

## Background

- Plant parasitic nematodes cause an estimated **157 billion dollars** in plant losses [1] [3]
- Root knot nematodes (RKN) inject **effector proteins** into plant roots, siphoning plant nutrients [2, 3]
- Plant immune system can recognize nematode effectors and trigger cell death to starve nematode - nematode effector becomes an **avirulence gene** [3, 5]
- RKN evades plant immune system by dropping avirulence gene [2]
- Can this “dropped” avirulence gene in root knot nematodes be identified?**



Fig. 1. *Meloidogyne incognita*

## Objectives & Obstacles

### Objectives

- Test designated primer sets to amplify genes of interest through high fidelity and real-time polymerase chain reaction (HiFi & qPCR)
- Extract DNA and RNA from *M. incognita* and *Nicotiana benthamiana*
- Inoculate *N. benthamiana* with plasmid pSfinx-mYFP through Agrobacterium-mediated transformation (AMT) to test transfection in plants [4]

### Obstacles

- RKNs are polyploidy- this could result in amplification of a copy of the gene of interest rather than the gene itself
- Effector proteins are miniscule within RKNs, which could result in faulty amplification
- Extracted DNA from juvenile stage 2 may have more effector proteins present than juvenile stage 1 or egg mass stage (see fig. 2)

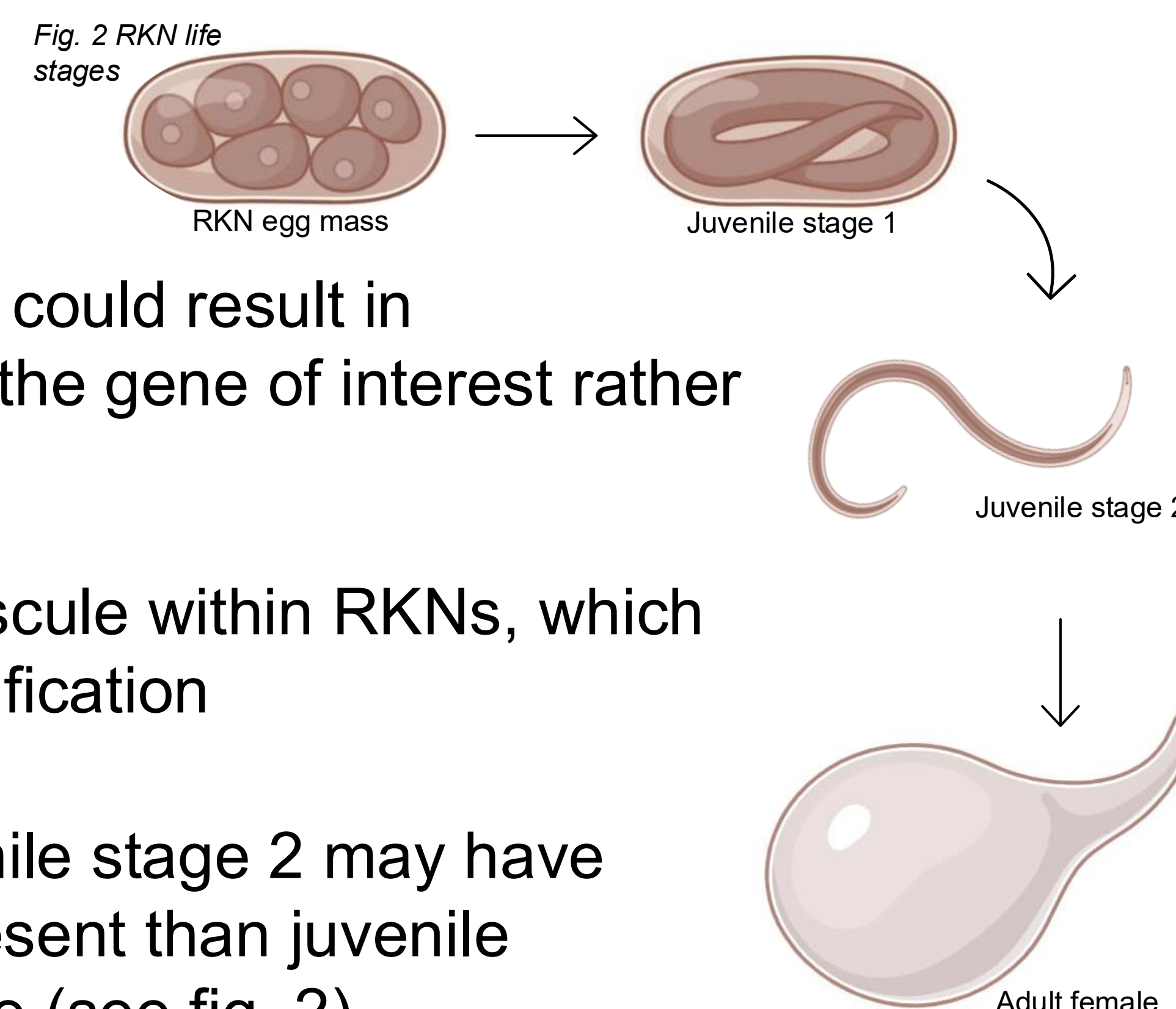


Fig. 2 RKN life stages

## DNA & RNA Extraction

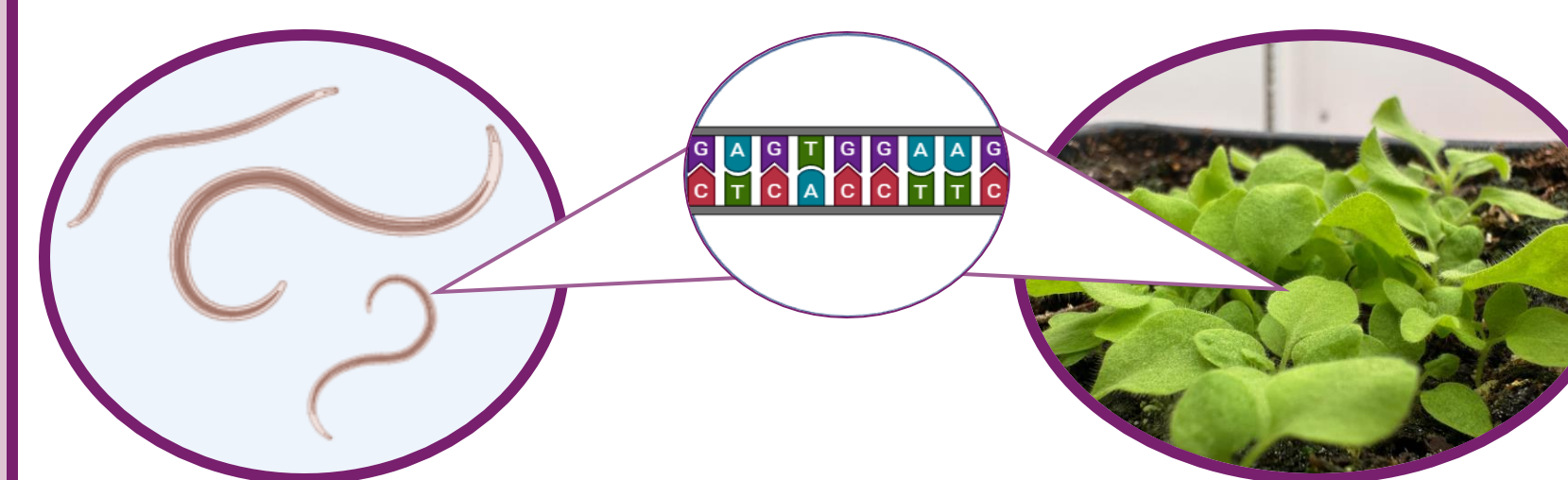
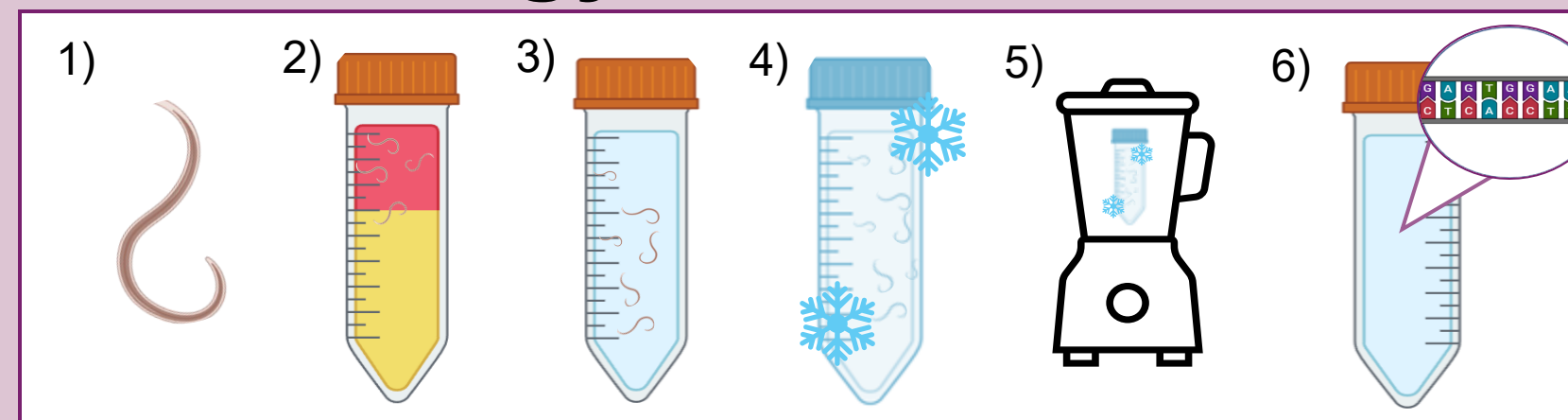


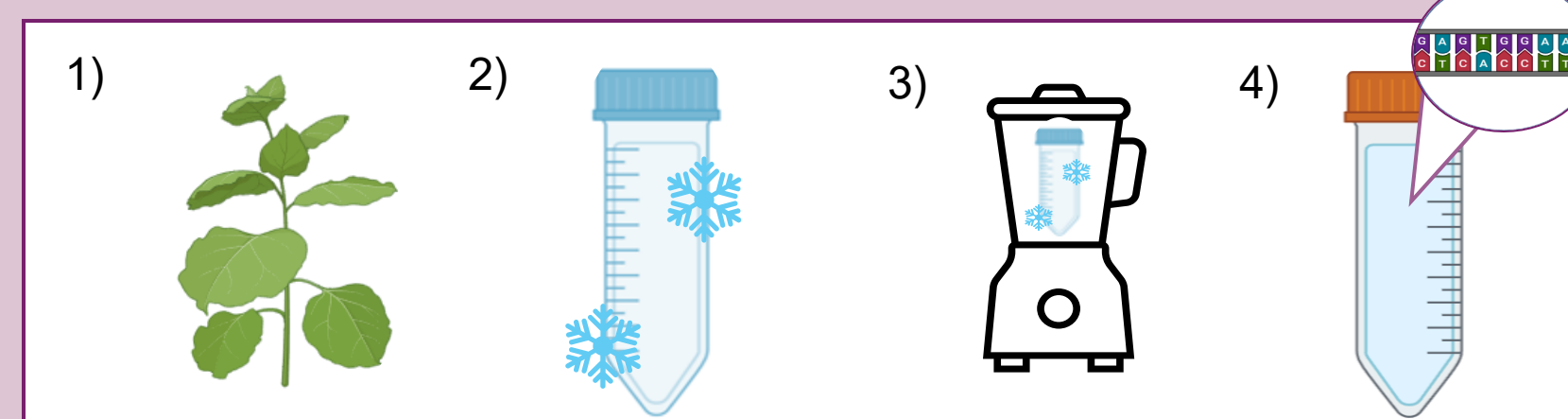
Fig 3. *Meloidogyne* spp. (left), *N. benthamiana* (right)

### Meloidogyne DNA Extraction



1. Collected J2 from tomato plant roots
2. Separated nematodes with sucrose gradient
3. Concentrated nematodes into one tube
4. Froze nematodes using liquid nitrogen (LN<sub>2</sub>)
5. Milled frozen nematodes
6. Extracted DNA using Qiagen DNeasy Blood and Tissue Kit

### “Benth” RNA Extraction



1. Collected youngest leaves
2. Froze plant tissue using liquid nitrogen
3. Milled frozen plant tissue
4. Extracted RNA using Qiagen RNeasy Mini Kit

## Materials & Methods

### HiFi PCR & Real-Time PCR

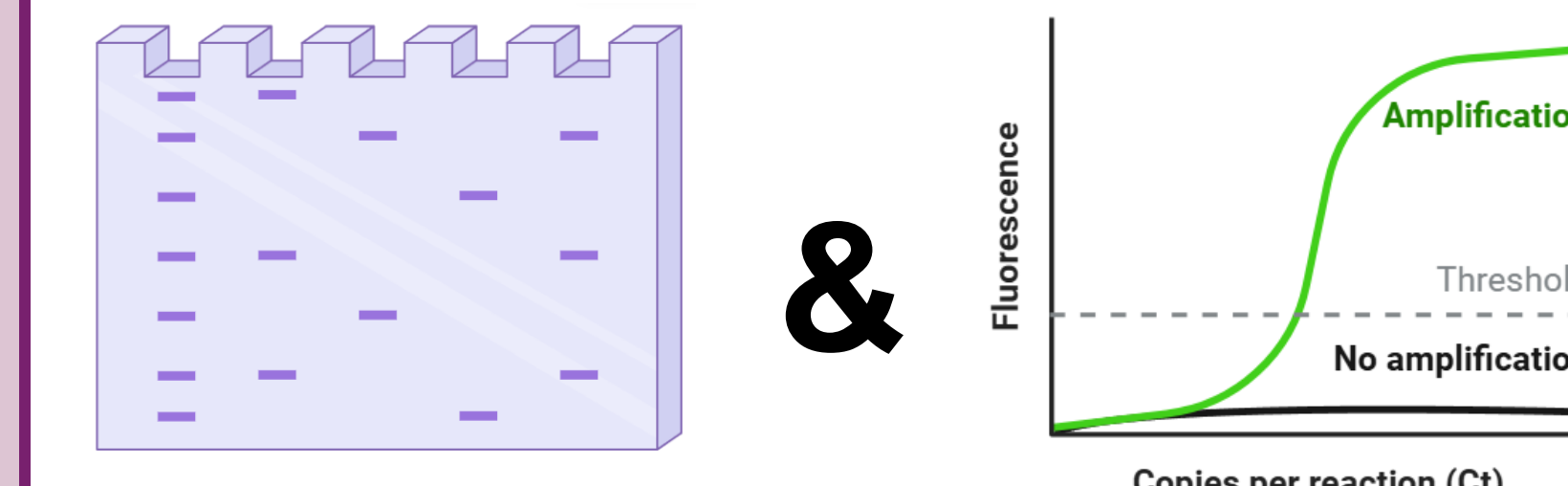
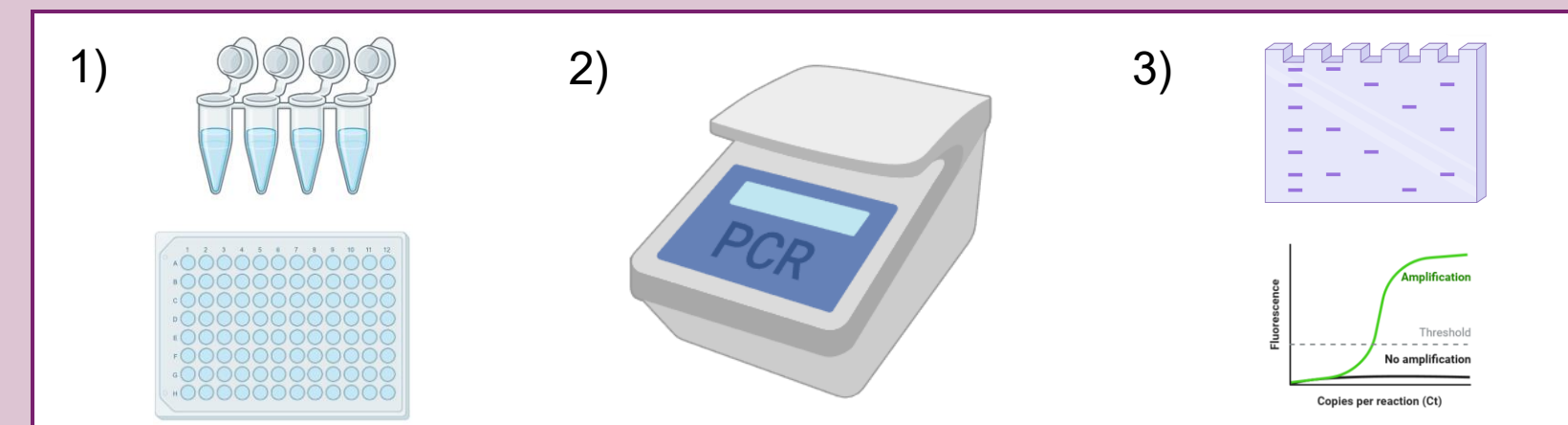


Fig 4. agarose gel example (right), qPCR Cq graph example (left)

### HiFi & Real-Time PCR



**HiFi PCR processes tested the amplification ability of primer candidates g22041, g22050, and g22089**

1. Loaded PCR tubes with NEB Q5 High-Fidelity 2X Master Mix, primers, and template nematode DNA
2. PCR was run using thermal cycler with specific annealing temperatures
3. Analyzed amplification with agarose gel electrophoresis and contrast photos

**qPCR processes quantified RKN, Charleston bell pepper, and “benth” cDNA**

1. Loaded 96 well plate with house-keeping primers/target primers, cDNA, and SYBR green fluorescent dye
2. qPCR was run using thermal cycler
3. Analyzed fluorescent content and determined expression of target genes

### AMT & Leaf Inoculation

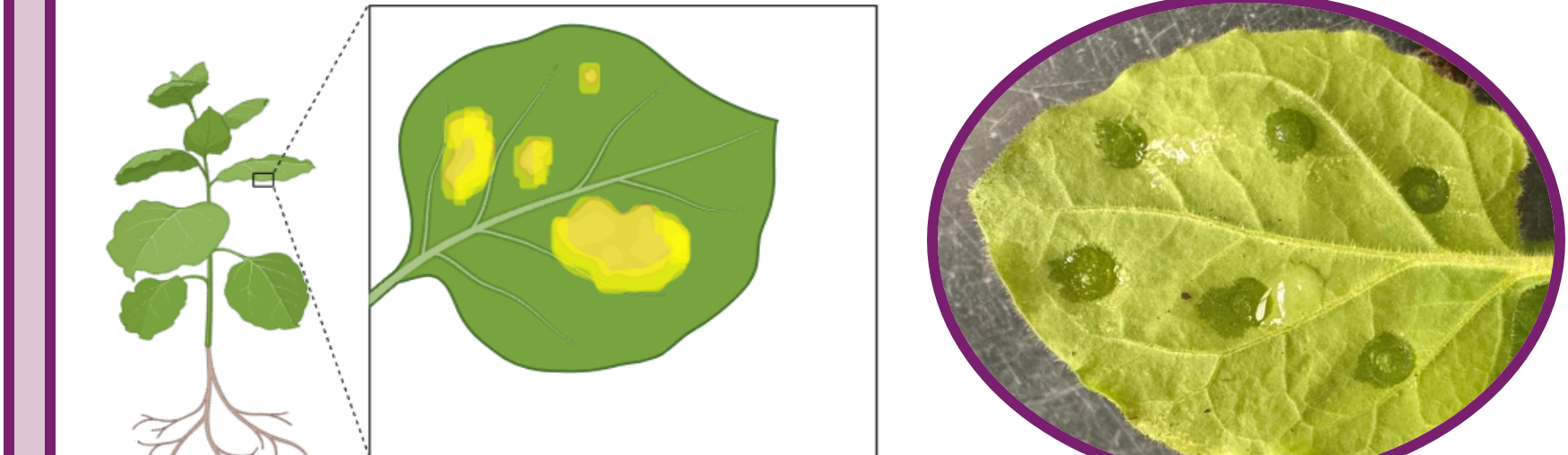
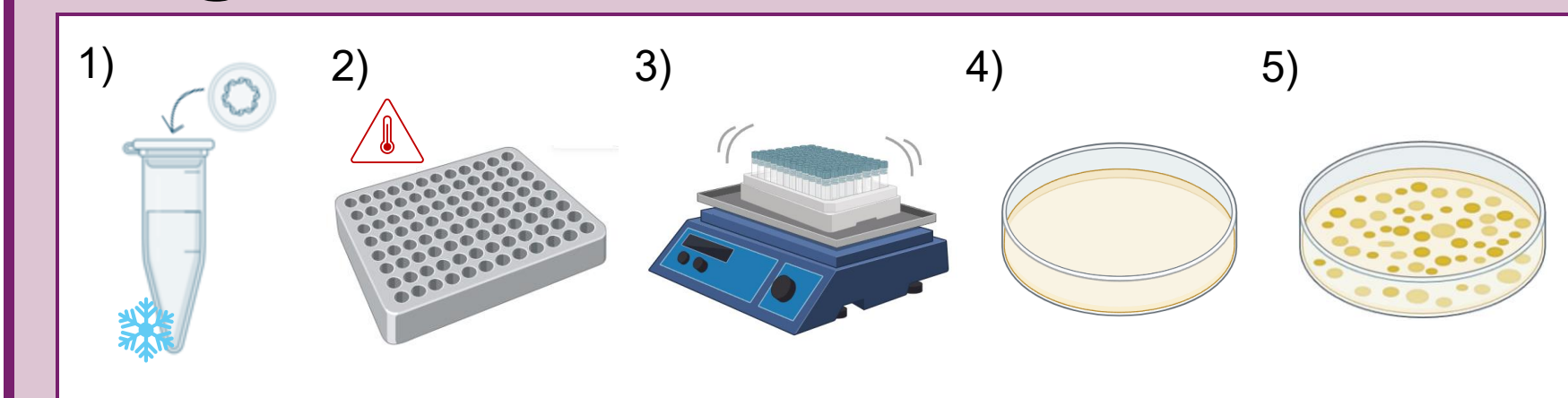


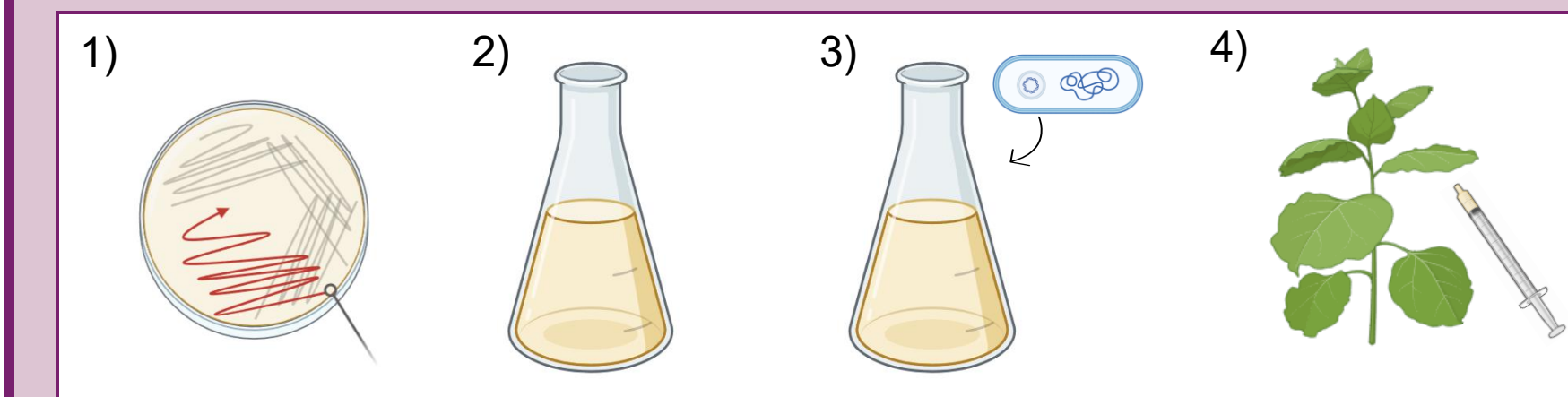
Fig. 5 *N. benthamiana* inoculated with *Agrobacterium* (left)

### Agrobacterium Transformation



1. Mixed pSfinx-mYFP plasmid and *Agrobacterium* in 1.5ml tube then froze in LN<sub>2</sub>
2. Heated mixture at 37°C on heating block
3. Placed in incubating shaker at 28°C
4. Spread mixture over LBK plate
5. Let incubate for two days for visible colonies

### Leaf Inoculation



1. Streaked single colonies from AMT
2. Prepared fresh media consisting of MgCl<sub>2</sub>, MES, and Acetosyringone
3. Mixed transformed Agro with transformation media- shook for two hours
4. Injected mixture with needleless syringe into underside of young leaves

## Results & Discussion

### A target avirulence gene may have amplified

- A band resulted after adjusting PCR annealing temperatures and re-extracting DNA for primer g22041
- The predicted band size was 1094 bp- the band is around that measurement

### What's next?

- Next step: confirming validity of gene by cloning and sequencing
- End goal: cloning amplified gene and transmitting to pepper plants via pSfinx-mYFP- viruses act as vector
- mYFP must successfully infect benth test plants before inoculating pepper plants

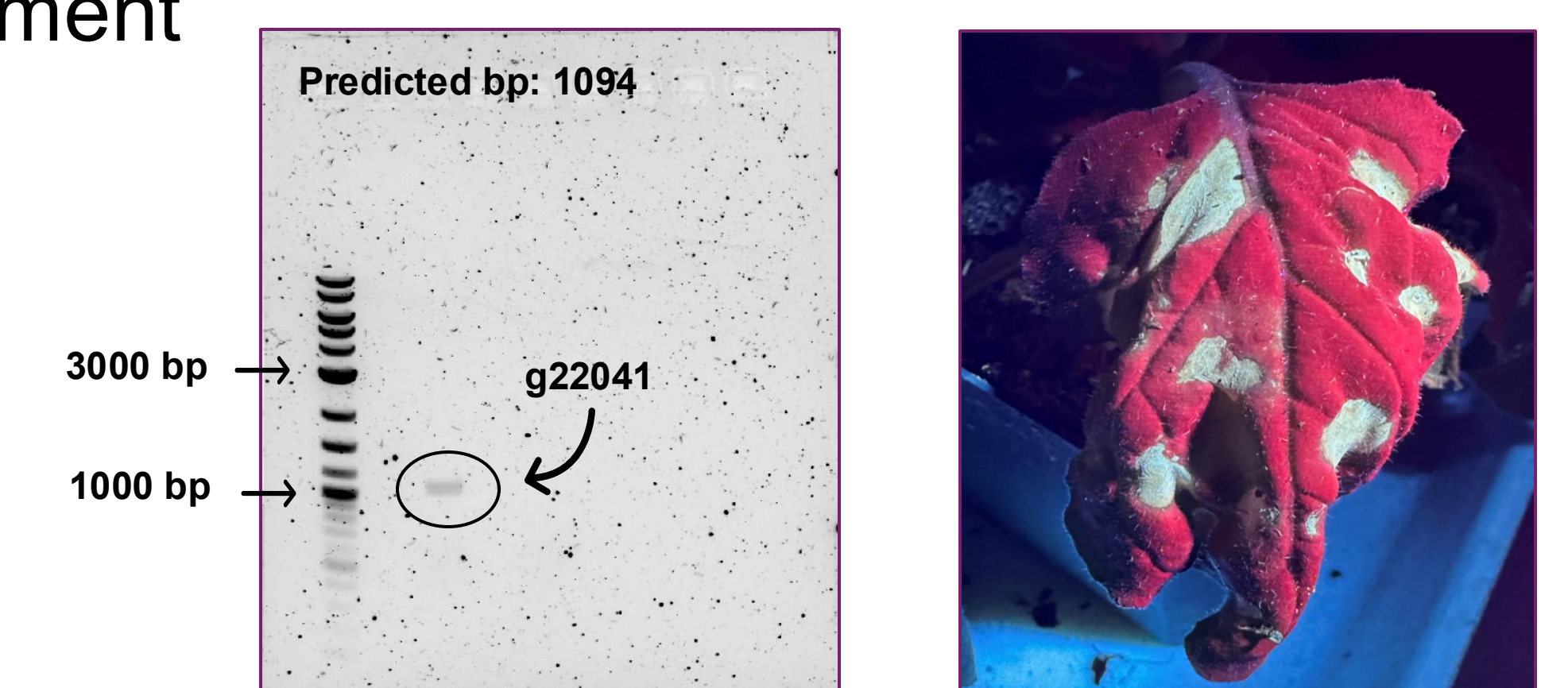


Fig. 6 & 7 (respectively): agarose gel results g22041 primer set; benth plant inoculated with plasmid mYFP for two weeks