Understanding the Genes in SC/SI Individuals of *Physalis acutifolia*

Transcriptome of *Physalis acutifolia*

Purpose: To have a published transcriptome (which currently doesn't exist), to be able to untangle pleiotropic genes (Mulato-Brito, 2007), and sort out housekeeping genes.

Approach:

1. RNA extraction of SC individual of leaf, fruit, flower, root, and bud.
2. Sequence this in the Nanopore with a coverage of 80x.
    a. This would require 1 flow cell for all 6 samples.

Genome size ~3.5 Gb.
Estimate transcriptome to be 1% of that (0.035 GB x 80 = 2.8 Gb) for one tissue. 6 different tissues at ~16.8 Gb.
Nanopore ranges from 10-20 Gb.

1. De Novo Assembly
    a. Steps below
2. Annotation of transcriptome
3. Potentially publish at: ???

Differentially Expressed Genes (DEG) between SC/SI *Physalis acutifolia*

Purpose: To understand the genes that regulate SI within this species.

Approach:

1. RNA extraction of unpollinated styles.
    a. Practice with 2 samples. 1 SC and I SI of one individual with 5 styles pooled.
        i. Optimize to obtain good yield.
    b. Do 2 samples with pollinated SC/SI and compare with unpollinated.
        i. After talking with Pat she said there wasn't much difference with pollinated and unpollinated styles.

Reevaluate methods

2. Run Scotty - Power Analysis for RNA Seq Experiments with the samples to reevaluated number of samples needed.
    a. Continue with 3 populations (1 SC and 2 SI). 3 individual plants with 5 styles pooled together.

*Evaluate data and revise methods if needed.

    a. Finish with the next 7 populations -- 3 individual plants with 5 styles pooled together.

Total Extractions: 32 extractions

3. De Novo Assembly
4. Determine DEG
5. qPCR on DEG
5. Potentially publish results at: AJB, Genetics

Sequencing Protocol

1. Extract RNA: Qiagen Plant RNEasy Kit
    a. Qiagen DNA Cleanup during extraction
2. Check Quality:
    a. Nanodrop for quality
    b. Bioanalyzer
3. Clean-up RNA: NEBNext Ploy(A) mRNA Magnetic Isolation Module
4. cDNA Synthesis: Native Barcoding Expansion 1-12 (PCR-free) and add in oligo(dT)/Random hexamer primers
5. Library prep and sequencing: Oxford Nanpore protocols and sequence using MinION SpotON Flow Cell

Computational Protocol

1. Sequence Processing: Basecalling using Albacore (Nanopore tech.)
2. Demultiplexing using porechop
3. DeNovo Assembling: BWA, SAMtools, SOAPdenovo-Trans
4. Determine read depth: External RNA Controls Consortium RNA Spike-In Mix
5. Transcript analysis: PANTHER Overrepresentation with Gene Ontology database
6. DEseq R to determine DEG

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1. With DEG do qPCR

Cost Breakdown

NEBNext Poly(A) mRNA magnetic isolation (24 samples)
$67 x 2 = $134

Nanopore Kit
$1000.00 x 2 = $2000.00

SuperScript IV (50 reactions)
$488.00

PerfeCTa SYBR® Green SuperMix
$194.60

Gene Specific Primers (~10 genes including control primers)
$150.00

Shipping Cost
$150

Total: $3116.60