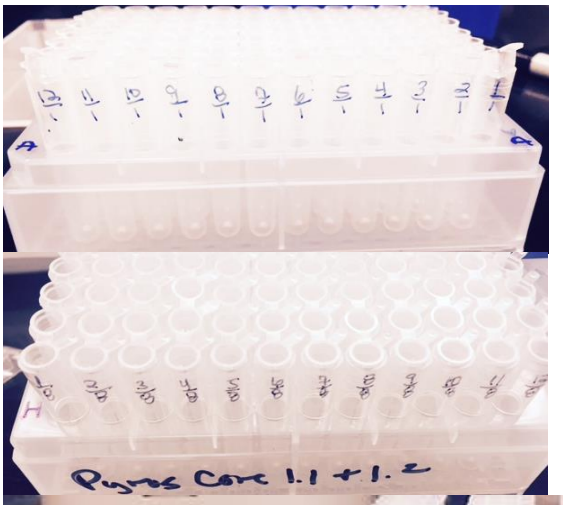
Silica Tissue Collection Protocol for Bassil Lab

I. Materials Needed

1. 96-Well Polypropylene Tubes Racked, Corning® 8-Tubes strips (12/rack)
   VWR# 29442-612
2. Corning® 8-Cap Strips, Sterile Caps for cluster tubes
   VWR# 29442-614
3. Standard 96-well PCR plate
   (We use Semi-Skirted 96-well PCR plate BioExpress# T-3085-1)
4. 6-12 mesh standard silica granules
   VWR Cat# AA44387-A1

II. Before Collecting

1. **Label cluster tube racks**
   Label position A1-A12 of the 8 strip cluster tubes in the 96 well rack 1/1, 2/1, 3/1….12/1.
   Label position H1-H12 of the tubes 1/8,2/8,3/8….12/8.



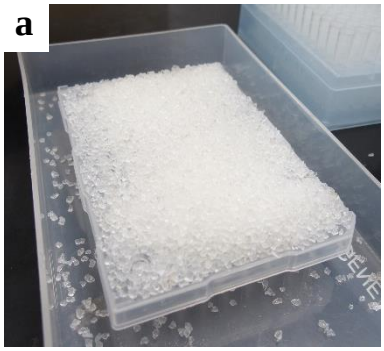
a. You will need two cluster tube rack boxes per 96 samples to collect.
   - Use black sharpie to label the first box with the collection name and **Box 1.1**
   - The duplicate box should be labeled using blue sharpie and designated **Box 1.2**

If you are collecting 192 samples, the first 96 samples rack is box 1.1 and its duplicate rack is box 1.2; the second 96 samples rack will be labeled box 2.1 and its duplicate rack is box 2.2.

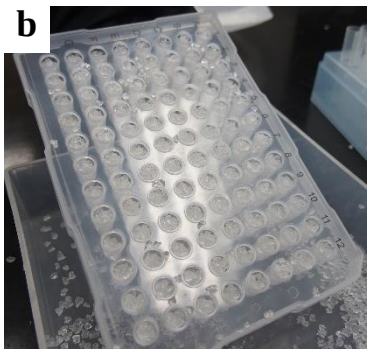
For ease of collecting, we pair the duplicate columns next to each other in a rack

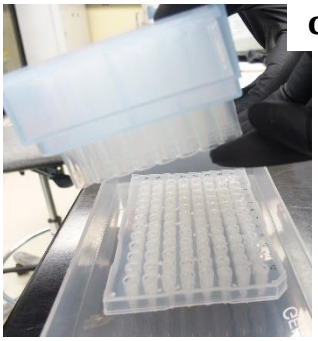
2. **Fill cluster tubes with plain 6-12 mesh silica gel.**
(Video demonstration**:** https://youtu.be/tdiqguIZ9Hg)
    a. Take a standard 96-well PCR plate and fill the wells with 6-12 mesh.
    b. Brush off excess silica.
    c. Then place 1.1 ml strip tubes in their rack over the PCR plate aligning each of the 1.1-ml collection tubes over the wells of the PCR plate.

a



b



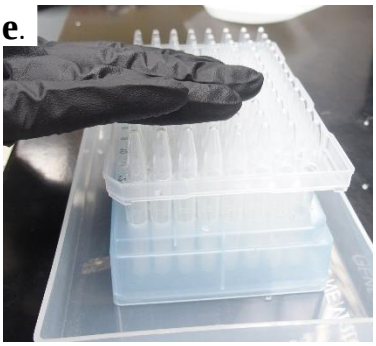


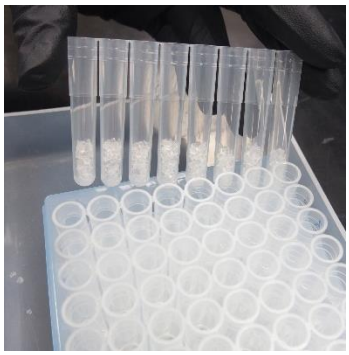
c

    d. Gripping firmly on both sides of the sandwhiched rack and PCR plate, we flip these so that the silica falls into the collection tubes.
    e. Tap the bottom of the PCR plate to encourage trapped silica to fall into the cluster tubes.

d

e.





3. **Cap the collection tubes** with 8-strip caps to prevent contamination and moisture entering wells.

Note: Only uncap the cluster tubes you are in the process of sampling into in order to prevent the silica gel from absorbing moisture from the atmosphere and losing its desiccating function. You also want to avoid adding extra moisture to the tubes so if the leaves are wet pat them dry with a paper towel before putting them in the well.

4. **Prepare Collection sheet (list and map)**

   a. **Electronic Sample List**

   We will need you to send us a detailed paper and electronic spreadsheet of the samples collected and shipped. Please include: inventory number, name, location collected from, species and any pedigree information available.

   **Excel Template is attached to the email*

Example:

tissue plate ID/#	Well	NCGR local #	plant name	fld. Loc	taxon	pedigree
NCGR pyrus 1.1, 1.2	A1	1097.001	Harvest Queen	PF31-05	Pyrus communis L.	Bartlett x (Barseck x Bartlett)
NCGR pyrus 1.1, 1.2	B1	112.001	Butirra di Roma	PF07-25	Pyrus communis L.	Butirra Clairgeau x Williams (magnetic pollen)
NCGR pyrus 1.1, 1.2	C1	206.001	Eletta Morettini	PF04-23	Pyrus communis L.	Butirra Hardy x Passe Crassane
NCGR pyrus 1.1, 1.2	D1	26.001	Ayer	PF51-13	Pyrus communis L.	Chance seedling
NCGR pyrus 1.1, 1.2	E1	313.001	Kalle	PF09-21	Pyrus communis L.	Clapp Favorite mutant
NCGR pyrus 1.1, 1.2	F1	1104.002	Jubileer D'Ar	PF28-03	Pyrus communis L.	Clapp Favorite x Klementina
NCGR pyrus 1.1, 1.2	G1	482.002	Reimer Red	PF48-13	Pyrus communis L.	Comice x Max Red Bartlett
NCGR pyrus 1.1, 1.2	H1	25.001	Aurora	PF01-23	Pyrus communis L.	Margherite Marillat x Bartlett
NCGR pyrus 1.1, 1.2	A2	709.001	OHxF 51	PF14-39	Pyrus communis L.	Old Home x Farmingdale seedling selection
NCGR pyrus 1.1, 1.2	B2	103.001	Brandy	PF07-27	Pyrus communis L.	Parentage unknown
NCGR pyrus 1.1, 1.2	C2	217.004	Eureka	PF24-09	Pyrus hybrid	Seckel x Kieffer
NCGR pyrus 1.1, 1.2	D2	1631.001	Clapp Favorite	PF16-15	Pyrus communis L.	Flemish Beauty x Bartlett

   b. **Electronic Collection Plate Map**

   We create a plate map of the tissue to collect prior to collecting, but you can print a blank collection map and fill in the information as you collect.

   i. Please collect tissue starting from the A well of a column and moving down to the H well; return to the top of the next column and move down again.

   ii. Leave well H12 blank.

   Note: Collection sheet of individuals from a population has a different plate map.

Date Col.			Cluster tube Box ID:								TC Col.Box 1.1-1.2
Collected By:				Genus:				Sample Prep: Silica Desication of Tissue			
1	9	17	25	33	41	49	57	65	73	81	89
A1 S1	A2 S9	A3	A4	A5	A6	A7	A8	A9	A10	A11	A12
2	10	18	26	34	42	50	58	66	74	82	90
B1 S2	B2 S10	B3	B4	B5	B6	B7	B8	B9	B10	B11	B12
3	11	19	27	35	43	51	59	67	75	83	91
C1 S3	C2 S11	C3	C4	C5	C6	C7	C8	C9	C10	C11	C12
4	12	20	28	36	44	52	60	68	76	84	92
D1 S4	D2 S12	D3	D4	D5	D6	D7	D8	D9	D10	D11	D12
5	13	21	29	37	45	53	61	69	77	85	93
E1 S5	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
6	14	22	30	38	46	54	62	70	78	86	94
F1 S6	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
7	15	23	31	39	47	55	63	71	79	87	95
G1 S7	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
8	16	24	32	40	48	56	64	72	80	88	96
H1 S8	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	H12 EMPTY

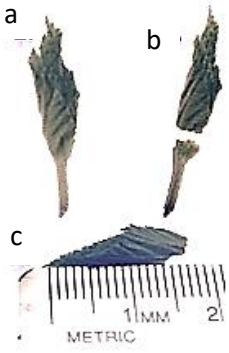
III. Collecting

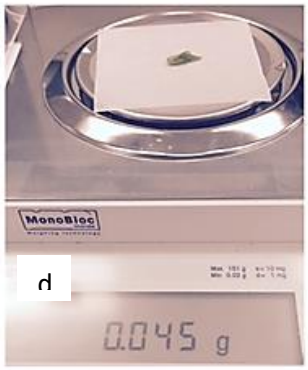
1. Morning hours are the best times to collect especially if the day is going to be hot.
   Remember
   a. Double check that you are sampling from the correct plant (we always send two people for collecting) and if you are recording as you go write down the plant information in the plate map before you collect to avoid mistakes.
   b. Keep lids on cluster tubes when not sampling and close the tubes as soon as you finish sampling a column to keep the silica from absorbing excess moisture.
   c. Pat dry wet leaves before adding them to the tube.
2. Collect the youngest actively expanding leaf tissue possible (unopened leaves are best, example below)

a. Actively growing leaf.
b. Remove stem
c. ~2 mm length, but it will also depend on how thick the leaf is.
d. We suggest weighing a few different types of leaves to get an idea of what 30-50mg of tissue looks like.

✱ NO MORE THAN 50 mg✱

We will be using filter based DNA extraction Kit (101318-938 Omega E-Z 96® Plant DNA Kit) so if there is too much material it will clog the filter.





IV. Shipping

1. Ship samples on **Monday, Tuesday or Wednesday** so that the samples arrive when we are open.

   Shipping Address:
   (removed since they are not service providers)

2. **We must be expecting your samples**- Please email us the tracking number for your package and the 'Bassil Electronic Tissue Collection Sheet' (list and map).