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Flushing plant samples in order to determine maximum hydraulic conductivity

Materials

 = Tygon 3601 tubing (1/4 in ID, 1/16 in wall) [this composes the majority of the tubing in the system]

 = Tygon 3603 tubing (1/2 in ID, 1/8 in wall) [this composes the wider tubing that samples are loaded into with the aid of latex tubing grommets]

 = Nalgene Three-way stopcock PP/TFE 6470-0004 4mm size

 = Nalgene Two-way stopcock PP/TFE 6460-0004 4mm size

Hose clamps:
For narrow diameter tubing: Cole-Parmer Snp-4 hose clamp (06832-04)
For wider diameter tubing (areas of the system where samples are loaded): Cole-Parmer Snp-12 hose clamp (06832-12)

Plastic tubing connectors 5-1 Connectors (58018) – these are used in the system to connect the places in the conductivity and flushing manifolds where samples are usually loaded. When the system is being bleached, these connectors close the openings and allow for all of the tubing and connections to be bleached.

In-line filter Calyx Capsule Nylon, 0.1 micron, 3/8 in barb, DCN010006

Some miscellaneous sizes of latex tubing may be needed to make grommets that will fit a wide range of sample diameters.

Extra hose clamp sizes are probably needed in some parts of the system (i.e. connected to the captive air tank and the filter)—there are sets available that contain a few pieces of each of many different sizes.

We are currently using ultra-filtered (0.1 micron filter) 20mM KCl solution.

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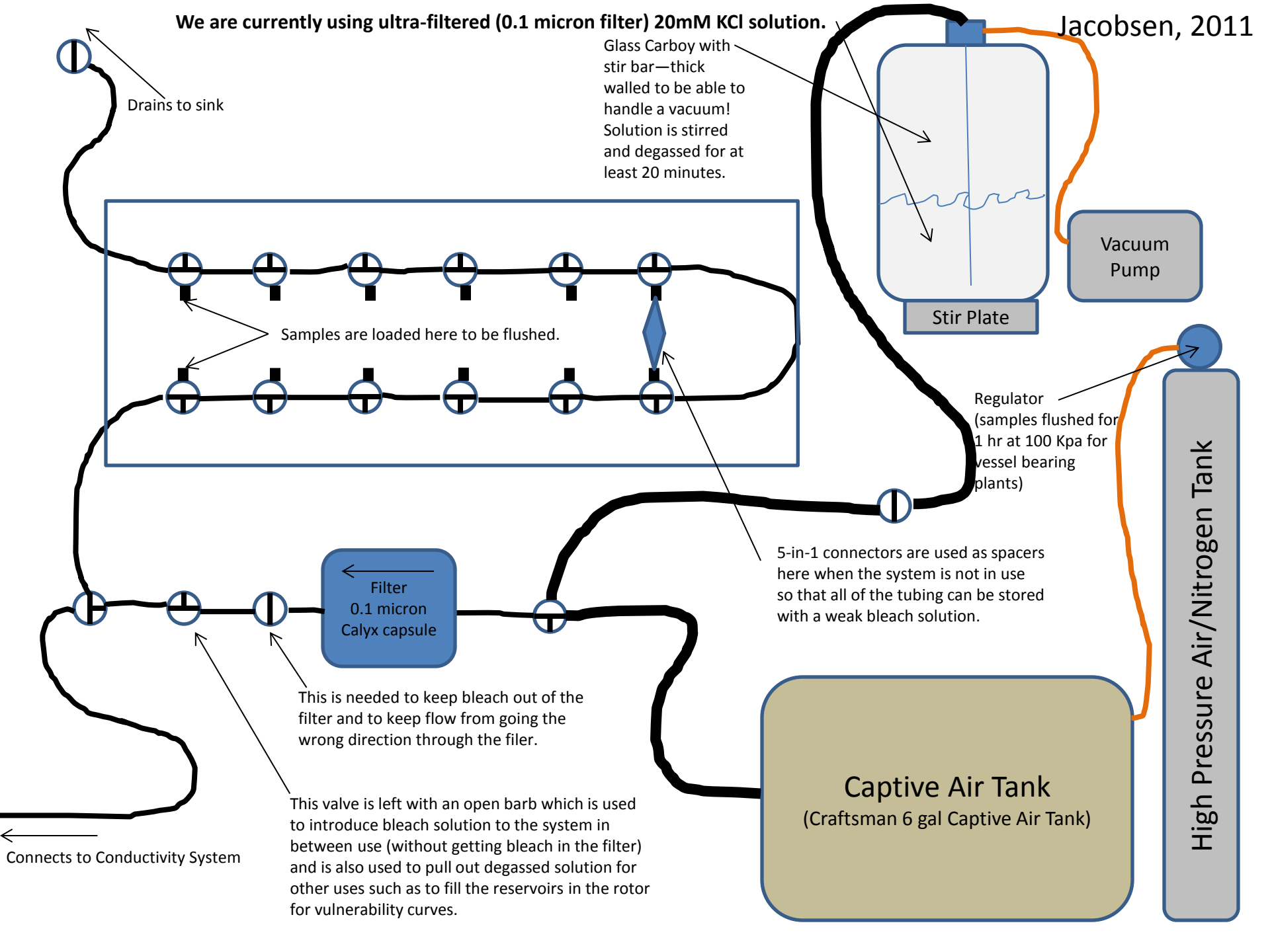

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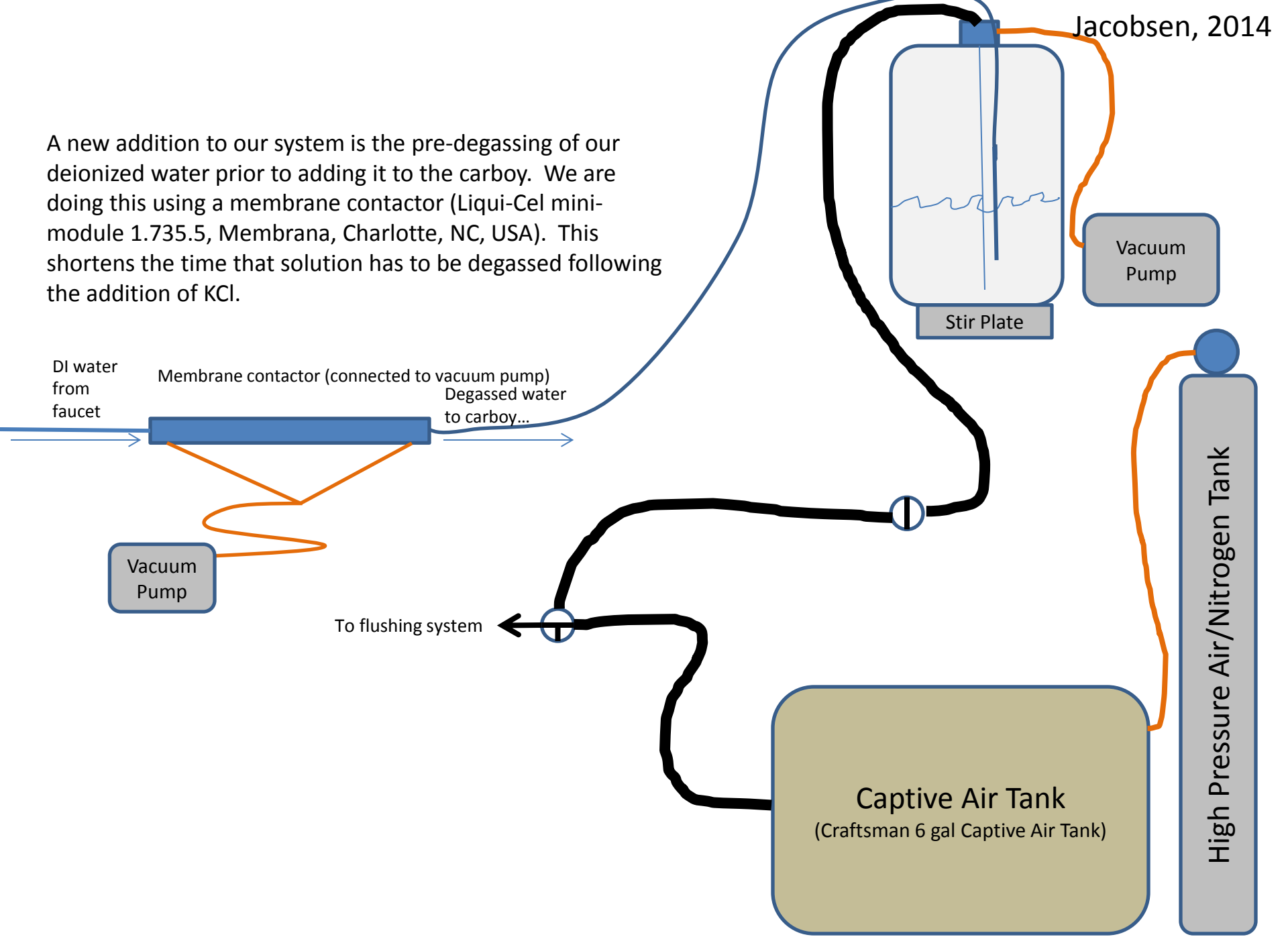
A new addition to our system is the pre-degassing of our deionized water prior to adding it to the carboy. We are doing this using a membrane contactor (Liqui-Cel mini-module 1.735.5, Membrana, Charlotte, NC, USA). This shortens the time that solution has to be degassed following the addition of KCl.

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Kimwipes
SAMCO TRANSFER PIPETS
CAT. NO. 212
QTY.
1ml
Dust Off

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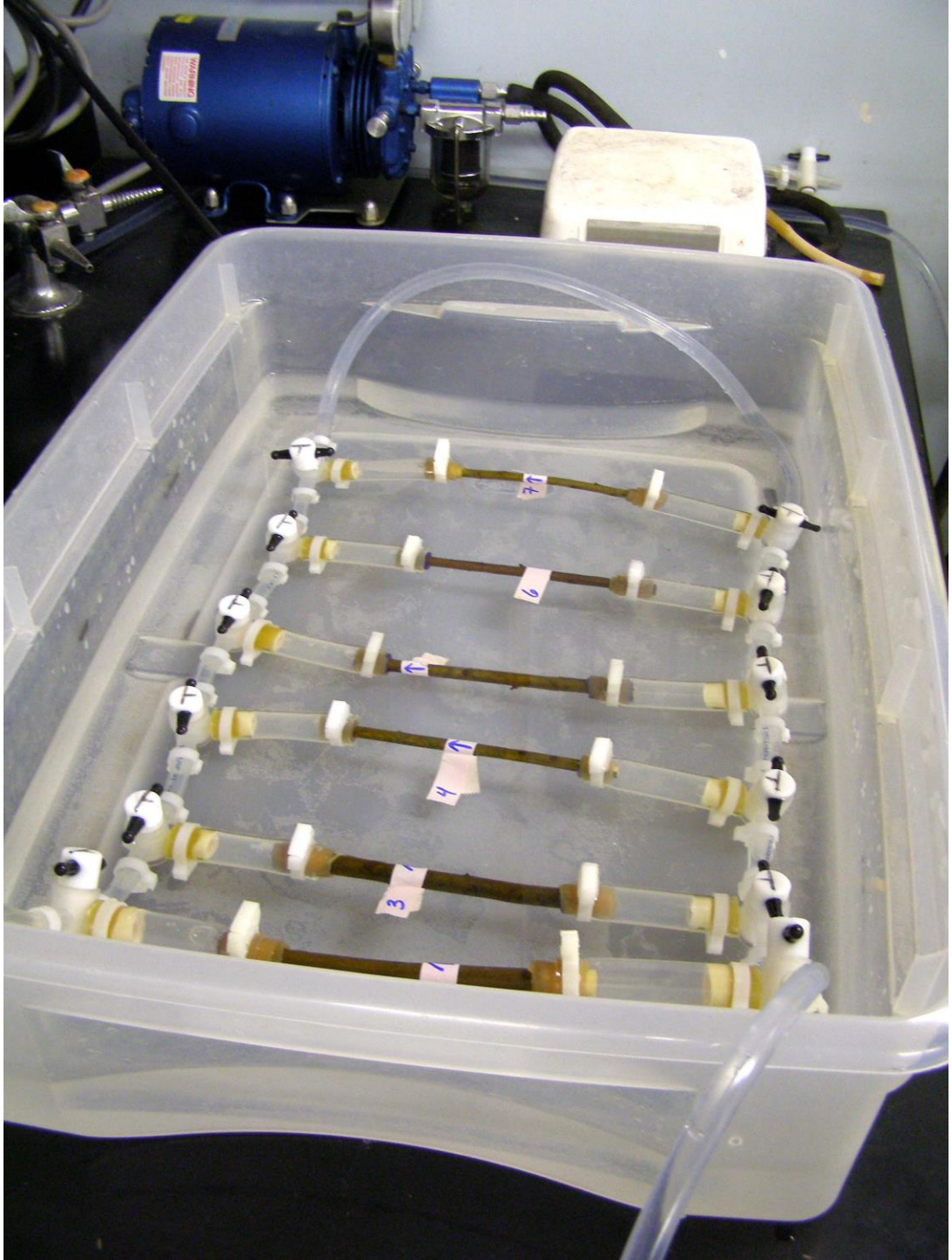

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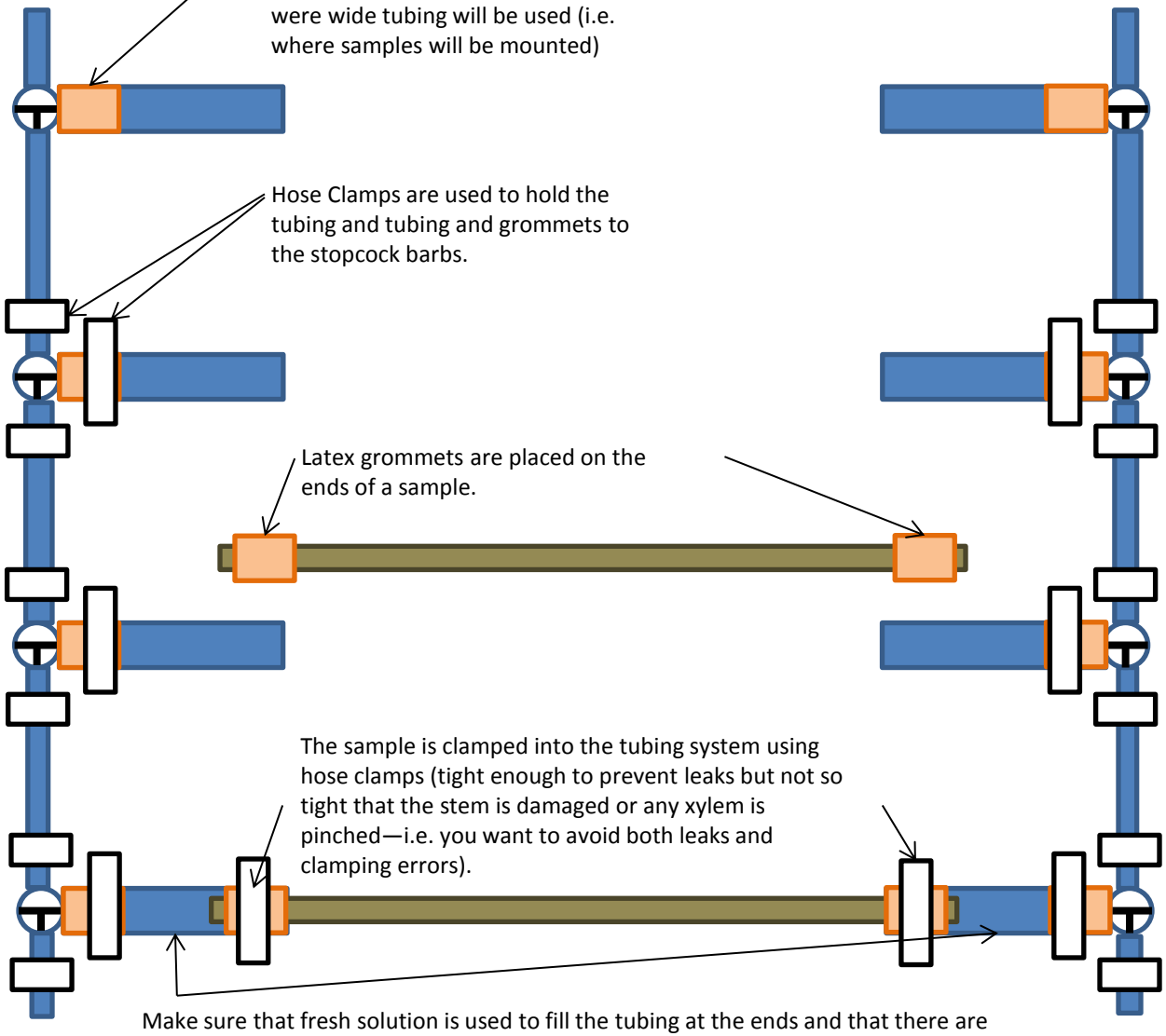
Assembling the system and loading samples:



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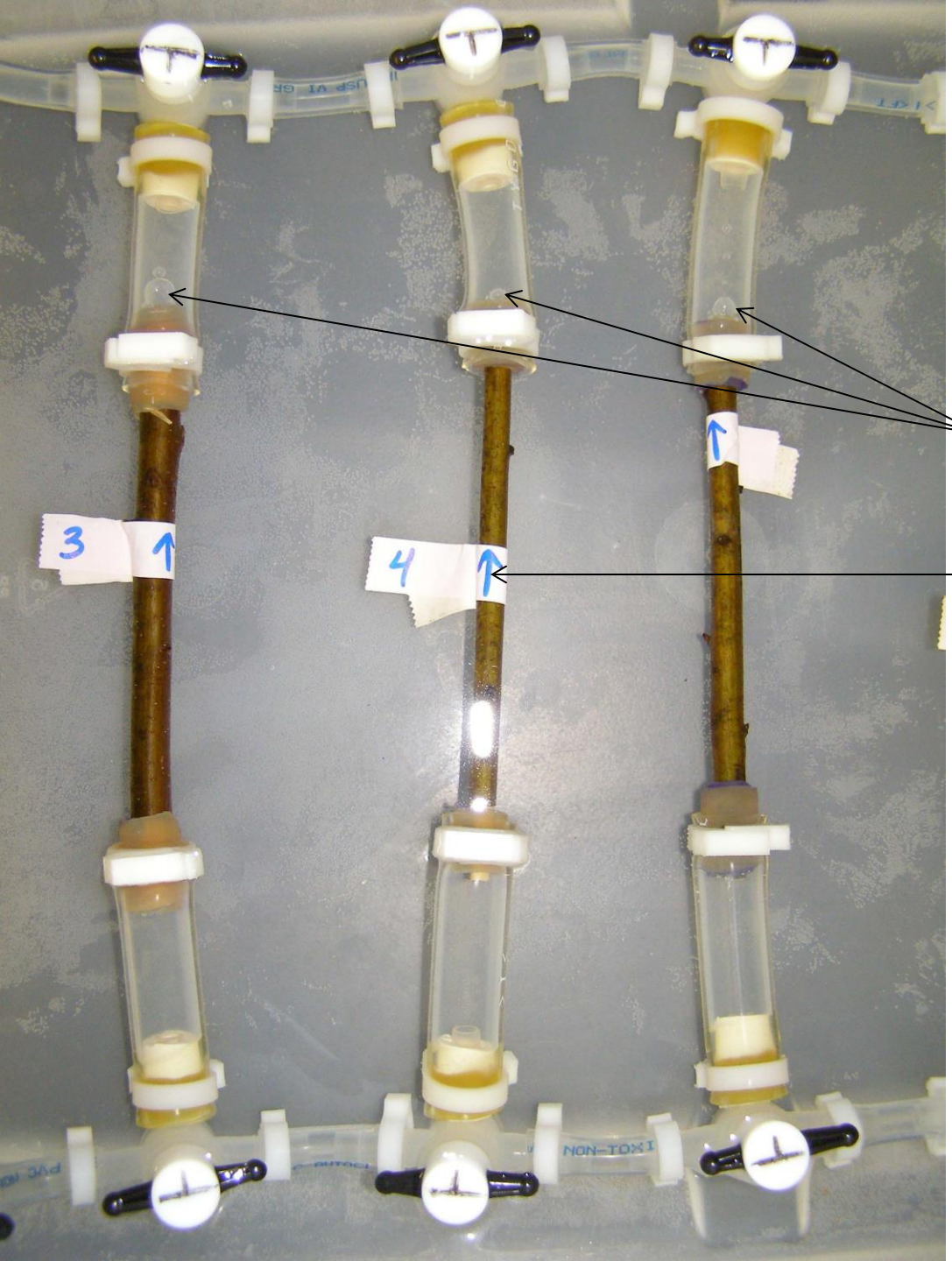

There are no air bubbles in the system when stems are loaded, but bubbles are often visible on the far end of samples during flushing as emboli are pushed out of the xylem.

Note: solution is always moved through stems in the same direction that water would move in the intact plant. Arrows are marked on samples so that we can easily keep track of the direction of flow.

We use stem and root samples that are 6-10 mm in diameter and try to get as close to 8 mm as possible. That is the sample size that fits best within this tubing system, although with different sized latex grommets there is flexibility in the system.

If samples are much larger than this size range, 3 samples will not fit in the rotor together when centrifuge-based vulnerability curves are being constructed.

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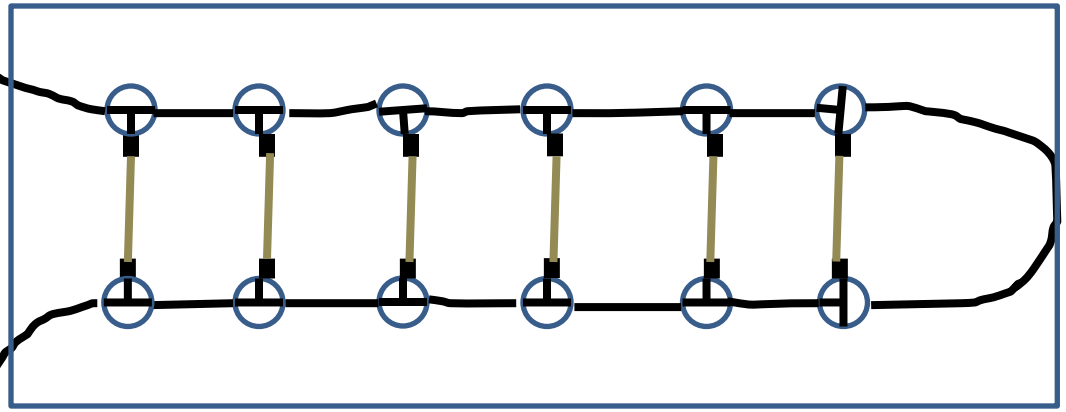
Stopcocks are shown as they would be during flushing (after solution as already been degassed and loaded into the captive air tank).
Vesselled samples are flushed for 1 hr at 100 kPa.
Samples usually reach Kmax in 20-60 min of flushing.



Stir Plate

Vacuum Pump

Filter
0.1 micron
Calyx capsule

Captive Air Tank
(Craftsman 6 gal Captive Air Tank)

High Pressure Air/Nitrogen Tank

Note: before flushing, make sure that the system has been flushed with new degassed solution, there are no bubbles in the tubing, and that the captive air tank and filter have been "burped".

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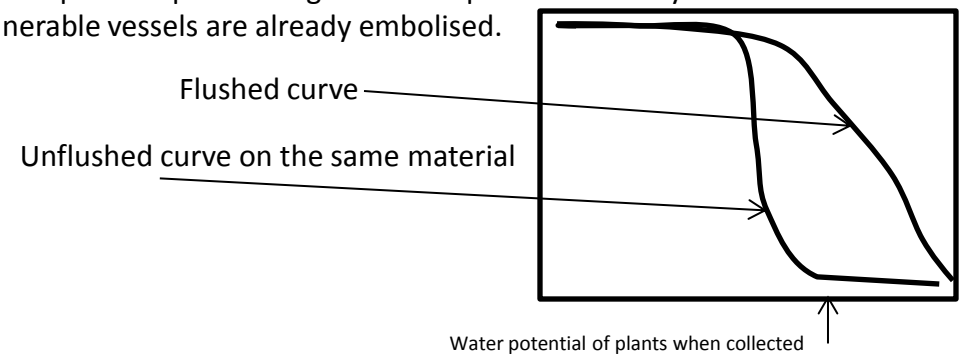
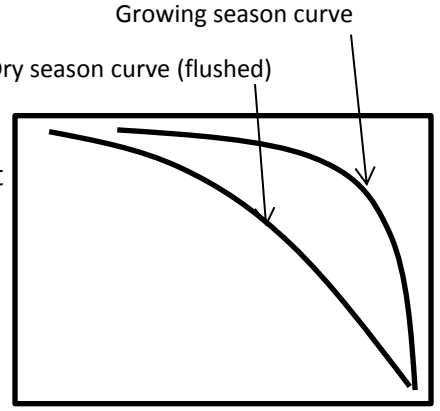
A note on flushing:

Flushing allows for you to construct an entire vulnerability curve from the "max" at 0 MPa to much more negative water potentials. If stems are not flushed, then the level of embolism in measured samples will not start to increase until the most negative water potential experienced by the xylem in the field is surpassed (assuming that water potentials have not been high enough for refilling to occur). Unflushed samples will produce sigmoidal-shaped vulnerability curves because of a delay in the increase in embolism in the stem because vulnerable vessels are already embolised.

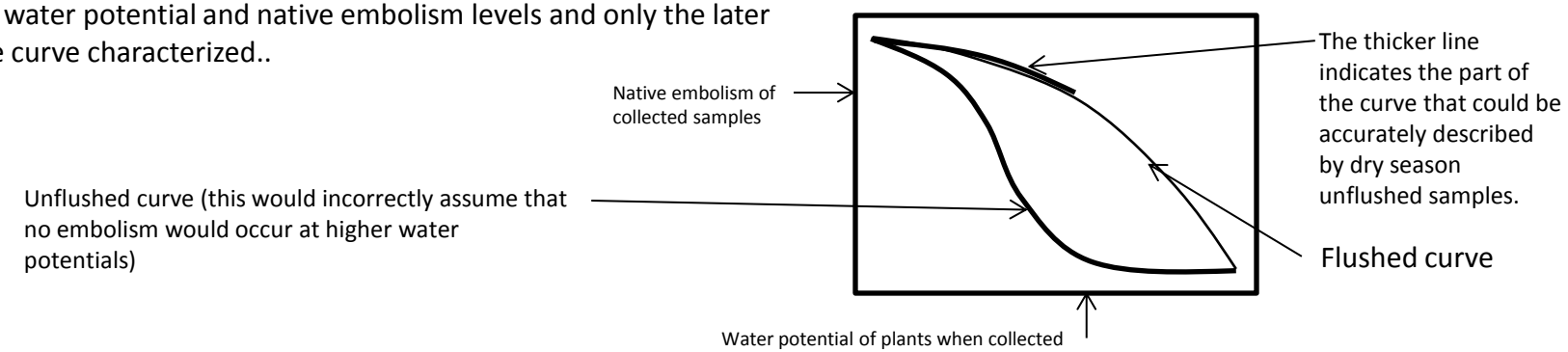


In my lab, we flush all samples prior to constructing vulnerability curves.

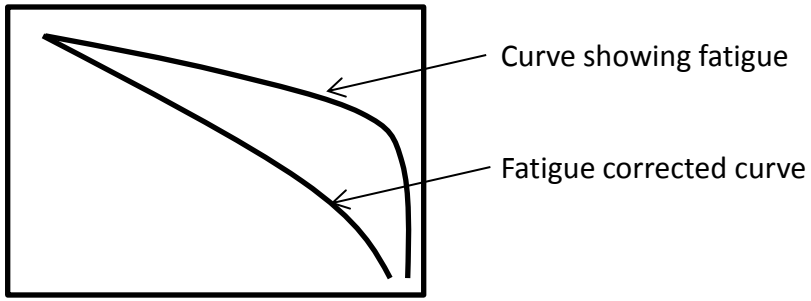
An alternative to flushing, is to collect samples when plants are hydrated and only to collect newly grown tissue. This allows you to start with near zero levels of embolism without flushing, but has the disadvantage of requiring the measurement of growing and non-hardened tissue which may be more susceptible to cavitation (cavitation resistance can change quite dramatically over the course of the growing season).



Finally, if samples are collected from plants that have already experienced drier conditions, samples must be flushed or curves must be "started" at the native water potential and native embolism levels and only the later part of the curve characterized..



Flushing may fill vessels that are damaged or would not be functional in the intact plants. These vessels embolize at very high water potentials and are termed "fatigued" vessels. Curves are sometimes "corrected" for this fatigue by using the Kh from a slightly negative water potential (-0.25 or -0.5 Mpa) as the Kmax instead of the post-flush value. This removes these highly vulnerable (and likely non-functional) vessels from the curve.



See the following paper for more information about cavitation fatigue.

Cavitation Fatigue. Embolism and Refilling Cycles Can Weaken the Cavitation Resistance of Xylem[1]

Uwe G. Hacke*, Volker Stiller, John S. Sperry, Jarmila Pittermann, and Katherine A. McCulloh
Department of Biology, University of Utah, Salt Lake City, Utah 84112

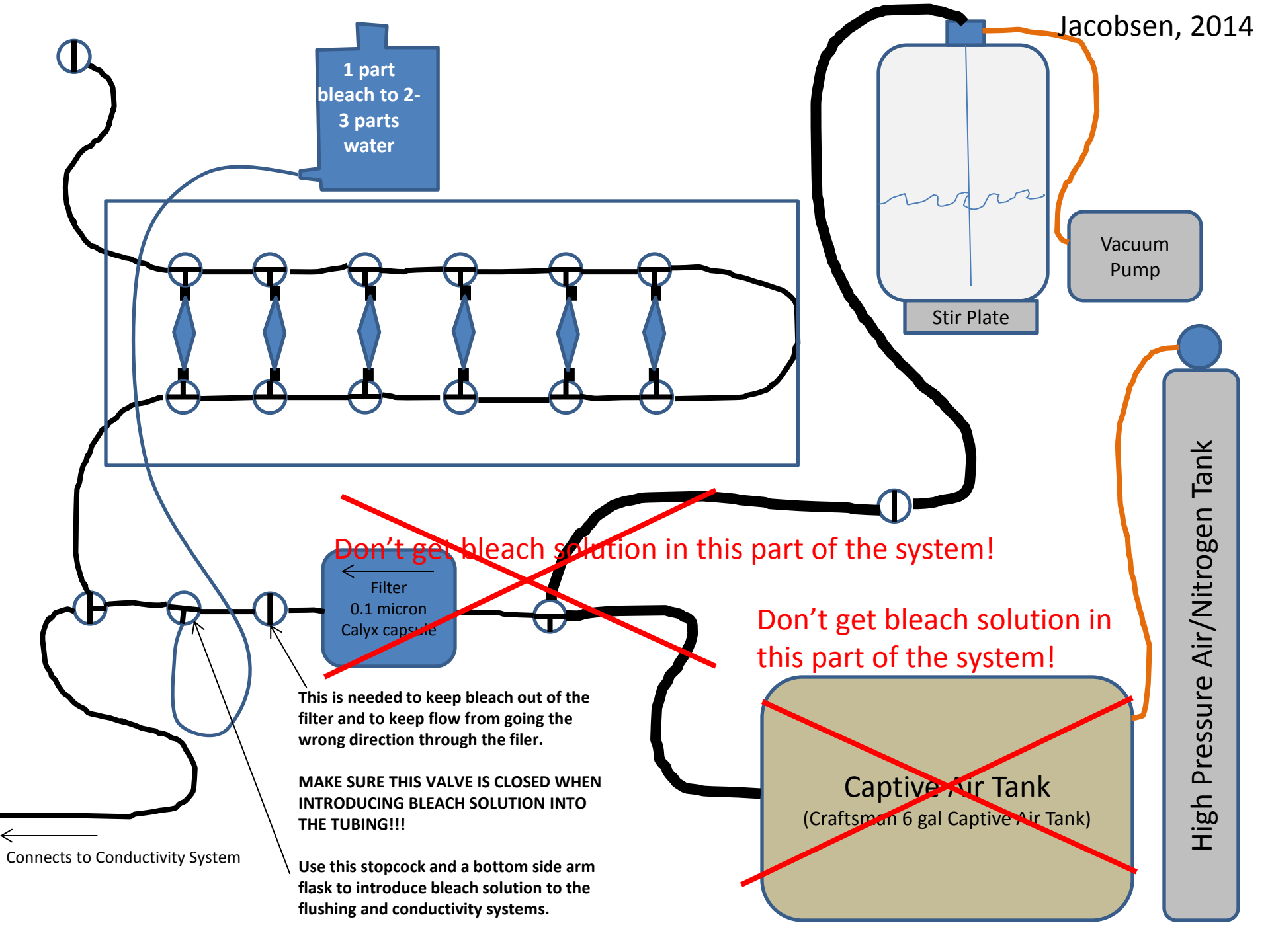
Plant Physiology, February 2001, Vol. 125, pp. 779–786, www.plantphysiol.org

Maintaining your flushing system:

Overtime, microbes are prone to building up within the tubing of the flushing system. This may lead to problems with sample clogging. We always sterilize our system at least once a week, using a dilute (1:3) bleach solution. The system is drained and the bleach solution introduced to the tubing. We let the system sit overnight with the bleach solution in the tubing before draining it, flushing it with deionized water, and then flushing it again with our degassed conductivity solution.

Check your system regularly to make sure that stopcocks are intact, latex grommets have not oxidized, and all of the hose clamps are intact and properly closed. All of these parts and sections of tubing should be replaced overtime as they break or become damaged.

Replace your filter regularly.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1 part bleach to 2-3 parts water
Vacuum Pump
Stir Plate
High Pressure Air/Nitrogen Tank
Don't get bleach solution in this part of the system!
Filter
0.1 micron
Calyx capsule
Don't get bleach solution in this part of the system!
Captive Air Tank
(Craftsman 6 gal Captive Air Tank)
This is needed to keep bleach out of the filter and to keep flow from going the wrong direction through the filer.
MAKE SURE THIS VALVE IS CLOSED WHEN INTRODUCING BLEACH SOLUTION INTO THE TUBING!!!
Use this stopcock and a bottom side arm flask to introduce bleach solution to the flushing and conductivity systems.
Connects to Conductivity System