
HOT SHAKER

Content

Why Turf?

- Provides a safe playing surface for recreation.
- Provides aesthetic pleasure in lawns and landscapes.
- Prevents soil erosion.
- Keeps soil surface covered, cool and decreases direct evaporation.
- Turfgrasses cover 1.9% of the U.S. land area, occupying three-times more than any irrigated crop.

Research Problem: Drought Tolerance & Dollar Spot Resistance

- Climate change is disrupting weather patterns, leading to extreme weather events, unpredictable water availability, exacerbating water scarcity and contaminating water supplies.
- Water management must play a central role in adapting to the worst effects of climate change and reducing greenhouse gases.

Research Problem: Drought Tolerance & Dollar Spot Resistance

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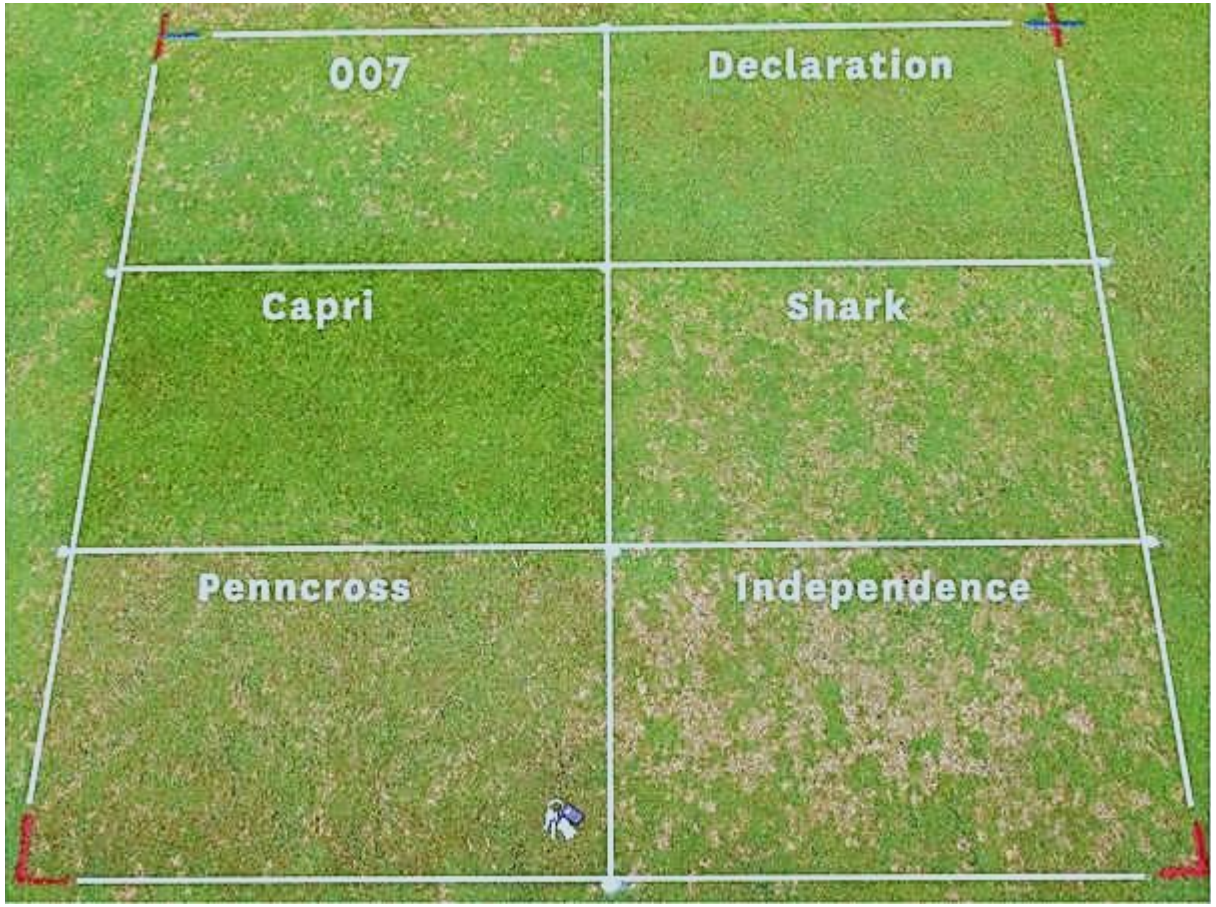
- 54% of golf course managers execute calendar-based fungicide application, i.e. every 2 weeks from beginning in May to ending in September or October, to control dollar spot
- An average annual cost of US $15,000 per golf course is spent to control foliar diseases on golf courses

Research Rationale:

- Developing resistant turf cultivars is a promising method to control dollar spot while reducing fungicide application.
- Development of specific markers that can be used to select dollar spot resistant cultivars for breeding program.



Overview of the Research Project:

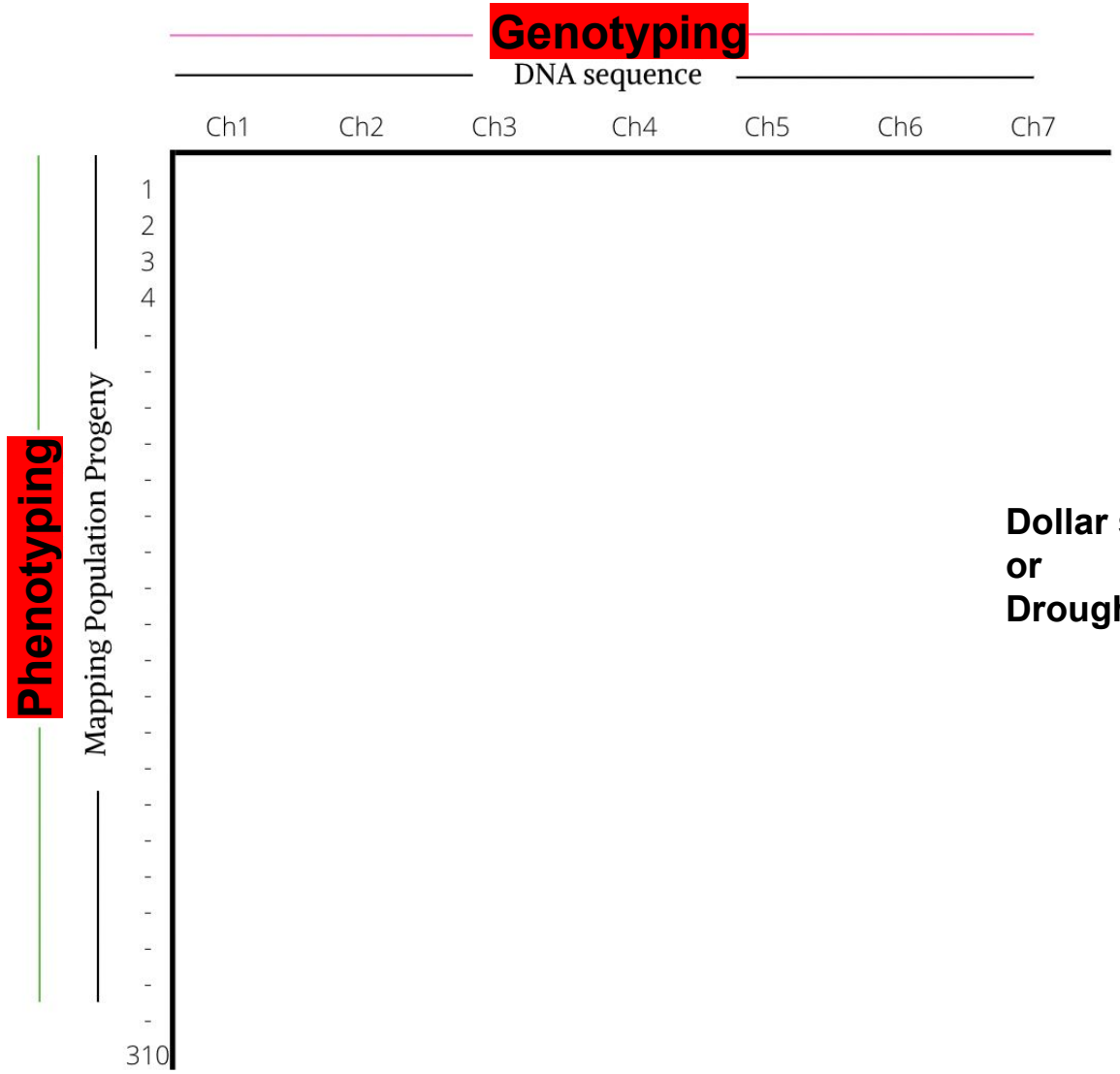
Identification of genomic regions and/or target genes associated with dollar spot resistance and drought tolerance

Colonial

Resistant
- dollar spot
- drought

Creeping

Susceptible
- dollar spot
- drought



Dollar spot disease
or
Drought stress

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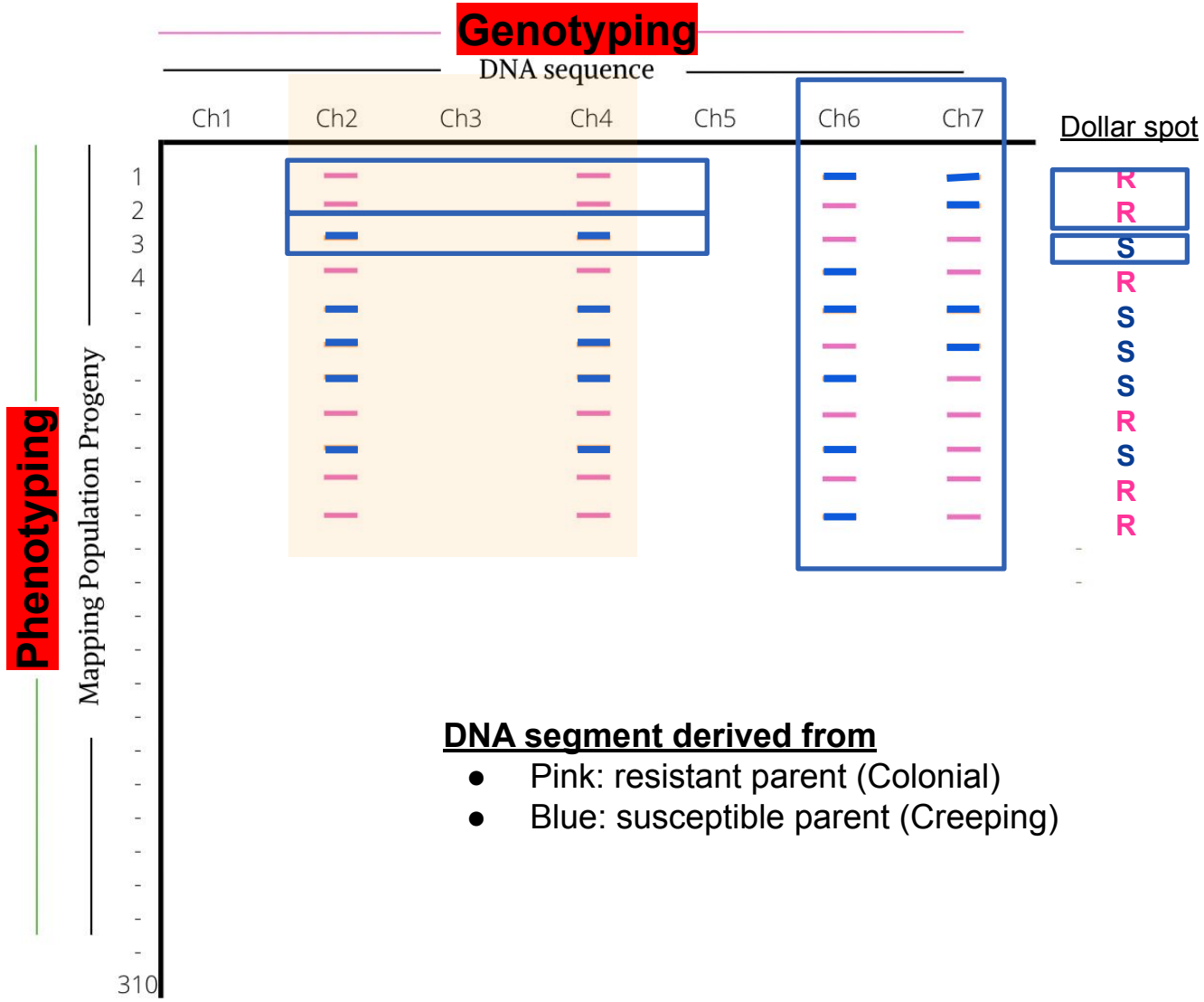
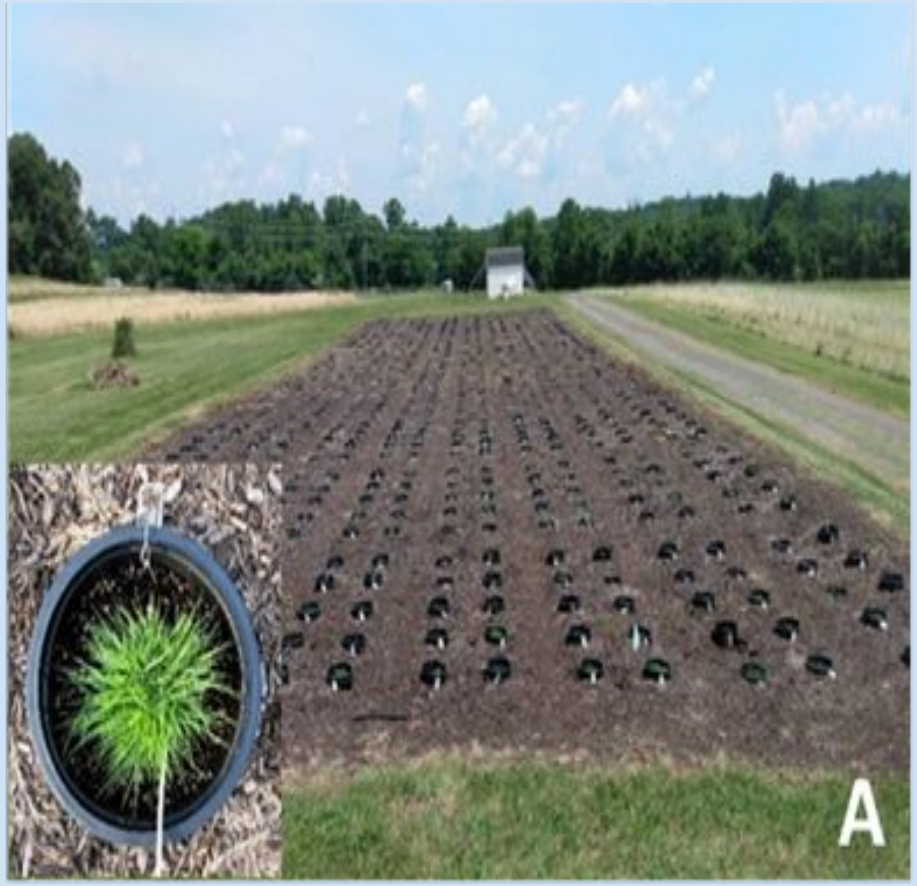


DNA segment derived from
- Pink: resistant parent (Colonial)
- Blue: susceptible parent (Creeping)

Content (Phenotyping Project)

- Phenotyping morphological traits of 310 mapping progenies in the greenhouse and field
- Installation of automatic raspberry pi system for temporal progression of drought stress and dollar spot disease among 310 mapping progenies

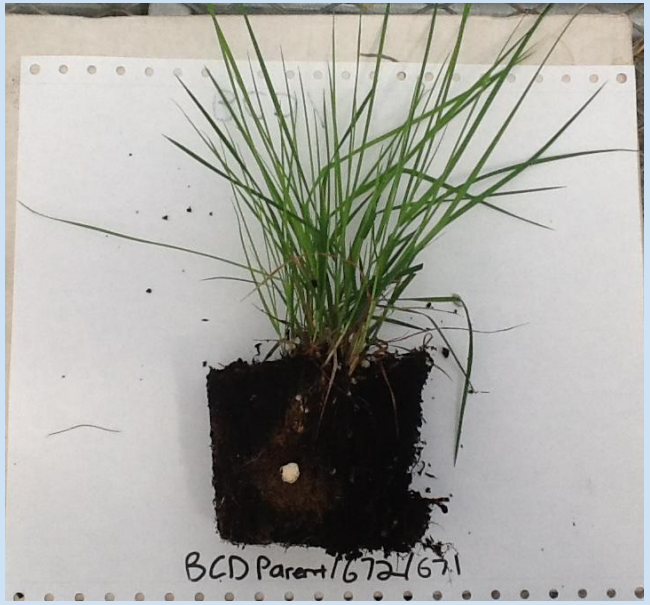
FNPRU Turf Field



FNPRU Turf Greenhouse

Mapping parents

- Hybrid population derived from a cross between Providence (creeping) and BCD (colonial) bentgrass
  - **Providence**
    - Creeping growth phenotype
    - High-yield production
  - **BCD**
    - Colonial growth phenotype
    - Drought tolerant
    - Dollar spot resistant



Field Images

- Field images were taken of each individual plant plotted within the field.

Morphological Trait of Hybrid Mapping Population

For more objective and accurate phenotyping

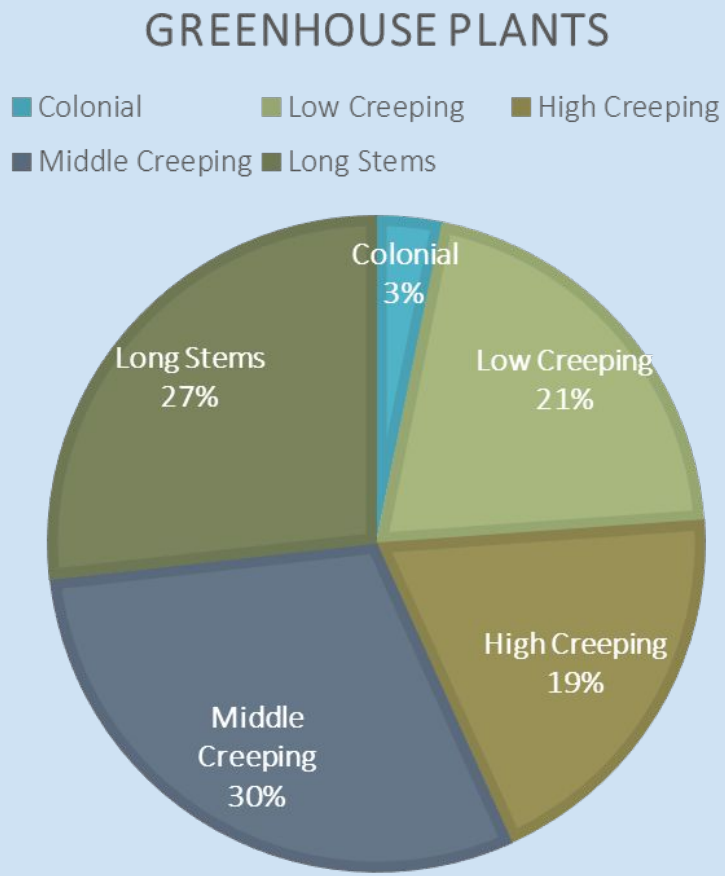
ImageJ program: Counting the number of green pixels within a field image

Segregation of Morphological Phenotypes in Greenhouse

Segregation of Morphological Phenotypes in Greenhouse

- Greenhouse plants were segregated into five phenotypes
  - Middle Creeping-93
  - Low Creeping-64
  - High Creeping-60
  - Long Stems-83
  - Colonial-10

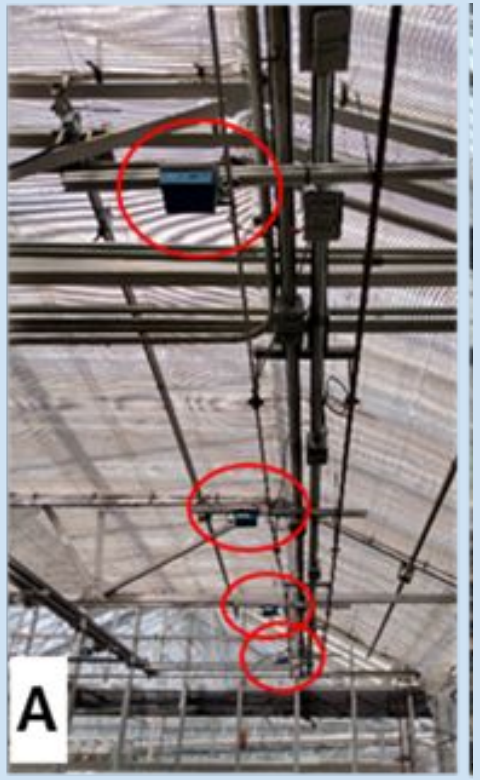
Segregation of Morphological Phenotypes in Greenhouse



Research goal for this phenotyping project

- Evaluate the genetic variations in drought tolerance and dollar spot resistance of hybrid plant in relation to their phenotypic differences.

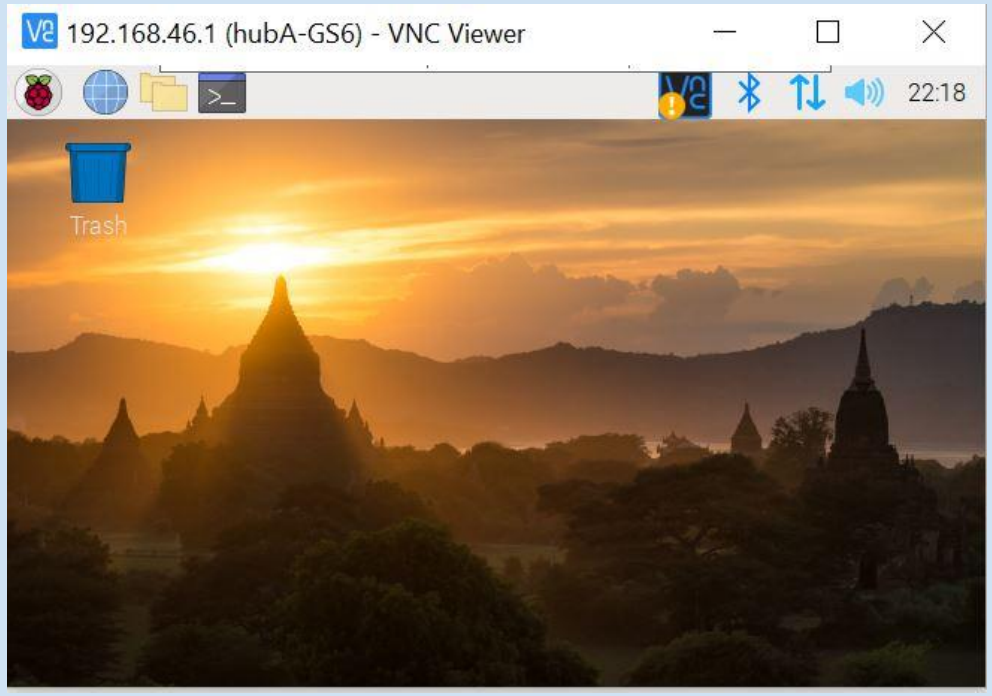
- Install the Raspberry pi system and use it for the temporal progression of drought stress and dollar spot disease.



Setting up Raspberry Pi

- Use the VNC viewer application to connected to the central Raspberry pi and see the virtual desktop.

- Create a running schedule using crontab and the snap_all.sh -t command within image acquisition file.




```
23 # m h  dom mon dow   command
24 28 23 * * * ./snap_all.sh -t
25 # 26 00 * * * /home/pi/Pictures/temp
26 # 00 18 * * * python /home/pi/PhotoAcq.py
27 # 00 21 * * * python /home/pi/PhotoAcq.py
28 #0 14 * * *  python /home/pi/PhotoAcq.py
29 #0 13 * * * /home/pi/snap_all.sh
30 # 0 6-20 * * * /home/pi/snap_all.sh #UNCOMMENT LATER FOR DIURNAL PHOTOS
```

Crontab

- Crontab/cron is a job scheduler application allowing one to schedule the frequency of a task.
- The basic format for cron is minutes, hours, day of month, month and day of week.
- Using the crontab guru at https://crontab.guru/ may be used to help plan cron schedules.

Command

* The script contains the raspistill command that tells a raspberry pi to capture a picture

```
56          echo "Acquiring images from GS6 blocks 1-10..."
57          ssh pi@192.168.46.25 raspistill -o /home/pi/Pictures/gs6block1_$cur
   rdate.jpg
58          raspistill -o /home/pi/Pictures/gs6block2_$currdate.jpg
59          ssh pi@192.168.46.97 raspistill -o /home/pi/Pictures/gs6block3_$cur
   rdate.jpg
```

- The snap_all.sh -t command utilizes the snap_all.sh script that commands all the raspberry pis to:
  - Take a picture
  - Save pictures with the current date

- Then the pictures are stored within a temp folder for eventual retrieval or viewing.

Future Directions (Phenotyping Project)

Phenotyping the visible and invisible stress symptoms using automatic raspberry pi as well as hyperspectral imaging system

To evaluate genotypic differences among ~310 mapping progenies

- Temporal progression of drought stress
- Temporal progression of dollar spot disease

Content (Genotyping Project)

- DNA extraction of 310 mapping progenies including two parents using several DNA extraction methods
- PCR (Polymerase Chain Reaction) and/or GBS (Genotyping By Sequencing) to find target genomic regions that are associated with traits of interest (e.g. dollar spot resistance, drought tolerance, etc)

Colonial

Creeping

Overview:

FTA Card Protocol

Pros: labor and cost efficient, will be used for PCR.

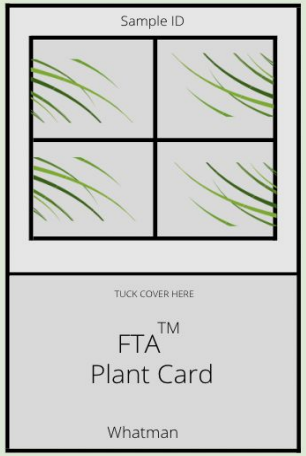
Cons: does not produce good enough quality for sequencing.

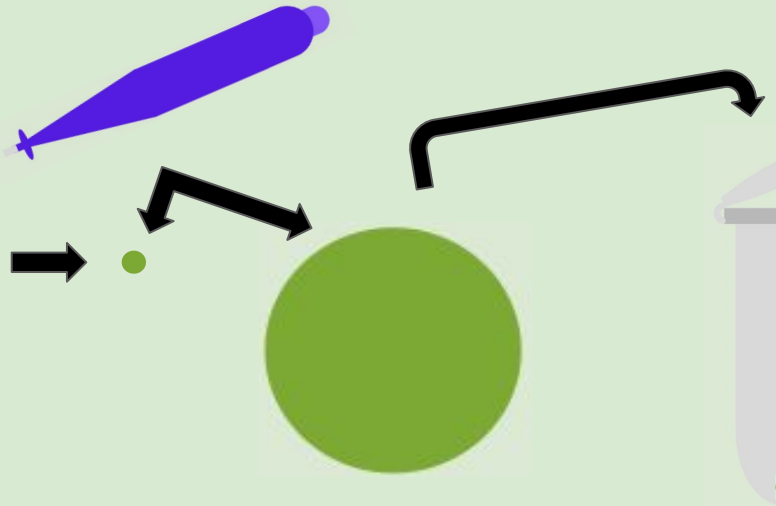
DNeasy Plant Mini Kit Protocol

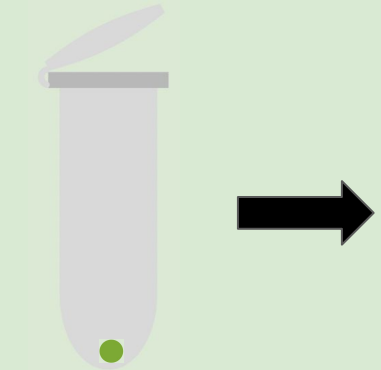
Pros: produces good DNA quality for sequencing, will be used for GBS.

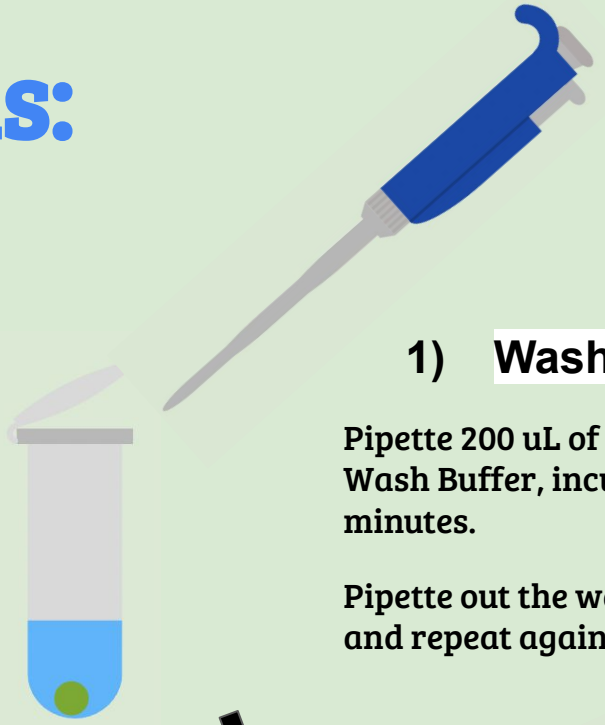
Cons: takes up time and more expensive.

DNA Extraction from FTA cards:





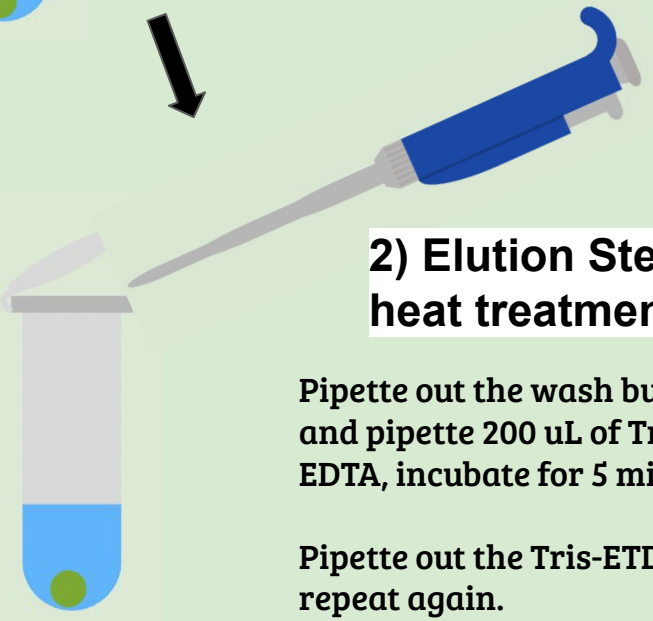




1) Wash Step

Pipette 200 uL of QIAcard FTA Wash Buffer, incubate for 5 minutes.

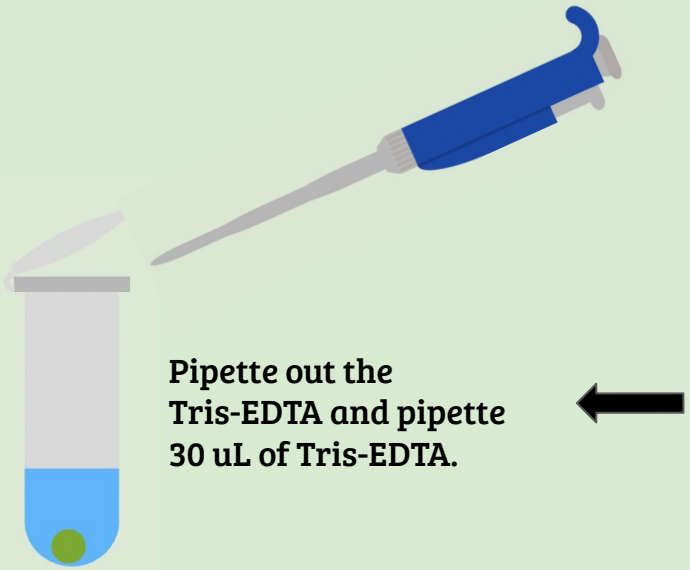
Pipette out the wash buffer and repeat again.



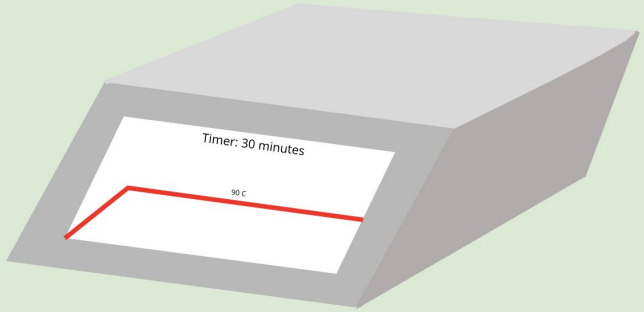
2) Elution Step (with heat treatment)

Pipette out the wash buffer and pipette 200 uL of Tris EDTA, incubate for 5 minutes.

Pipette out the Tris-ETDA and repeat again.



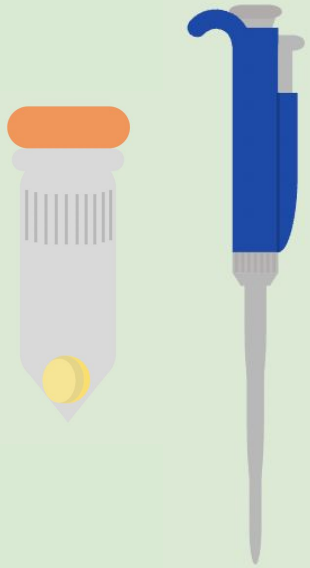
Pipette out the Tris-EDTA and pipette 30 uL of Tris-EDTA.



Heat treatment for 30 minutes at 90 C.

DNeasy Mini Kit Protocol:

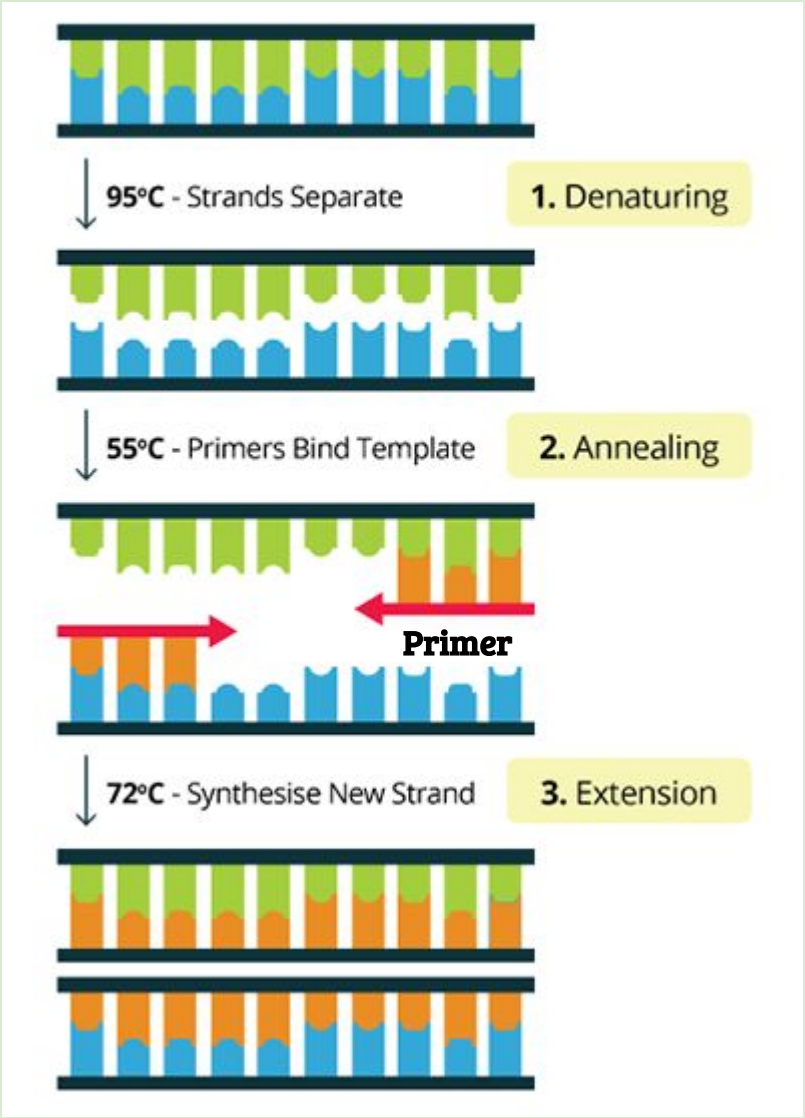
1) Each plant sample was placed in a tube with a bead and sand.
2) Labeled thoroughly.
3) Tubes were then placed in a machine and ground into powder.
4) Utilized the DNeasy Plant Mini Kit to extract plant DNA.
5) Each sample was then read by a Nanodrop machine.



DNA Extraction:

1) Cell Lysis - utilizing chemical mixtures to disrupt exterior of cell and release DNA content.
1) DNA Precipitation - to precipitate DNA into a visible solid form.
1) DNA Wash - to remove contamination and increase purity.

What is PCR?

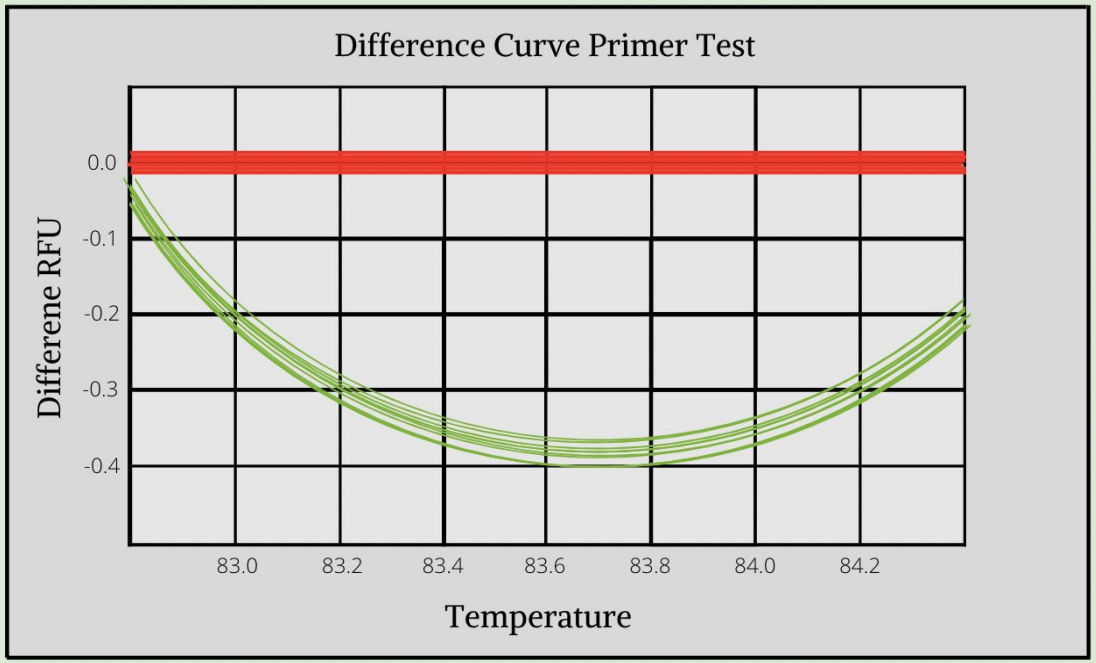


Using short synthetic DNA fragments called primers to select a segment of the genome to be amplified, and then multiple rounds of DNA synthesis to amplify that segment.

- **Denaturing**: Hydrogen bonds of DNA samples break, separating the DNA into single strands.
- **Annealing**: DNA primers and DNA polymerase enzymes bind to single stranded DNAs.
- **Extension**: New complementary strand of DNA is formed (new duplicate). DNA sequence is doubled each time PCR is conducted.

First Primer PCR Test:

	1	2	3	4	5	6	7	8	9	10	11	12
A	BCD	241	314	339	357	404	415	427	443	453	482	491
B	Prov	254	316	344	359	406	416	428	444	454	483	492
C	203	257	325	351	362	408	417	431	445	456	484	493
D	204	258	328	352	382	409	418	432	446	457	485	494
E	205	260	330	353	399	410	420	433	447	461	487	495
F	207	271	331	354	400	411	421	437	448	479	488	497
G	209	280	333	355	402	412	423	438	449	480	489	499
H	240	313	334	356	403	414	424	439	452	481	490	500



Group One (RED): 45% vs. Group Two (GREEN): 49%

Future Directions (Genotyping Project)

- PCR with more parent-specific markers
- GBS (Genotyping By Sequencing) to get SNP (Single Nucleotide Polymorphism) information

Future Research

Germplasm

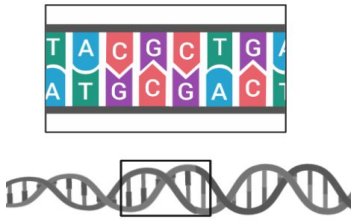
Bridging From Genome To Phenome

Functional Validation

Mapping population

*Bentgrass hybrid population generated by Dr. Warnke

Genotyping



- Target regions associated with dollar spot resistance and drought stress

Phenotyping

- Visible phenotypes - automated raspberry pi
- Invisible phenotypes - hyperspectral imaging system

Gene editing

*CRISPR-Cas9 system

Breeding for Superior Turf Lines

Special Thanks To

Scott Warnke
Jin Barnaby
Josh Marvel
Margaret Pooler
Kevin Conrad
Scott Aker

Christine Moore, Fran Kolpack, Jamie Byers, Stef Washington

Thank you for guiding us and providing us with this unforgettable experience.