

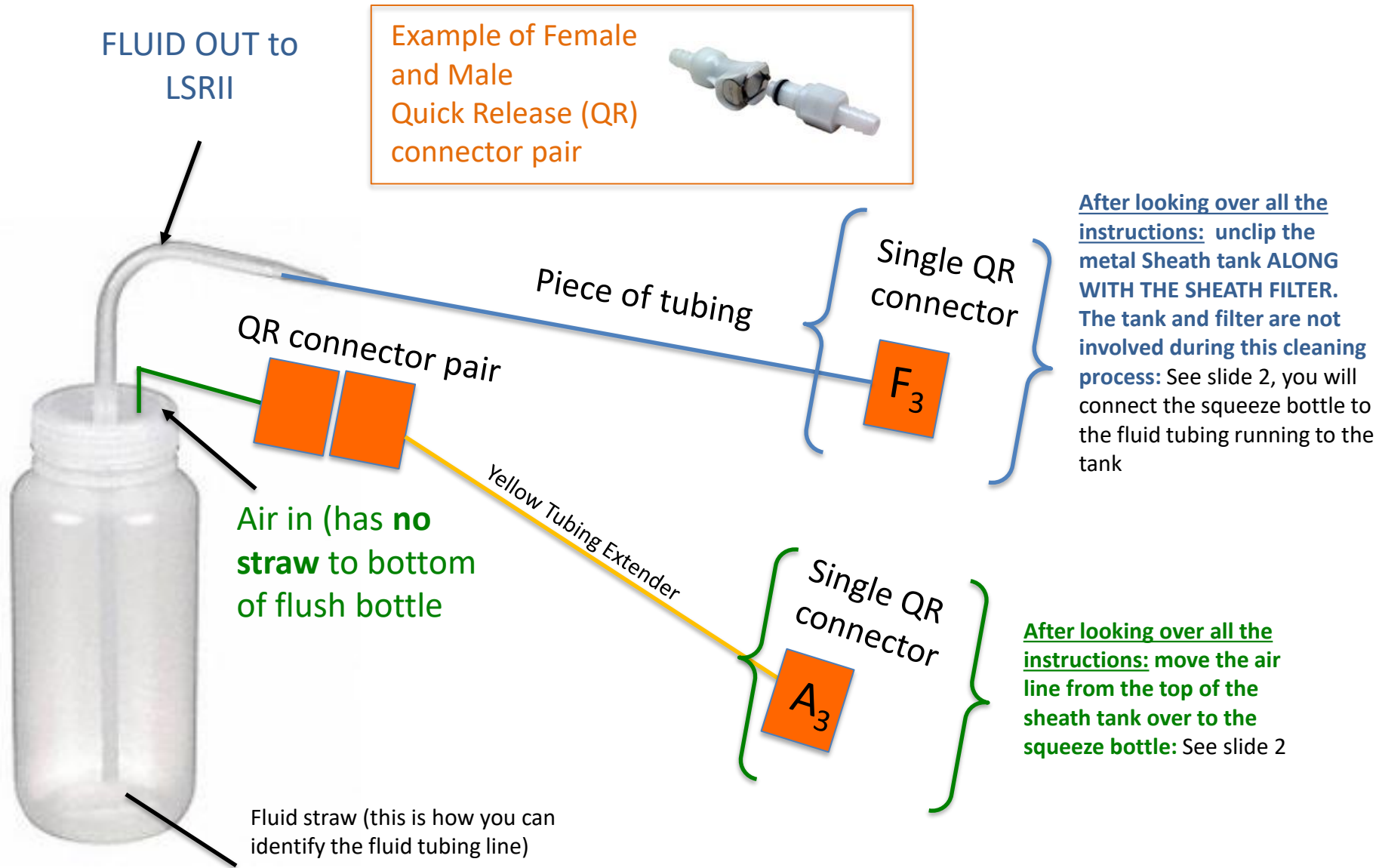
LSRII Flow Cell Cleaning

You will need:

- The 250ml **Redi-Flush squeeze bottle** (along with the extender tubing on the air line)
- **Tape** to label connectors if you are worried about getting confused
- **Autoclaved MQ water** (and syringe filtered MQ to run on the SIT, and to check instrument is clean)
- **50ml falcon or measuring cylinder** for making up solutions as you go (eg on shelf above analysis workstation)
- **10% bleach** in MQ H₂O
- **30% Contrad 70 detergent** in MQ H₂O (Contrad kept under sink in sort room)
- **FACS tubes** (if not by the LSRII-1, check the bulk box from next to LSRII-2).
- **Sterile FACS tubes** for preparing CS&T beads (dust free – these come capped or uncapped in sleeves, look in the sort room).
- **CS&T beads** (prepare fresh before running): these aren't cheap and are best run fresh so try to make sure the instrument is clean before diluting them for running (1 drop in 500ul syringe filtered PBS or MQ H₂O; *they are a little more stable in PBS*).

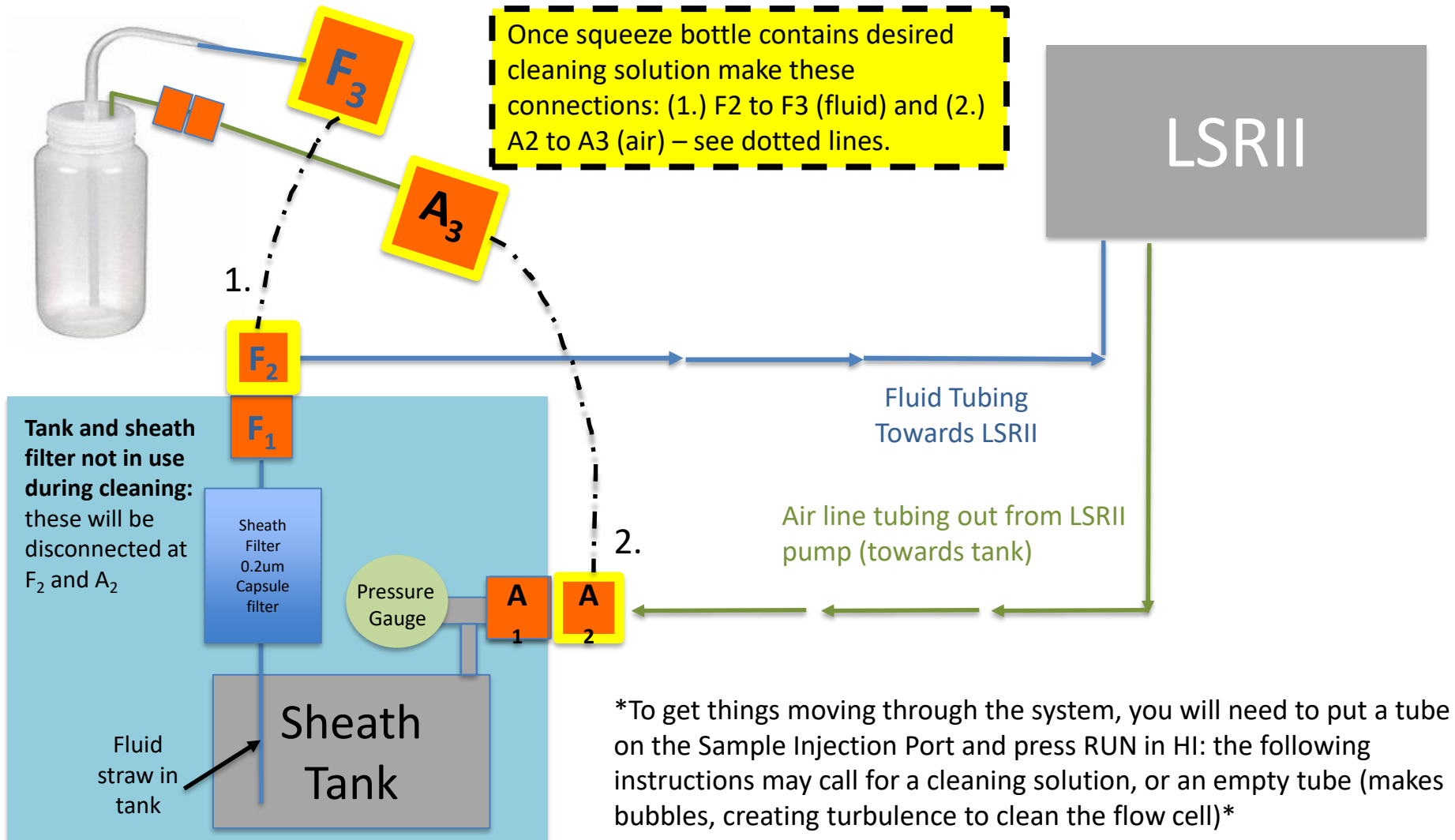
The Redi-Flush squeeze bottle system

*Read through, do not disconnect until looking at slide 2 and **labeling any tubing parts with tape** for yourself if needed. Tape is located on the shelf above the analysis workstation by the entryway*



Connecting the Redi-Flush system

This slide shows how to connect the a squeeze bottle of cleaning solution



Example steps for troubleshooting

These are the types of cleaning actions that can be tried for debris on the flow cell

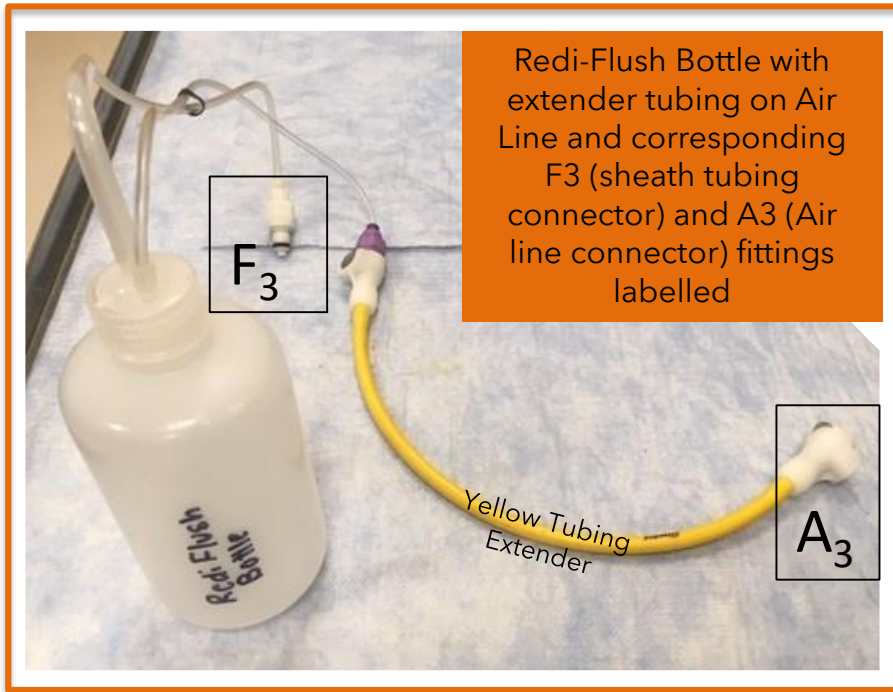
For all of these steps, make use of the PRIME button 5 times or periodically if desired, then leave in RUN in HI:

1. **Bleach:** 10% Bleach in squeeze bottle + SIT (run in HI with solution in FACS tube on Sample Injection Port). *Always follow with water (bubbling rinse (2) then with water on the SIT (3) until event rate drops in Diva, before attempting to run CS&T. Run bleach no more than 30min as it is corrosive.*
2. **Bubbling water rinse:** Autoclaved MQ H₂O (eg from autoclaved sheath carboy) in bottle + empty tube on the SIT to produce a good flushing action on the Flow Cell. *E.g. Run for 5-10min then go to (3)*
3. **Water rinse:** Same as (2) but with H₂O on SIT, perform *until events in QC template less than 0-20 evts/sec then looking at CS&T*
4. **Bubbling detergent wash:** 30% Contrad detergent in bottle but empty tube on SIT in RUN (must follow with water, e.g. (2) then (3), running H₂O to get the air out). *E.g. Run for 15min before considering (5) if needed, or going to (2) or (3)*
5. **Detergent soak:** 30% Contrad in bottle + water in SIT: *Option to RUN for a while (15min) and then immediately do a bubbling detergent wash (4) then water rinse (2 and 3), or turn off LSRll and leave Overnight (O/N) before continuing (at 2 then 3) In both cases follow with (2) and (3).*
6. Last step: Reconnect sheath tank/filter again, and keep running MQ H₂O *while monitoring event rate in Diva* Don't be alarmed if event rate is high initially, it will drop with time. *After event rate drops: Record a CS&T file in the template then execute CS&T in Diva*

Note: If CS&T looks terrible after cleaning, it is likely to be one of the following:

- (i) Cleaning reagents are still in the system: Detergents are viscous and can take time to run clear so keep running water (eg after reconnecting everything to the sheath tank; run MQ in FACS tube for up to 1h while returning to check periodically)
- (ii) Something is still baked on the flow cell so O/N detergent soak is likely needed if not already done; consult with core staff

Examples of Redi-Flush bottle anatomy & location

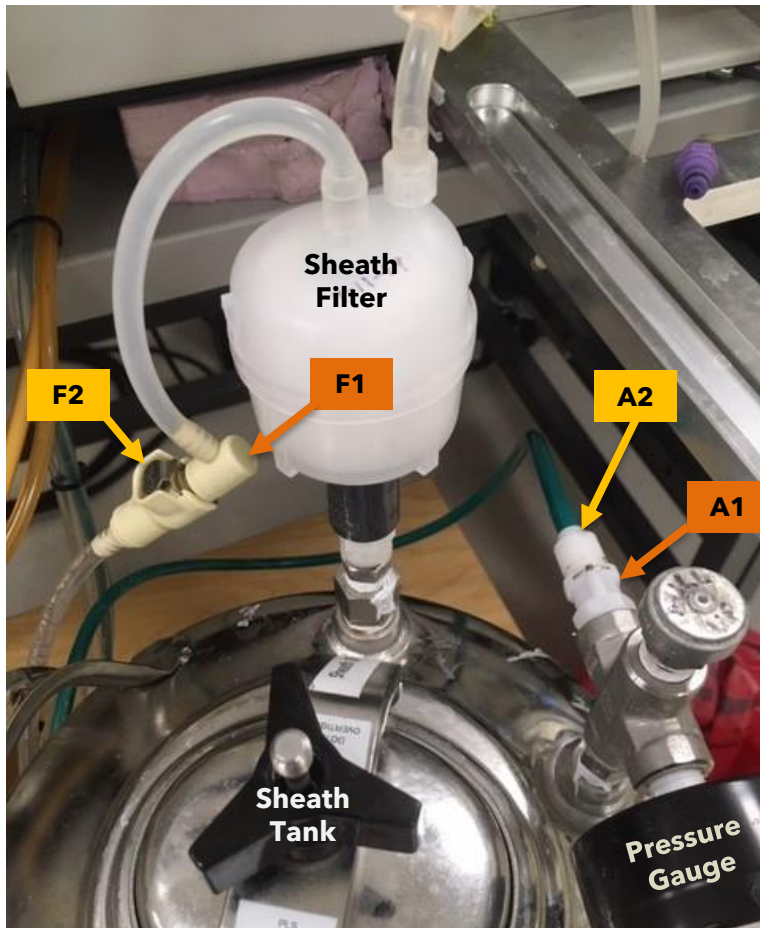


Redi-Flush Bottle and Contrad 70 located under sink in FCCF



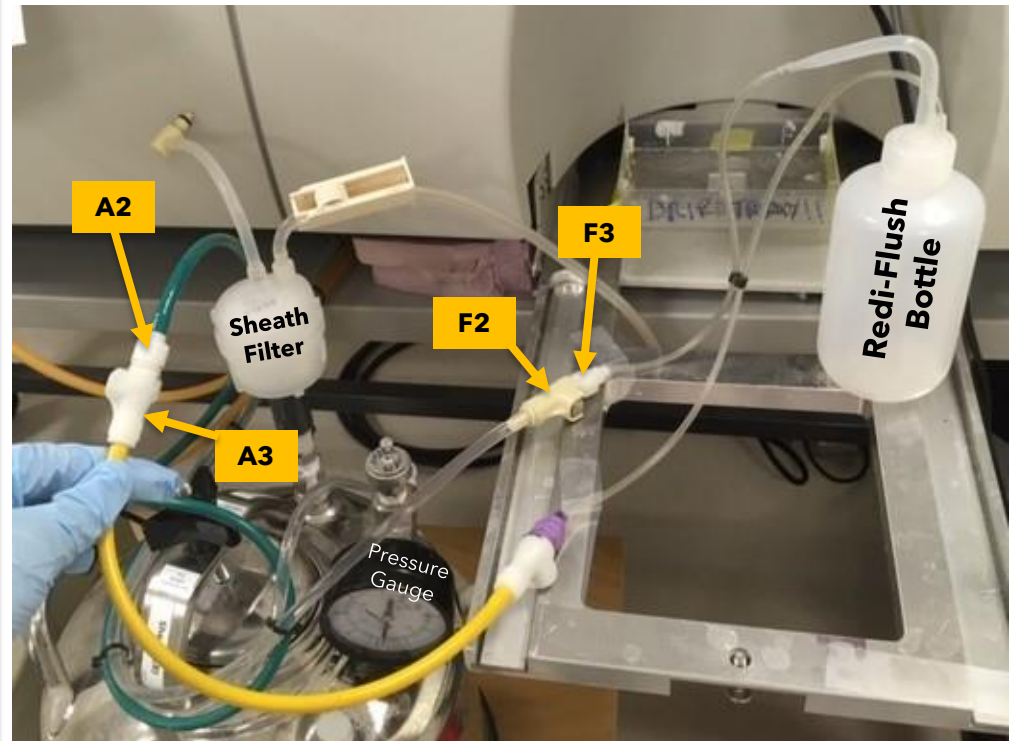
Examples of normal Set-Up vs Set-up with Redi-Flush Bottle System

Picture 1: Tank/Connectors Normal Set-Up



Please note previous statement from Slide 3:
Tank and sheath filter not in use during cleaning: these will be disconnected at F₂ (Sheath Line) and A₂ (Air Line)

Picture 2: Set-up with the Redi-Flush Bottle System



Please note previous statement from Slide 3: Once squeeze bottle contains desired cleaning solution make these connections: (1.) F₂ to F₃ (fluid) and (2.) A₂ to A₃ (air) – see dotted lines on diagram in Slide 3 indicating the new connections.