Cell Imaging Preparation General Procedure (For MEF cells mainly, Steven Chang, Modified by Shaoying Lu)

The goal is to control the cell density so that it will be exactly one day over-confluent 3 days before imaging. More than three days before imaging,

(Check with somebody who has experience with MEF transfection for the exact cell concentration.)
For passing MEF cells, when the cells are about one day over confluent, 1:30 pass ratio reaches over-confluence in approximately 5 days.1:10 pass ratio reaches over-confluence in approximately 3 days. This can vary a lot depends on how much cell you have before passing.
Four days before imaging**:** If the cells are already over-confluent, pass approximately 70%~80% of the cells to a new medium dish so it would be over-confluent again on the next day. This is, however, not recommended because it is hard to control cell density accurately.

Three days before imaging
1. The cells should be just one day over confluent. For MEF cells, this means that cells are touching one another with **no space** in between and the cells are **rounded** instead of being long and spread out under non-confluent condition.
2. When the cells are just one day over confluent, pass cells in the late afternoon. Use the 10%FBS DMEM –P/S medium. When passing, resuspend the pellet in **3mL medium** and add **3 drops** for each small dish (using pipette-aid and set it to slow). This usually gives a good cell density for transfection on the next day.

Two days before imaging
1. In the morning, the cells in the small dish should be 70%~80% confluent. If it is more confluent, it should be okay too.
2. Do transfection in the morning following the protocol.
3. The suggested amount of DNA for MEF cells when it is approximately 80% confluent:
KRas-Src: 3~4μg per small dish 2 ml growth medium
For the suspension cells NK92 $1x10^6$ cells in 2 ml medium, use 3 ug DNA, 9 ul lipofectamine 2000 (Invitrogen Catalog Number 11668-027).
(Convert μg to μL using the concentration of the DNA)
(Adjust the amount when you have a lot more or a lot less amount of cells)
4. DNA was added and gently mixed with 100 μL Optimum (Invitrogen). For each μg of DNA added, two μL of Lipofectamine was added to a separate vial that contains 100 μL Optimum. After 5 minutes

of incubation, the Lipofectamine-Optimum was applied to the DNA-Optimum and gently mixed. The mixture was further incubated in room temperature for 20 minutes to allow the formation of DNA-Lipofectamine complex. After 20 minutes, the DNA-Lipofectamine complex was added to the 35mm dish that contains

MEFs while swirl gently to mix. The cells were incubated in a 37°C incubator for 5.5 hours before changing

the medium to 0.5% FBS supplied DMEM with penicillin/straptomyosin.(longer is okay).

1. Prepare fibronectin coated glass bottom dishes (Cell E&G Catalog number GBD00002-200) in the morning. FN stock (1 mg/ml) was diluted with PBS to obtain the low (2.5 mg/ml), median (10 mg/ml) and high (20 mg/ml) FN concentration. Each dish was coated with 200 ml of the diluted FN at the glass-bottom hole of the dish. The dishes were incubated in a 37°C incubator for 4 hours before using to ensure sufficient precipitation of FN.

2. Pass cells onto the glass bottom dishes in the late afternoon. Use 0.5%FBS DMEM + P/S medium.

During the centrifuge step of passing cells, vacuum out the excess liquid on the glass bottom dish before passing cells onto it.

3. Only pass the cells to the center glass well of the dish. When finish passing, leave the dish in the incubator and add 2mL

medium when the cells are attached to the glass. (It takes approximately 30 minutes for cells to attach).

4. Suggested passing ratio when the transfected cells are about 70% confluent in the small dish:
For normal dishes: 1:15 ~ 1:20
For confluent dishes (wound-healing): 1:4~1:6
(Adjust the ratio when you have different amount of cells)

Imaging day
If cells are passed on the imaging day, wait for at least three hours before imaging those dishes.