

The Daffodil DNA Project Overview

The YouTube Video Playlist can be viewed at

<https://www.youtube.com/playlist?list=PLTGQIG5a-YcchKzulfECjNp059q8qeIOC>

STEP 1. DNA Extraction

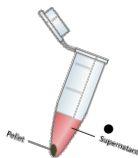
Preparing the plant sample



- We will freeze some of the leaves of the daffodil overnight, and then grind the leaf material using mortar and pestle. This mechanically breaks open our daffodil plant cells releasing nuclei (chromosome containing organelles).
- We will add our leaf material to an Eppendorf tube along with **AP1 lysis buffer** (an additional step to 'chemically' break open the cells) and **RNAase** (to remove RNA). This is mixed with the vortex and incubated in a water bath to produce a lysate (plant cell smoothie!)

Getting the DNA and removing the stuff we don't want.

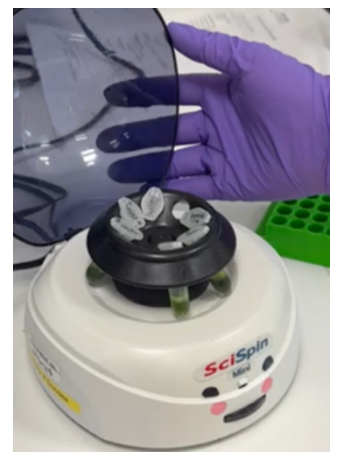
- **Precipitating unwanted cell stuff (P3)** - To our lysate we will add P3 buffer which precipitates proteins and polysaccharides we don't want, then centrifuge. The pellet contains cellular components we don't want and so we remove the supernatant using a pipette.



- **Separating unwanted stuff (spin column)** - We put the supernatant into the purple (QIAshredder) spin column and centrifuge. A filter in the spin column catches everything else we don't want and leaves our DNA in the bottom of the collection tube.

- **Cleaning our DNA (AW1 & AW2 buffer and new spin column)** We take our DNA, mix this with AW1 buffer, add to a the clear (DNeasy Mini) spin column and centrifuge. This time the DNA sticks to the filter and we dispose of the liquid in the catch tube. An additional rinse using AW2 cleans the DNA.

- **Releasing the DNA (AE buffer)** - Lastly, we add AE Elution buffer to very center of the filter to release DNA. After a short incubation at room temperature we centrifuge, then discard the spin column. Yay! We have DNA!



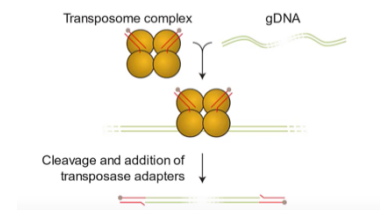
STEP 2. Choosing the Best DNA

- We will use gel electrophoresis to identify which group has extracted the most, and cleanest DNA... good luck!

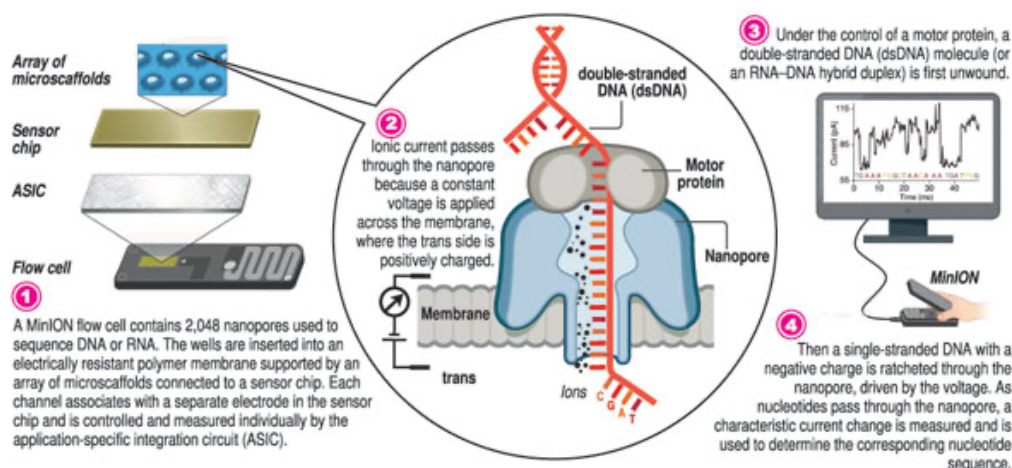
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STEP 3. DNA Library Preparation

- We need to cut the DNA into small fragments, then attach additional smaller pieces of DNA (adapters) onto the end of our fragments. Another set of sequencing adapters are then added to our DNA fragments. These will help our fragments pass through the nanopores used to sequence the DNA.
- **Preparing the reagents** - Some of the reagents have been kept in the freezer. These need to be thawed, briefly centrifuged to push everything to the bottom of the tube, mixed by pipetting up and down and then stored on ice.
- **DNA Cutting and adapter attachment** - We add daffodil DNA and fragmentation mix (FRA) to a PCR tube, then place it into a thermocycler. Enzymes activate at different temperatures to cut the DNA and add primer out first set of adapters.
- **Sequencing adapter attachment** - The PCR tube is removed, cooled, and the rapid adapters reagent (RAP) is added to attach the sequencing adapters. The reaction incubated at room temperature until ready to load.



STEP 4. DNA Sequencing using MinION Oxford Nanopore Technology



- **Preparing the Minion** – The MinION is the DNA sequencer. A flow cell is inserted into the MinION which contains the nanopores.
- **DNA Library preparation** – The DNA library is prepared by mixing the best DNA sample with buffers, loading beads and water.
- **Loading the Flow Cell** - the Flow Cell is loaded with DNA.
- **Sequencing** – The Minion is connected to the computer and sequencing is launched using MinKNOW software. This will take several days!

STEP 5. Base Calling using Guppy

Code used in the video: "C:\Program Files\OxfordNanopore\ont-guppy-cpu\bin\guppy_basecaller.exe" --input_path C:\Users\Science\Documents\30MAR22FURNESS\30mar22furness\30mar22furness\20220330_1607_MN32710_FLG001_9a1e9e66\fast5 --save_path C:\Users\Science\Documents\30MAR22FURNESS\30mar22furness\30mar22furness\30MAR22Furness --flowcell FLO-FLG001 --kit SQK-RAD004

However if you are importing the files into a folder such as "test", you would need to locate this folder within the directory (eg Users\Science\Documents may need to change): "C:\Program Files\OxfordNanopore\ont-guppy-cpu\bin\guppy_basecaller.exe" --input_path C:\Users\Science\Documents\test --save_path C:\Users\Science\Documents\test\basecalled --flowcell FLO-FLG001 --kit SQK-RAD004

Guppy program can be downloaded from community section at:

<https://nanoporetech.com/community>

STEP 6. Assembling the DNA sequence with Geneious Prime

Geneious Prime program can be downloaded at:

<https://www.geneious.com/download>

STEP 7. Viewing and Analyzing the DNA Sequence in Jalview

Jalview is one of the most widely used programs for visualizing and analyzing DNA, RNA and protein multiple sequence alignments. It uses an interactive multi-window interface for viewing sequences, trees, and three-dimensional structures. It has a number of analysis tools for aligning sequences, measuring similarities and comparing structures.

Jalview can be downloaded at: <https://www.jalview.org/download>

For help getting started using Jalview please visit the Jalview website:

<https://www.jalview.org/help/getting-started>

<https://www.jalview.org/training/training-videos>

- **Jalview Video: Trimming the Sequence Assembly using Jalview**

The sequences used in this video can be accessed from:

<https://www.ncbi.nlm.nih.gov/nuccore/MH706763>

- **Jalview Video: Reviewing the Assembled Sequences using Jalview**

- **Jalview Video: Create a Tree of Daffodil Chloroplast DNA Sequences**

The sequences used in this video can be accessed from:

<https://www.ncbi.nlm.nih.gov/nuccore/MH706763>

<https://dag.compbio.dundee.ac.uk/daffodils>

STEP 8. Viewing the Daffodil Chloroplast Genome using OGDRAW

OGDRAW web page can be accessed at: [https://chlorobox.mpimp-](https://chlorobox.mpimp-golm.mpg.de/OGDraw.html)

[golm.mpg.de/OGDraw.html](https://chlorobox.mpimp-golm.mpg.de/OGDraw.html)