    - 200 µL Buffer AL (mix well)
    - 200 µL 96–100% ethanol (mix again)
9. **Transfer lysate (~600 µL total)** to DNeasy spin column in 2 × 300 µL steps.
    - Centrifuge at **6,000 × g (8,000 rpm) for 1 min**, discard flow-through.
10. **Wash 1:**
    - Add 500 µL Buffer AW1
    - Centrifuge 6,000 × g for 1 min
    - Discard flow-through
11. **Wash 2:**
    - Add 500 µL Buffer AW2
    - Centrifuge at **20,000 × g (14,000 rpm) for 3 min**
    - Discard flow-through
12. **Dry spin:**
    - (Optional) Repeat spin for 1 min to ensure all buffer is removed
13. **Elute DNA:**
    - Add 100 µL Buffer AE directly to membrane
    - Incubate 1–2 min at room temp

- Centrifuge at 6,000 × g for 1 min into clean 1.5 mL tube
- Optional: re-elute with same or new 100 µL

From Onna village farm: 3 leaf samples and one soil sample preserved in ethanol From Nanjo farm: 3 leaf samples preserved in ethanol and 3 Q-tip samples (rubbed on different leaves) preserved in AE buffer From Trafru farm: 3 leaf samples preserved in ethanol and 3 Q-tip samples (rubbed on different leaves) preserved in AE buffer Results: 18S: Very good bands from all samples => we have 17 samples to send for sequencing, including the PCR blank



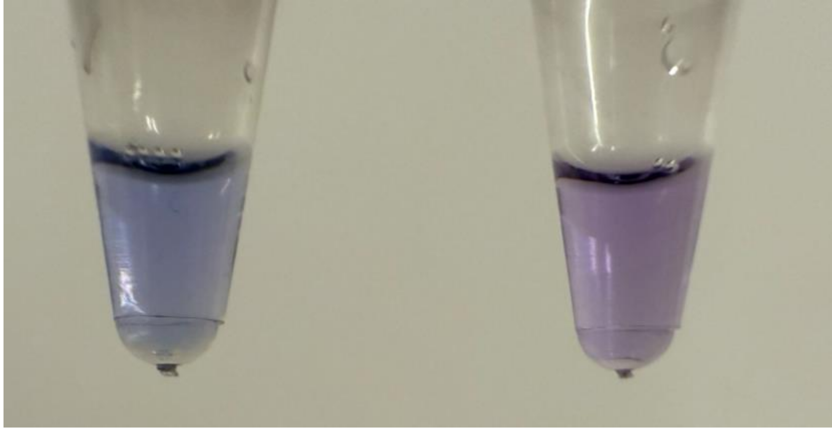
COI: We obtained bands from the samples that were preserved in AE buffer only. So basically, 3 samples from Nanjo farm (good bands), and 3 samples from Trafru (weak bands) => we have 7 samples to send for sequencing including the PCR blank
*Concern: no samples from Onna village farm were preserved in AE buffer so we don’t have any samples that amplified with COI from this farm


2025/08/01 19:43:40
COI
iGem
PCR 2025/7/31
Gel 2025/8/1
eDNA

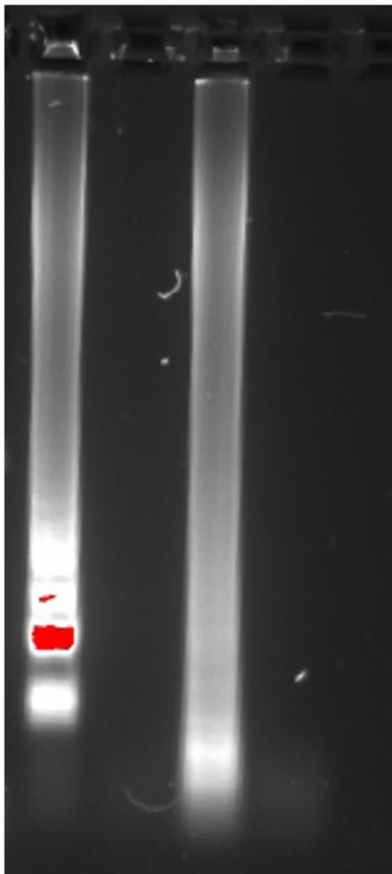
2025/09/23 LAMP reaction using thrips DNA.
Prepared a reaction mix as follows:

Component	25 µl Reaction	Final Conc
10X ThermoPol Buffer	2.5 µl	1X (contains 2 mM $MgSO_4$)
$MgSO_4$ (100 mM)	1.5 µl	6 mM (8 mM total)
dNTP Mix (10 mM)	3.5 µl	1.4 mM each
FIP/BIP Primers (25X)	1 µl	1.6 µM
F3/B3 Primers (25X)	1 µl	0.2 µM
LoopF/B Primers (25X)	1 µl	0.4 µM
Bst DNA Polymerase, Large Fragment (8,000 U/ml)	1 µl	320 U/ml
DNA Sample	1 µl (2-3 ng)	> 10 copies or more
Either phenol red or hydroxy naphthol blue	1-5 µl	
Nuclease-free Water	to 25 µl	
Total Reaction Volume		25 µl

Incubate at 63 C for 60 min. Here is the result after the incubation.



Run samples on a gel with SybrSafe. 1st lane - LAMP reaction without a dye, 2st - Negative control without a dye, 3rd - LAMP reaction with hydroxy napthol blue, 4th - Negative control with hydroxy naphtol blue.



For phenol red no color change was observed.

Also load the 10 ul of reaction mix with DNA onto Whatman Grade 1 paper squares of ~5mm length and width, however paper quickly absorbs the reaction so color is very bleak.

PCR with water samples collected by blue light trap

Sample: 2 water samples from blue light trap, 2 insect samples, positive control, negative control

Extract DNA from 2 insect samples by DNeasy Blood & Tissue Kit according to the protocol (25/06/11)

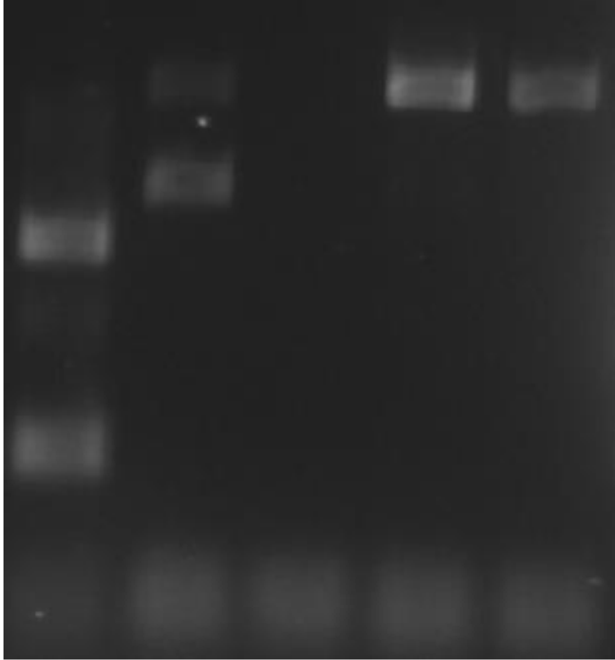
PCR reaction mix prep (same as 25/06/25)

- 12.5 ul of Phusion Plus Master Mix
- 1 ul of 20uM of each ThripsITSF (20 pmol)
- 1 ul of 20uM of each ThripsITSR (20 pmol)
- 1 ul of 20uM of each SdITSF3 (20 pmol)
- 1 ul of 20uM of each SdITSF7 (20 pmol)
- 5 ul of each sample
- 3.5 of H2O

PCR conditions (same as 25/06/25):

Initial denaturation	94°C	9 min	
Denaturation	94°C	30 sec	} 35 cycles
Annealing	52°C	30 sec	
Extension	72°C	1 min	
Final extension	72°C	7 min	

Gel image:



First is positive control. Second lane and third lane is water sample #1 and #2. Fourth and fifth lane are extracted DNA from insects collected by blue light LED trap #1 and #2.

Result: different bands for trap water samples and insect DNA extraction system from positive control indicating 200 and 500 bp bands.