GUV formation protocol:

Materials:
- Ethanol, DI water and Kimwipes for cleaning
- 5-10 µl glass pipettes)
- Vacuum desiccator
- Electroformation chamber
- 4040A function generator
- Desired lipids dissolved in chloroform at 2mg/ml concentration
- Buffer: typically H20 or low salt solution (<10mM), Sucrose okay to 0.5 M.
- O-ring
- Vacuum Grease
- Copper strips
- Tape

Preparation:
- Clean the chamber with ethanol and DI water using Kimwipes alternating the solvents at least twice to make sure any grease or sugars are removed from the slides
- Make sure the ITO slides in the chamber are firmly in place and tape the copper conductor strips so that one is in contact at the top and bottom of each slide.
- Using a glass syringe apply a 2-3 µl (@ 2mg/ml conc.) droplet of the lipid/chloroform solution in the center of the ITO slide on the shorter side of the chamber. The layer should appear greenish under white light reflection.
- Quickly (within a minute) place the whole chamber in the vacuum desiccator, desiccate for at least 30 mins, preferably 1.5 hrs. This is to remove all of the chloroform from the film.
- Meanwhile, clean the O-ring using a Kimwipe and ethanol. Use Que-tip or grease slide to apply a thin layer of vacuum grease to one side of the O-ring.
- Remove the chamber from the desiccator and place the O-ring (greased side down) approximately in the center of the short side of the chamber, directly over the greenish film.
- Using a 200 µl pipette, place 75 µl of buffer solution in the O-ring.
- Put the top half of the chamber on using the alignment pins, ensure there are no bubbles (very small bubbles are ok).

Procedure:
Attach the BNC connector to the chamber.
- Set the voltage amplitude between to 1V peak-to-peak (never exceed 5V as this will damage the ITO coating.) Set the driving frequency to 14Hz for 120 minutes then increase to 2V peak-to-peak and decrease frequency to 2Hz run for 50 min.
- Wait and watch as vesicles begin to form over the course of 2-3 hours. Blistering of the lipid layer should be noticeable within a few minutes.
- When the formation is done, turn off the equipment and remove chamber from microscope. Using pipette take out ~50 µl of the solution from the chamber into glass vial already filled with ~0.5 ml desired buffer. *Slowly* mix this solution in the vial. Depending on the yield, a 10:1 dilution of this solution may be in order. The vesicles are now ready! They are good for ~24 hours.

Reference:

Protocol for Electroformation of Giant Unilamellar Vesicles (GUVs)
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