Standard Operating Procedure
(Laboratory Practices)

•**Cell Sorting Protocol: Lentivirus infected cells**
Lab/Location(s): Jimmy Fund 415
Principle Investigator: John Daley
Protocol Name & Number: Sorting of Lentivirus infected cells
Current Revision Date 05/11/2015
Description of Possible Risks: Lentiviruses can deliver a significant amount of viral RNA into the DNA of the host cell and have the unique ability among retroviruses of being able to infect non-dividing cells, so they are one of the most efficient methods of a gene delivery vector. HIV, SIV, and FIV are all examples of lentiviruses. **Lentivirus Sorting Procedure:**

Cell infected with lenti viral particles can be sorted on the either BD FACSAria II SORP cell sorters housed within the Baker BioProtect III containment hood only.

1) Prior to sorter:
2) Fill Sheath tank
3) Add Bleach to the waste tank to ensure a final 10% solution post sort waste
4) Fill Spray bottle with fresh 10% Bleach solution
5) Researchers must use universal safety precautions; wear disposable lab coats and gloves.

Procedures during sorting / analysis:
1) Researchers must use universal safety precautions; wear lab coats and gloves.
2) BioProtect III hood must have the glass doors closed. Once sorting has started access is permitted through the sash opening.
3) BioProtect III hood blower switch must be on. This will engage the Air Pressure Alarm used to indicate if there is a high or low air flow condition present for more than 3 seconds.
4) Samples must be filtered by researcher and solid caps placed on tubes immediately prior to sorting to minimize clogs and ice buckets must be placed on BD FACSAria II table inside of hood.
5) Sample tubes should be filled with maximum amount of sample to minimize loading and unloading samples.
6) Enable "sweet spot" to insure stream stability.
7) Install correct collection chamber, open sort collection chamber and align sort streams
8) Close sort collection chamber door prior to starting sort
9) Solid **(not blue filter cap)** test tube caps must remain on the samples. **(especially when vortexing)**
10) Samples may only be uncapped immediately prior to placement on the sample holder
11) Collection tubes must be capped prior to removal from the sort collection chamber
12) Collections tubes must be wiped down with 10% bleach prior to removal from the sort collection chamber
13) At the conclusion of the sort the Operator must disinfect the sample tubing.
    a. Operator must run 5 minutes of 10% Bleach followed by
    b. Run 3 minutes of BD FACSRinse
    c. Run 3 minutes of ddH_20
14) Disinfecting tubes must be capped and disposed of in the biohazard waste bag

Flow cell Nozzle Obstruction cleaning procedure:

In the event the Lentiviral cell population clogs the sorter (tubing or nozzle)

1) Operator will stop the sort stream: turn off the stream using the button labeled with a '✓' on the Breakoff window. This will shut off the stream, unload the sample and close the aspirator door. Alternatively, pressing the Large Red Emergency Stop Button to the left of the sample stage will also stop the sheath and sample stream.
2) Re-cap the sample tube and wipe exterior of the tube with 10% bleach and place in ice bucket
3) Open aspirator drawer using software controls
4) Evacuate collection chamber for 60 seconds; increase Aerosol Management System (AMS) evacuation rate to 100%vacuum. **MUST WAIT 60 SECONDS**
5) Remove and re-cap collection tubes and wipe exterior of tube with 10% bleach and place in ice bucket
6) Close aspirator drawer using software controls.
7) Operator will then perform the BD Clean Flow Cell Procedure with ddH20 3 times
   a. Open aspirator drawer using software controls
   b. Evacuate collection chamber for 60 seconds
   c. Place ddH20 on sample station
   d. Diva Software: Select from the menu - Instrument > Cleaning Modes > Clean Flow Cell.
   e. Repeat twice
8) Restart the sort stream
9) In the event the nozzle obstruction remains as evidenced by an incorrect droplet image formation, the Operator must open aspirator drawer wait 3 minutes to allow aerosols in the sort chamber to be evacuated.

10) Operator will decontaminate the sort chamber with 10% bleach spray and Super Sani Cloths. With stream turned off, open the sort block chamber door and dry plates and surfaces as needed.
9) Remove the nozzle; wipe nozzle with SaniWipes, place the nozzle in ddH20 for in a15cc conical tube with 1ml ddH_20, cap and ultrasonicate for 60 seconds.
11) Set AMS unit to 20% vacuum, Replace nozzle, and ensure all chamber doors are closed then restart sort stream, verify that correct droplet stream image is present.
12) All tubes and wipes must be disposed of in biohazard bag.

Post-Sorting procedure: Decontamination Procedure:

After cell sorting is complete the operator is responsible for disinfecting the areas used for Lentivirus cell sorting.

1) Disinfect sample lines using a freshly made 10% bleach solution as follows.
    a. Fill a tube with a volume of 10% bleach equal to or greater than the volume of sample that was sorted and place on the sample stage.
    c. Run Bleach for 3 minutes Flow Rate: 11
    d. Fill a tube with DI water, to equal or greater than volume of the bleach tube run previously, run for 3 minutes Flow Rate: 11

The following areas must be disinfected with **10% Bleach** and the **Super Sani-cloth** wipes to clean accordingly

- Sort chamber
- Sample holder
- Immediate surrounding surfaces
- Sort Chamber and Sample Station doors
- Computer workstation area and key board

• Contact the Flow Cytometry Facility (617-632-3179) or Suzan Lazo 617-632-4571 for more information.