Appendix

A. Materials

A.1 Cells

- Madin-Darby canine kidney (MDCK) cells. More information available here.
- hTERT-HME1 (HME) cells. More information available here.

A.2 Reactants

- Dulbecco's Modified Eagle's Medium - high glucose, with 4500 mg/L glucose, L-glutamine, sodium pyruvate, and sodium bicarbonate, liquid, sterile-filtered, suitable for cell culture. Product number: D6429. Product brand: SIGMA. Specification sheet.
- Penicillin streptomycin sol (10,000 U/mL), catalog # 15140122. Product brand: Gibco. Description & specifications.
- Fetal Bovine Serum (FBS). More info here.
- Phosphate-Buffered Saline (PBS), pH 7.4, liquid. Prepared in the own laboratory, but it is the same as the one provided by Gibco, with catalog # 10010031: Description & specifications.
- SiR-DNA (SC007), Spirochrome. Product information.
- Trypsin-EDTA Solution 1X - 0.05 % trypsin, 0.02 % EDTA, trypsin gamma irradiated by SER-TAIN Process, in Hanks Balanced Salt Solution. Product brand: Sigma. Product # 59417C. Specification sheet.
- Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 555 from Thermo Fisher Scientific, catalog # A-21422, RRID AB2535844. Data sheet.
- Alexa fluor 568. Catalog # A12380. Product brand: Life Technologies. Description & specifications.
- Goat anti-Mouse IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 633 from Thermo Fisher Scientific, catalog # A-21050, RRID AB2535718. Data sheet.

- Mammary Epithelial Cell Growth Medium (MECGM) KIT. Product brand: PromoCell. Product # C-21110. Description.
- Soybean Trypsin Inhibitor, powder. Product brand: Gibco. Catalog # 17075029. Description & specifications.
- Bovine Serum Albumin (BSA) - cold ethanol fraction, pH 5.2, 96 %. Product brand: Sigma. Product # A4503. Specification sheet.
- Dimethyl sulfoxide (DMSO) - sterile-filtered, BioPerformance Certified, meets EP, USP testing specifications, suitable for hybridoma. Product brand: Sigma. Product # D2438. Specification sheet.
- GATA-3, concentrated and prediluted monoclonal antibody. Product brand: Biocare Medical. Catalog # CM 405 A, B. Specification sheet.
- Dow SYLGARD^{TM} 184 Silicone Encapsulant Clear 0.5 kg Kit (includes base and curing agent). Product brand: Dow. Description & details.
- Pluronic© F-127 - powder, BioReagent, suitable for cell culture. Product brand: sigma. Product # P2443. Specification sheet.
- Photoresist AZ© 125 nXT A negative. Product brand: Microchemicals. Technical datasheet.
- Developer AZ© 726 MIF. Product brand: Microchemicals. Technical datasheet.

A.3 Machinery

- Hood
- Pipettor.
- Incubator: binder CO2 (CB series).
- Centrifugator: Rotanta 460R.
- LEICA DMIL.
- Integra vacusafe (aspirator for discarding biological liquids).
- Fridges and freezers.
- Plasma cleaner.

A.4 Others

- Glass pipettes.
- Micro-pipettes and tips.
- Flasks.
- Petri dishes.
- Wells.
- Filters.

B. Protocols

B.1 Passaging MDCK cells

Materials: DMEM, Trypsin, PBS, flask with cell culture, pipettes, tube.

1. Clean everything with ethanol.
2. Bring reactants into the hood.
3. Remove flask containing the cell culture from the incubator and check it is confluent under the microscope.
4. Remove flask contents.
5. Wash with 5 mL PBS. To do so, drop the amount of PBS in the flask and slightly tilt it to make sure the whole surface is washed.
6. Remove PBS.
7. Introduce 3 mL Trypsin into the flask, making sure it spreads throughout the surface. Let incubate for 10 – 15 minutes. Before proceding, check under microscope cells have been detached.
8. Tilt the flask and wash the surface with 5 mL DMEM to make sure all cells are detached. Empty the flask contents into a tube for centrifugation.
9. Centrifuge for 4 minutes, at 1500 rpm and around 20 C. Once this is done all cells should be visibly packed at the bottom of the tube - the liquid part is known as supernatant.
10. Remove supernatant liquid from the tube, leaving a small quantity just to make sure cells are not sucked away.
11. Add 6 mL DMEM into a new flask and 4 mL into the centrifuged tube. Resuspend the medium in the tube up and down about 10 times to homogenize the cells.
12. Take 2 mL from the homogenized medium in the tube, take these into the flask and resuspend again.
13. Without extracting the pipette, lay the flask flat and make sure the liquid spreads through the whole surface. Usually, there are some spots left uncovered, when this

happens pipette some medium and drop where needed.
14. Place the flask in the incubator, reactants back in the fridge, properly discard old flask and tube and clean the hood with ethanol.

B.2 Preparing trypsin inhibitor

Materials: Soybean trypsin inhibitor, PBS, filter, syringe, tube.
Trypsin inhibitor is necessary for passaging HME cells.

1. Weight 0.01 grams of the soybean trypsin inhibitor powder in a container, for every 10 ml of solution (that is a 1:1000 ratio).
2. Inside a hood, fill a tube with 5 ml PBS.
3. Drop 1 ml of PBS in the soybean container, then shake by flipping it up and down several times.
4. Empty the PBS and soybean mixture in the tube with PBS, making sure to pipette up and down so as to drop all the soybean that may be left in the pipette tip.
5. Wash the soybean container with PBS and drop its contents again in the tube with PBS. Do this until the tube is filled with 10 ml of solution (or the amount required).
6. Once that is done, get a syringe and connect it to a filter. Drop the contents of the tube in the syringe and, these, into a new tube. This step is necessary to filter out possible impurities from weighting the soybean in non-sterile conditions.

B.3 Passaging HME cells

Materials: MECGM, Trypsin inhibitor, PBS, flask with cell culture, pipettes, tube.
Note: When carrying the flask containing cells, it is important not to tilt it too much.

1. Clean everything with ethanol.
2. Bring reactants into the hood.
3. Remove flask containing the cell culture from the incubator and check it is confluent under the microscope.
4. Remove flask contents.
5. Wash with 5 mL PBS. To do so, drop the amount of PBS in the flask and slightly tilt it to make sure the whole surface is washed.
6. Remove PBS.
7. Introduce 2 mL Trypsin into the flask, making sure it spreads throughout the surface. Let incubate for 10 - 15 minutes. Before proceding, check under microscope cells have been detached.
8. Tilt the flask and wash the surface with 2 mL Trypsin inhibitor to make sure all cells are detached. Empty the flask contents into a tube for centrifugation.
9. Centrifuge for 5 minutes, at 1200 rpm and around 20 C. Once this is done all cells should be visibly packed at the bottom of the tube – the liquid part is known as supernatant.
10. Remove supernatant liquid from the tube, leaving a small quantity just to make sure cells are not sucked away.
11. Add 6 mL MECGM into a new flask and 4 mL into the centrifuged tube. Resuspend the medium in the tube up and down about 10 times to homogenize the cells.

12. Take 2 mL from the homogenized medium in the tube, take these into the flask and resuspend again.
13. Without extracting the pipette, lay the flask flat and make sure the liquid spreads through the whole surface. Usually, there are some spots left uncovered, when this happens pipette some medium and drop where needed.
14. Place the flask in the incubator, reactants back in the fridge, properly discard old flask and tube and clean the hood with ethanol.

B.4 Exchanging medium

Even if cells are not confluent, they may need a new medium that replenishes nutrients and keeps pH at an adequate level. This is perceptible when the medium includes phenol red, a molecule capable of indicating pH level[i]. A sign of cells needing a medium exchange is its medium appears orange rather than pinkish red.
Materials: flask containing cell culture, medium, pipettes, hood, microscope and incubator.

1. Bring flask with cells from incubator into the hood and remove its contents.
2. Place the required amount of medium (MECGM for HME cells) in the flask. Such amount should be the same as the amount already present.
3. Check under microscope that cells remain attached, then bring the flask back into the incubator.

B.5 Preparing collagen samples

Materials: collagen, medium (usually PBS or DMEM), 10+1 buffer, eppendorf vial, pipettes.

1. First off, calculate the amount of chemicals needed. This will depend on V_t the total amount of volume needed for the experiment (always make this larger than the required volume), C_o the original collagen concentration (clear stated in the bottle lable) and C_w the concentration one wants to prepare. Use the following formulas to obtain $V_{col} = (C_w/C_o) \cdot V_{col}$ the volume of collagen needed, $V_{buf} = 0.1 \cdot V_{col}$ the volume of buffer and V_{med} the volume of medium:

$$\begin{aligned} V_t &= V_{col} + V_{buf} + V_{med} \\ &= \frac{C_w}{C_o} \cdot V_t + 0.1 \cdot V_{col} + V_{med} \\ &= 1.1 \frac{C_w}{C_o} \cdot V_t + V_{med}. \end{aligned}$$

Thus, the only unknown is the volume of medium:

$$V_{med} = \left(1 - 1.1 \frac{C_w}{C_o}\right) \cdot V_t.$$

[i] National Center for Biotechnology Information. PubChem Database. Phenol red, CID=4766, nih.gov (accessed on Mar. 26, 2019)

Notice that one cannot use a higher volume of collagen concentration than the original, otherwise the equation above would yield negative volumes.
2. Begin by introducing V_{med} in an eppendorf vial.
3. Next, add the 10+1 buffer, with a different pipette tip and making sure to wash it (pipetting up and down) to remove the whole volume of buffer.
4. Now introduce the collagen (again, usig a new pipette tip) and gently homogenize the mixture by pipetting five times up and down.
5. The mix is now ready to be introduced in container such as well slides. Just pipette the required amount into the new container (leaving some space between the upper liquid surface and lid).
6. The collagen should take about one hour to polymerize if incubated at 37 °C. If the collagen has polymerized correctly, it will not move in the container independently of how much it is tilted (even if upside down).

B.6 PDMS coating

Materials: PDMS, activator, pipette and tips, cup, spoon, scale, vacuum chamber, oven.

1. Pour the required amount of PDMS, directly from its container, into a cup. For a Petri dish, 5 grams should be enough. Pipettes are not recommended, since the PDMS is highly viscous.
2. Take 1/10 of activator volume with respect to the amount of PDMS taken, i.e. if using 5 grams of PDMS, take 500 μl of activator.
3. Mix well with a spoon.
4. Place the cup into a vacuum chamber for 30 minutes. This process is known as degasing.
   - During this process some foam may form on the solution surface. If this happens let some air into the chamber until the foam is gone.
5. Let in the oven at 80 °C for an hour.

B.7 Labeling with SIR-DNA

The cell culture to be labeled should have been split from its main container into something more practical, e.g. a petri dish or wells. This can be done during passaging: After centrifugation and removal of supernatant fluid, homogenize cells with medium. Part of this homogenized medium shall be used to keep the cell line in a main container and another part to use in labeling. Wash the Petri dish with DMEM (i.e. cover the surface with DMEM and then remove it) and then seed cell medium, add DMEM and homogenize. Let incubate until confluent. Once cells are confluent one may proceed to labeling. Materials: SIR-DNA, cell culture, medium, eppendorf vial, wells for observation.

1. Check confluency under microscope.
2. Remove medium in container, then add in it an appropriate amount of medium and remove this one.
3. In an eppendorf, place a small amount of medium (usually between 2 and 4 mL).
4. The amount of SIR-DNA required is 1/1000 part of the medium's volume. Pipette this amount from directly into the medium, making sure to wash any remaining

amount in the pipette tip by pipetting up and down a few times.
5. Place the needed amount of SIR-DNA-medium mixture in the container for observation. For example, 200 μL.
6. In that container, add a small amount of cell medium. For example, 200 μL.
7. After an hour of incubation, cells should be ready to be observed.

B.8 PDMS stamp

Materials: Sylgar 184 Silicon elastomer kit with base and curing agent, pipette, scale, vacuum chamber, cup, spoon, wafer, parafilm.

1. Pour PDMS into a plastic cup, directly from its container. 10 grams are enough to obtain stamps from a wafer.
2. Pipette activator into cup. The volume required is 1/10th of the PDMS amount taken, i.e. for 10 g of PDMS, take 1 ml activator.
3. Mix well with a plastic spoon.
4. Place the cup in a vacuum chamber for 30 minutes.
5. Wrap the cup with parafilm for transportation. Place under a hood, along the wafer.
6. Dispense the mixture on the wafer, making sure it spreads through the wafer surface (especially where the masks have been printed on).
7. Let it in an 80 °C oven for 2 hours.
8. After a few minutes (15) of cooling down, the PDMS may be peeled off with a pair of tweezers. To make the peeling as smooth as possible, one may cut around the patterns with a scalpel and remove the PDMS that is not on the patterns. This way, the PDMS stamp may be lifted off with ease in just one pulling. It is important to store these in a petri dish to avoid dust deposition and, also, placing it with the pattern looking upwards (so the structures don't collapse under the PDMS weight).
9. Store at room temperature.

B.9 Pluronics solution

Materials: Pluronics F127, distilled water, scale, tube, agitator.

1. The ratio of pluronics to water for a single dish is 0.2:100.
2. In order to reduce the number of times preparing this solution, a 50 ml total volume may be used. Thus, one needs to weight 5 grams of pluronics and have 50 ml of distilled water.
3. Because pluronics take a long time to dissolve (about two days), one should first add half of the total volume of distilled water to the tube with the pluronics solute and let it on an agitator for a couple of days.
4. Once the pluronics are dissolved, add the rest of the water and swirl the tube. It must be noted that the solution tends to gelate at temperatures above 10 °C, so storage in a fridge is required.