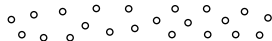


A

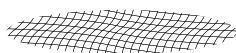
Seal with waxy film



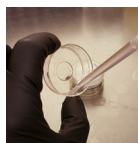
35 mm Chamber Bottom



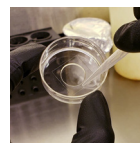
Glass Beads

Chamber Top
(or tape bottom)

Nylon Mesh

Chamber Base
(or bottom with
glass out)35 mm
Chamber Lid**B****Steps 4.1-4.5:**

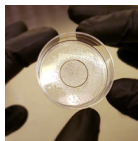
- Prepare solution for bead loading
- Transfer as much medium into a conical tube and save

**Step 4.6:**

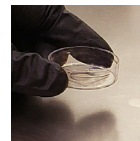
- Tilt dish 45° and aspirate remaining medium without touching center of dish

**Step 4.7:**

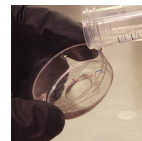
- Dispense solution to center of dish
- Place bead loader on top of dish
- Dispense beads with a small firm tap

**Step 4.8:**

- Check dish for a single monolayer of beads
- Dispense more beads if needed

**Step 4.9:**

- Tap the dish 10-15 times firmly

**Step 4.10:**

- Tilt dish 45° and pour in medium to wash beads away from center
- Gently aspirate floating beads

C**Plate Cells****Bead Load****Incubate****Change Media****Image****1****2****3****4****5**

~24 h

<5 min

0.5-2 h

<5min

Varies

- Incubation after plating may vary on cell type
- Pre-bead loading treatments such as transfection can be preformed here

- It is best to work fast to avoid drying of cells

- For bead-loaded plasmids, extend the incubation to allow for expression

- Change to a medium suitable for imaging

- Additional procedures such as fixing and staining can be performed before imaging