FtsZ purification protocol
B. subtilis and E. coli FtsZs included

Day 1: Induction

1. Grow Ec or Bs FtsZ in pET 21b(+) in C41(DE3) cells in LB + Amp^{100} broth at 37°C overnight from freshly transformed single colony.
2. The next morning, inoculate 1 L LB + Amp^{100} broth 1:100 (10 mL) with o/n culture.
3. Grow cells at 37°C until OD_{600} ~ 0.6. Induce with ITPG to final concentration = 1 mM.
4. Grow cells an additional 4 hrs. at 37°C.
5. Harvest cells by centrifugation. Spin cells at 5000xg for 10 min at 4°C.
6. Take cell pellet and wash 1X with 10 mL FtsZ induction buffer.
7. Spin re-suspended cell pellet down at 7500xg for 10 min at 4°C.
8. Remove sup and keep pellet and store at -80°C (good for at least 1 year) or proceed to purification.

Day 2: Purification

1. Take 1 frozen cell pellet from 1 L o/n culture. Thaw on ice with 10 mL FtsZ induction buffer plus protease inhibitor (recommended using AEBSF at final concentration of 1 mM for final re-suspension volume).
2. Re-suspend pellet on ice and bring volume up to 30 mL with FtsZ induction buffer.
3. Lyse cells by French Press 2X at 10,000 Psi. Alternatively, cells can also be sonicated on ice 5X for 10 sec intervals with 30 sec rest in between.
4. Clear lysate by spinning at 160,000xg for 45 minutes at 4°C.
5. Take sup and precipitate FtsZ with concentrated solution of ammonium sulfate. For Ec FtsZ, add a volume of $AmSO_4$ equal to 0.25 of the volume of the sup to bring final $AmSO_4$ to 20%. For Bs FtsZ, add a volume of $AmSO_4$ equal to 0.43 of the volume of the sup to bring final $AmSO_4$ concentration to 30%. (For example, for Bs FtsZ if the sup volume is 25 mL, add 10.75 mL $AmSO_4$ directly to it). Incubate on ice for 20 min.
6. Spin the solution at 10,000xg for 10 min at 4°C. Keep the sup and transfer to new vessel and disregard the pellet.
7. For Ec FtsZ, add 0. 14 the volume of the sup to bring the final $AmSO_4$ % to 30%. For Bs FtsZ, add 0.16 of the volume of the sup of $AmSO_4$ to the sup (brings $AmSO_4$ to 40%). Incubate on ice for 20 min. Then spin again at 10,000xg as above.
8. Pour of the sup. Keep the pellet. For either Ec or Bs FtsZ, two options now: to both aliquot and store FtsZ, or further purify by anion exchange chromatography.

Store and Aliquot:

1. Re-suspend on ice final FtsZ pellet in 5 mL FtsZ polymerization buffer. Let pellet loosen up for ~15 minutes prior to pipetting.
2. Add 0.05 g sucrose (to final of 10%) and GDP to final concentration of 50 uM.
3. Aliquot 50 ul. Flash freeze on liquid N_2 and store at -80°C.

Anion Exchange purification

1. Re-suspend on ice final FtsZ pellet in FtsZ anion exchange buffer – low salt and bring volume up to 50 mL.
2. Apply the re-suspension to an anion exchange column. Size of the column should have at least 6 mL gel bed size (generally use Bio-Rad Uno6 or Uno12 or GE HealthCare MonoQ 10/100 column).

3. Run re-suspension over the column. Wash with at least 5 column volumes of FtsZ anion exchange buffer – low salt.
4. Then elute FtsZ off column using a 50-500 mM KCl gradient. FtsZ elutes in the 180-200 mM KCl range.
5. Check which fractions have FtsZ by SDS-PAGE
6. After gel, pool peak FtsZ fractions and dialyze against 1 L of FtsZ polymerization buffer (pH 6.5) + 1% sucrose. Dialyze overnight at 4°C.
7. The next morning, concentrate FtsZ using a spin column with proper MWCO or polyethylene glycol. Aliquot (~100 µL) into 0.65 mL tubes, then flash freeze on liquid N_2. Store at -80°C.
8. The next day, thaw all of the aliquots on ice. Remove protein from tubes (you can save them for re-use) and put into dialysis tubing. Dialyze overnight against 1 L of FtsZ polymerization buffer (pH 7.5) + 1% sucrose. Dialyze overnight at 4°C.
9. Final [FtsZ] should be ~100-300 uM. Add sucrose to a final % of 10% (0.05 g per 5 mL FtsZ) and GDP to final concentration of 50 uM. Aliquot and flash freeze on liquid N_2. Store at -80°C.

Buffers:
FtsZ Induction Buffer – 50 mM Tris pH 8.8, 100 mM NaCl, 1 mM EDTA
FtsZ Polymerization Buffer (pH 6.5) – 50 mM MES pH 6.5, 50 mM KCl, 2.5 mM $MgCl_2$, 1 mM EGTA
FtsZ Polymerization Buffer (pH 7.5) - 50 mM HEPES pH 7.5, 50 mM KCl, 2.5 mM $MgCl_2$, 1 mM EGTA
FtsZ Anion Exchange Buffer Low Salt – 50 mM Tris pH 8.5, 50 mM KCl, 1 mM EDTA, 1% sucrose
FtsZ Anion Exchange Buffer Low Salt – 50 mM Tris pH 8.5, 500 mM KCl, 1 mM EDTA, 1% sucrose

Materials:
100 mM GTP and GDP stock
Saturated Ammonium Sulfate solution
100 mM AEBSF stock
1 M IPTG