# POLYACRYLAMIDE GEL FABRICATION

Use **40% aqueous solution of acrylamide** and **2% aqueous solution of BIS** (BioRad).

Make Hepes buffers:
- 50ml of 10mM Hepes, pH=8.5, for making the gels
- 500ml of 50mM Hepes, pH=8.5, for washing the gels

Filter 10mM buffer with syringe and 0.2mm filter, and 50mM buffer with 500ml filter unit.
Always store @4°C.

Print out the gel titration table (in a case you change proportion or any other parameter).
Prepare as many 50 ml tubes as many gel kinds you are going to make.
Mark each tube with %acrylamide – %BIS (e.g., "5 – 0.1" for 5% acrylamide and 0.1% BIS gel).
Prepare one 50 ml tube for red bead sonnication.

Add acrylamide, BIS and 10mM Hepes into gel tube, mix gently, let equilibrate at $T_{room}$.
Add red beads (0.2µm diameter) and 500µl Hepes into red-bead tube (**once for each gel kind**)
In the meantime, wipe with kimwipe and air-blow all coverslips and arrange them:
- activated square (22mm No.1) coverslips on one rack
- round (12mm or 18 mm No.1) coverslips on another rack

Prepare the spacers for holding the coverslip assemblies.

Put gel tubes with the mix into vacuum chamber, sealing the lid.
Start the vacuum pump and slowly attach tubing to the lid.
Monitor vacuuming for first ~5 minutes, to make sure it doesn't bubble over.
When stable leave to vacuum for 15 more minutes.

In the meantime, fill small beaker with ice and place in it the tube with red beads and Hepes.
Take TEMED and ammonium persulfate from desiccator jar.
Prepare 10% ammonium persulfate in $H_2O^{dd}$ (0.1g for 1ml in little tube), triturate to dissolve.
When there is only 5 minutes left on vacuuming timer, start sonicating the beads @ lowest power setting.

After sonnication and vacuuming, gently mix (500+beads)µl from bead mixture into gel mix.
Stir very gently few times with glass bars, leave bars inside gel tubes.
Add ammonium persulfate and TEMED and stir gently with the bar.
Now you must be quick and careful.

Take square coverslip in your left hand, between thumb and index finger.
Pipette small amount of the mix (draw from the middle of tube) onto square coverslip, depending on which round coverslip you will use to sandwich it with:
- 12µl if using 12mm top coverslip
- 20µl if using 22mm top coverslip

Pick round coverslip with tweezers, place it on top of the drop, immediately turn upside down.
Place the sandwich on spacers, round coverslip hanging down.
Wait ~30min to x-link, checking frequently—ready when shrinks a bit away from the edges

When cured, lift sandwich with tweezers, turn proper side up again, place in 35mm dish.
Add 50mM Hepes to each dish with sandwich to soak.
Use one tweezers with curved tip and one with straight, very fine tip.
With curved tweezers press gently on the square coverslip close to gel edge.
Wedge straight tweezers into sandwich edge, lever against curved tweezers, and gently and steadily apply lifting force to the round coverslip.
When it detaches from gel, throw it away.
Repeat for each gel and stack all dishes into one big dish for washing on shaker.

Wash gels 3×10min in 50mM Hepes on shaker.
Store @4°C in PBS.

| unit | acrylamide | BIS | Red beads (0.2μm) | 10% ammonium persulfate | TEMED | 10mM Hepes (minus 500 μl) |
|---|---|---|---|---|---|---|
| % | **6** | **0.15** | **1:50** | **1:250** | **1:2000** | |
| μl | 750 | 375 | 100 | 20 | 2.5 | 3252.5 |
| % | **5** | **0.1** | **1:50** | **1:250** | **1:2000** | |
| μl | 625 | 250 | 100 | 20 | 2.5 | 3502.5 |
| % | **4.5** | **0.07** | **1:50** | **1:250** | **1:2000** | |
| μl | 562.5 | 175 | 100 | 20 | 2.5 | 3640 |

For 5 ml total volume

**Sonicate Red beads+500 μl Hepes**

H=5000 –A-B-Yb-ap-T-500[μl]

A[%]*5[ml]=40%*1000*a[μl]
B[%]*5[ml]=2%*1000*b[μl]

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