**Experiment**

1. **Harvest cells**
2. **Count cells**
3. **Aliquot in 1.5 mL tube**
4. **Spin down**
5. **Remove supernatant**
6. **Resuspend**
7. **Aspirate ~10 µL air in repeating pipette**
8. **Cool tip in gel on ice for 10 sec**
9. **Resuspend**
10. **Aspirate ~25 µL**

**Preparation**

1. **Thaw overnight**
2. **Add 50 µL water on observation window**
3. **12 x 8 wells**
4. **Cool tube with cell pellet**
5. **Add ECM**
6. **Resuspend**
7. **Avoid bubbles**
8. **Resuspend on ice**

**Materials**

- 225 µL ECM
- 12 mL medium
- 5 mL water
- 500K – 3M cells
- Repeating pipette
- 0.2 mL combitip

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**Day 0**

**Day 1**

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**Model**

9603-400-B
9603-400-W
Invert and inspect reflection for filling
Don't touch glass

**9**
- Prime pipette until dispensing
- Dispense 2 µL in 8-16 wells
- Eject remaining volume to tube to resuspend and cool back down
- Repeat step 7-9 until finished

**10**
- Incubate:
  - 37°C
  - 15 min

**11**
- Add 25 µL medium on outlets
  - 12 x 8 wells

**12**
- Incubate:
  - 37°C
  - 45 min

**13**
- Add 100 µL medium on inlets
  - 12 x 8 wells

**14**
- Place on Mimetas Perfusion Rocker for continuous perfusion

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