PHIRE direct PCR genotyping protocol for 96 plants x 2 flanking markers

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Read the Finnzyme instruction manual for the Phire kit first.

We typically design PCR primers with a length of 30nt, to deal with the high annealing temp during PCR.

1. Thaw 2x Phire buffer and forward/reverse (F/R) primer mix solutions (5µM of each primer). For **each** F/R primer mix, label a 1.7ml master mix tube and make a master PCR mix according to the following table. Add reagents in the order shown.

	1 column of a 384-well plate, 10µl rxn	**12 columns of a 384-well plate, 10µl rxn**
MilliQ water	44µl	**484µl**
2x Phire buffer	50µl	**550µl**
F/R primer mix	4µl	**44µl**
Phire polymerase	2µl	**22µl**
TOTAL	100µl	**1100µl**

2. After all reagents are added, mix gently by pipetting up and down a few times.

3. Using a repeating pipet, dispense 10µl of each master mix into a 384-well microtiter plate according to the following diagram. For each flat of 96 plants, only 12 of the 24 columns will be used.

			1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
plant A	**Marker 1**	**A**																								
	Marker 2	**B**																								
plant B	**Marker 1**	**C**																								
	Marker 2	**D**																								
plant C	**Marker 1**	**E**																								
	Marker 2	**F**																								
plant D	**Marker 1**	**G**																								
	Marker 2	**H**																								
plant E	**Marker 1**	**I**																								
	Marker 2	**J**																								
plant F	**Marker 1**	**K**																								
	Marker 2	**L**																								
plant G	**Marker 1**	**M**																								
	Marker 2	**N**																								
plant H	**Marker 1**	**O**																								
	Marker 2	**P**																								

4. Using scissors, remove half of a young leaf from the first plant to be genotyped (typically, plant A for any given column). Using a **clean** 0.5mm Harris Uni-Core punch and a **clean portion** of cutting mat, collect one leaf disk and eject it into Row A under the appropriate column. Collect a second leaf disk and eject it into Row B. Verify that the disks are in the solution and not stuck to the side of the well, or missing.

5. Place the used punch in a rack in a container with 2% bleach of sufficient depth to just cover the steel tips of the punch.

6. Use a clean punch and a clean portion of cutting mat for each additional sample.

7. When all leaf disks have been collected, put the 384-well plate into a thermocycler. Cover the plate with a FRESH single sheet of plate sealer (clear vinyl from a fabric store, cut to size, is the cheapest). Close the lid on the thermocycler.

8. Run the “phire” PCR program:

1 cycle of 98C x 5min
40 cycles of 98C x 5sec, 58C x 5sec, 72C x 20sec
1 cycle of 72C x 1min

The whole program takes just over an hour to complete. After PCR the plate may be stored at 4C for several days if necessary.