

# Tissue Collection and PCR

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## 1. Collect plant tissue

- a. Cut about 4cm leaf tissue with bleach-rinsed scissors
- b. Put tissue in one cell of Qiagen tissue collection plate (kept on ice)
- c. Rinse scissors with bleach, then water
- d. Leave 6 blanks in the collection plate as neg controls
- e. Cap collection plate and record sample ID addresses

## 2. Extract tissue

- a. Use Qiagen 96 Plant Extraction kit
- b. Cut dry or fresh tissue into tubes – use about 5-6cm of grass leaf and cut it into pieces around 1cm long
- c. Be careful not to cross-contaminate samples when putting on and taking off lids

## 3. Quantify tissue

- a. Run samples on a 2% agarose gel with “PCR Markers” ladder to estimate concentration
- b. Bring all samples to about 200ng/ul with ddH<sub>2</sub>O

## 4. PCR samples

- a. Set up a master mix containing primers, polymerase, and template:

<u>1x Reaction</u>	<u>Primer Mix (400uL)</u>
0.75uL primer mix	10uL Forward (100uM)
3.75uL HotStarTaq	9uL Reverse (100uM)
0.75uL DNA	1uL Fluor Reverse (100uM)
2.25uL ddH <sub>2</sub> O	380uL ddH <sub>2</sub> O

- b. Run PCR reaction on thermal cycler:

95°C 15min (HotStarTaq activation step)

- a. 95°C 45sec (denaturing)
- b. 60°C 1min (annealing)
- c. 72°C 1min (extension)

go to step ‘a’ 34 times

72°C 10min (final extension)

## 5. Run samples on ABI 310

- a. Melt 1 aliquot of HiDi Formamide (DMF, need 12uL per sample)
  - i. HiDi Formamide degrades quickly to formic acid, so must be stored at -20 until ready to use. It is aliquotted for 48rxns.
- b. Keep internal lane standard (ROX-500) on ice
  - i. Dilute enough ROX500 for your run 1:1 with ddH<sub>2</sub>O
- c. Add 12uL DMF and 1uL ROX-500 to each tube
- d. Denature on thermal cycler at 95°C for 5min, then store on ice
- e. Set up GeneScan 96well Sample Sheet
- f. Set gel temp to 60°C
- g. Set up GeneScan 96well Injection List

- h. Run samples
- i. (for more detailed instructions for the ABI310, see ABI310 Fragment Analysis Protocol)