

Standard Operating Procedure (Laboratory Practices)

Infectious Agent and Strain: Lentivirus

Lab/Location(s): Mayer 522a, Mayer 583

Principle Investigator: John Daley

Protocol Name & Number: Analysis of Lentivirus infected cells

Current Revision Date 03/23/2015

Description of Possible Risks:

- 1) 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 usage practice:

1. Cell infected with lenti viral particles will be analyzed on the designated instruments:
 - a. BD LSRFortessa analyzer
 - b. BD LSRFortessa X-20 analyzer
 - c. CantoII 583 HTS analyzer
 - d. CantoII 584 HTS analyzer

Researchers must run their Lenti Virus samples in 96 or 384-well micro titer plates on the HTS (High Throughput Sampler) attached to the above stated analyzers **ONLY**. You may not run Lentivirus on the CantoII Carousel. Usage of the HTS system allows for fully automated sample acquisition.

2. Researcher must use universal safety precautions; wear disposable lab coats and gloves.

96 Well Plate Procedure

Analyzer (BD LSR II) Disinfectant protocol for 96well micro titer plate is as follows:

- a) Last row on EACH 96 well plate must be treated as a disinfectant row
(no exceptions). This will sanitize the HTS tubing as well as the sample tubing on the analyzer.

Plate Loader Settings for Row: P Wells: 1-12

Sample Flow Rate: 3.0
Sample Volume: 175ul
Mixing Volume: 100
Mixing Speed: 200
Number of Mixes: 0
Wash Volume: 200ul

- Wells 1-6 will contain 200ul (10x) Bleach
- Wells 7-9 will contain 200ul BD FACSRinse
- Wells 10-12 200ul ddH₂O

384 Well Plate Procedure

Analyzer (BD LSR II) Disinfectant protocol for 384well micro titer plate is as follows.

- a) The last row on EACH 384 well plate must be treated as a disinfectant row

(**no exceptions**). This will sanitize the HTS tubing as well as the sample tubing on the analyzer.

Plate Loader Settings for Row: H Wells: 1-24

Sample Flow Rate: 3.0

Sample Volume: 90ul

Mixing Volume: 45

Mixing Speed: 200

Number of Mixes: 0

Wash Volume: 200ul

- Wells 1-12 will contain 100ul (10x) Bleach
- Wells 13-17 will contain 200ul BD FACSRinse
- Wells 18-24 200ul ddH₂O

Post-analyses procedure:

After each analysis the researcher is responsible for disinfecting the areas used for Lentiviral analysis work.

Please spray the following with **10% Bleach** and use the **Super Sani-cloth** wipes to clean accordingly

- Analyzer
- Analyzer Sample Station
- All affected areas
- The researcher is responsible for removal and disposal of their gloves; disposable lab coat and all wipes used to disinfect the analyzer and affected areas.
- Contact the Flow Cytometry Facility (617-632-3179) or Suzan Lazo-Kallanian 617-632-4571 for more information.