**Optimization of reverse transfection conditions for siRNA or sgRNA oligos**

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**Overview**

* **Day -1:**
  + SiRNA/sgRNA transfection controls are spotted and then air dried into a PerkinElmer Cell Carrier Ultra 384 well imaging plate using the ECHO525 acoustic liquid handler (D304).
  + The plate is then sealed, and stored at –30o C.
* **Day 0:**
  + Set up the reverse transfection.
  + Use the Multidrop dispenser (D304, cell culture hood) to add 20 ul of OPTIMEM
  + Use the ECHO525 to dispense RNAiMAX into wells already containing 20 ul of OPTIMEM.
  + Use Multidrop dispenser to add 20 ul of cells resuspended in 20 ul of OPTIMEM/20% FBS with cells.
  + Incubate at 37o C
* **Day + 2 or 3:**
  + Fix and Stain cells using the Bluewasher washer/dispenser (D304, chemical fume hood).
  + If not imaging right away, store plate at 4o C.
* **Day X:**
  + Warm plate to RT before imaging, otherwise condensation on bottom of plate will impede image acquisition.
  + Image plate.
  + Recover plate and store in YOUR fridge. Discard at your discretion.

**Reagents**

* siRNA controls are diluted to a 5 uM concentration (Stock).
* sgRNA controls (Synthego sgRNA kit v2) are resuspended at 3 uM concentration (Stock) in water. A working solution at 0.3 uM in water is prepared fresh before spotting. Do not freeze and thaw siRNA or sgRNA oligos solutions more than 3-4 times.
* RNAiMAX or other transfection reagent.

**Preparation**

* **Day1**: Set up ECHO 525 dispense protocol for siRNA.
  + Run a simulation of ECHO 525 transfer to ensure proper well dispenses and total volume used.
* **Day 2:** Set up ECHO 525 dispense protocol for RNAiMAX.
  + Run a simulation of ECHO 525 transfer to ensure proper well dispenses and total volume used.
* Set up Multidrop dispenser.
  + Make up solutions
  + Prime cassette
* Set up BlueCat Bluewasher.
  + Make up solutions
  + Prime Bluewasher

**Day 1:**

1. Add siRNA control (5 uM) or sgRNA controls (0.3 uM) to Labcyte source plate (LDV or PP, as appropriate)
   1. Be aware of the Dead volume and Maximum Volume per well for the LDV or PP source plate.
2. Centrifuge Labcyte source plate at RT for 2 minutes at 1200 RPM.
3. Conduct a survey of siRNA volume in Labcyte source plate to ensure there is adequate volume.
4. Spot 150 nl of siRNA 5 uM (0.8 pmoles per well, final conc. in 40 ul: 20 nM) or 325 nl of sgRNA 0.3 uM (0.08 pmoles per well, final conc. in 40 ul: 2 nM) per well of a 384- well imaging plate.
5. Air dry siRNA in the laminar air flow hood (usually takes 30 to 150 minutes).
6. Seal the plate with Alufoil and store at -30oC.

**Day 2:**

1. Thaw imaging plate at room temperature.
2. During this time, set up ECHO 525 for dispensing RNAiMAX from the Labcyte source plate with appropriate RNAiMAX volume.
3. For ONE set of controls (No siRNA, Neg siRNA, Pos siRNA; or No sgRNA, Neg sgRNA, Pos sgRNA) you will need 6.3 ul total to dispense a matrix for 25, 50, 75, 100, 125 and 150 nl RNAiMAX.
   1. See “RNAiMAX\_Optimization\_Template” Excel spreadsheet for layout.
4. Centrifuge SOURCE PLATE at RT for 2 minutes at 1200 RPM.
5. Centrifuge thawed IMAGE PLATE at RT for 2 minutes at 1200 RPM
6. Run a simulation of ECHO525 transfer to ensure proper well dispenses and total volume used.
7. Conduct a survey of RNiMAX volume in source plate to ensure there is adequate volume. Remember to calculate the 4 ul dead volume/well into your final total volume in the source plate.
8. Add 20 ul of OptiMEM to the IMAGE PLATE using the Multidrop. Aseptic techniques apply.
9. Start ECHO525 run.
   1. Click BLUE arrow.
   2. Click RUN on new window that appeared.
   3. Click START on new window that appeared.
10. Follow directions.
    1. For source plate window, give it a name (SOURCE\_PLATE) if desired.
    2. Once Destination plate is requested, first place image plate onto tray.
    3. Click the pointer in the BARCODE window so the barcode will register.
    4. Scan in barcode of your imaging plate. This action will cause the ECHO 525 to accept the destination plate.
11. Wait for ECHO525 to finish dispensing.
12. Follow window cues once finished.
13. Seal source plate (NOT your image plate) with AluFoil and return to minus 30.
14. Add the clear lid on your image plate.
15. Incubate image plate at RT for 30 minutes in the laminar flow hood.
16. During this incubation step, prep the cells for the multidrop dispenser.
17. Dispense 20 ul of cells into imaging plate.
18. Incubate at RT for 30 minutes.
19. Place plate at 37oC and incubate for 72 hours.

**Day 5:**

1. Fix the cells with PFA using the Bluewasher.
2. Stain the cells with DAPI or continue with your endpoint visualization assay.

**Day X:**

1. Acquire cell image with CV7000S or CV8000.