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RbCl Uber-Competent Cells

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We use this protocol in our group and it is working, though open to further improvement/modification

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Abstract

A protocol kindly provided to our team by the advisor to the 2018 MIT iGEM team (Dr. Brian Teague). It is modified slightly from that version (made by Stefan Maas in 1997) to fit our needs. Stefan Maas' protocol is similar to the Hanahan, D. protocol from 1983.

It worked pretty well to make good competent cells in Summer 2018 and we just used it again in Summer 2019.

Materials

MATERIALS

- MOPS **P212121**
- Manganese(II) chloride tetrahydrate **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**M3634
- Glycerol **Bio Basic Inc. Catalog #**GB0232.SIZE.500ml
- Rubidium chloride **Bio Basic Inc. Catalog #**RB0668.SIZE.25g
- Potassium acetate **Merck Millipore (EMD Millipore) Catalog #**1.04820.1000
- Calcium Chloride **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**C4904
- Potassium hydroxide **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**1050121000
- Acetic acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**695092

Make sure you also have all the glassware/plasticware you need (listed in the first step), as well as:

- SOB media + 20mM $MgCl_2$
- LB plates without antibiotic (2)
- LB media
- Plastic tube racks with foam inside
- Labeled boxes for storing 1.5mL tubes

Troubleshooting

Day 0

1 Autoclave the following materials: 2h

	Amount	Material	Comments
	2	2L Erlenmeyer flasks	For culturing cells, second one is if you mess up the first time
	2	500 mL bottles	For TfbI
	2	100 mL bottles	For TfbII
	2	Stir bars	For stirring buffers
	~200	1.5mL tubes in a glass beaker	For aliquoting competent cells (the whole beaker should just be covered

			in foil)
	1	500 mL graduated cylinder	For measuring media
	1	250 mL graduated cylinder	For QSing TfbI
	1	100 mL graduated cylinder	For QSing TfbII
	4	200 mL centrifuge bottles	For centrifuge steps, have different orange caps from other bottles

Make sure all bottles have their caps on loosely!

For best results, autoclave glassware 3/4-full with DI (to remove residual detergent that could interfere with bacterial growth or competency). Plasticware should be rinsed with DI 2-3x and then autoclaved on dry cycle. Any containers without caps should be covered securely in aluminum foil. Autoclave tape should be applied if necessary to foil or outside container walls. See the Autoclave protocol for more details.

2 Take a tube of comercially competent cells and put it on ice (Thermo-Fisher TOP10 preferred, we think). Make sure to use a tube of comercially competent cells to start, or you may have poorer results. Work near a flame to ensure sterility. 20m

Using a sterile pipet tip or a flamed and cooled inoculation loop, take a drop of the thawed competent cells and streak gently onto an LB plate. Put the plate upside-down (agar-side up) in the 37 °C incubator for 16-18 hours. Discard the tube of competent cells in bacterial waste (or use for a transformation if possible).

See the Streaking Cells protocol for more details.

2.1 If you work fast, you might be able to pick a chip of ice from the tube quickly, then return the tube to the -80°C freezer. However, we don't recommend this unless you're experienced, since once the competent cells start warming over -80°C, they lose competency.

Day 1

3 Make sure you take the plates from the incubator in the morning and leave them in the cold room until you are ready to do your overnight culture. 20m

Work near a flame to ensure sterility. Using a serological pipet, fill 2-3 14mL round-bottomed culture tubes with 5 mL of LB media. For each culture tube, pick a colony from your plate with a p200 tip and drop it into the media. Put the tubes on a rack and put the rack in the 37 °C shaker at 250rpm for 16-18 hours.

(You should be able to safely use your SOB + 20mM $MgCl_2$ for this step, that might actually be better. However, we've only tried this with LB).

Do NOT add any antibiotic to the culture media - the cells are not antibiotic-resistant and should not be either!

See the Overnight Culture protocol for more details.

Day 2

4 Work near a flame to ensure sterility. Add 800 µL fresh overnight culture to 400 mL medium in 2L flask. 5m
(We used LB but 2xYT and NZDT work, SOB + 20mM $MgCl_2$ is optimal).

Flask should be 5x the volume of the medium and covered with foil.

5 Shake the culture at 250 rpm at 37 °C for 3-4 hours until at A_{600} of 0.5-0.7 (for cuvettes of length 1 cm, A_{600}=OD_{600}).

Use the spectrophotometer and cuvettes at our bench (in Quartzy hopefully) for checking the OD (it has a cuvette length of 1cm). Make sure to blank with media. Swirl the culture before taking a 1mL aliquot. Do not return the aliquot to the flask. Work near a flame for sterility.

Note

This is the step where you are most likely to mess up! Start checking your culture after about 02:30:00 and then start checking every 10-15 minutes after that (depending on how much the culture grew). Note that while the culture initially takes a while to get to 0.1 or 0.2 OD_{600} (lag phase), the OD will start shooting up during the log (exponential phase).

6 While the cells are shaking, load foam racks and plastic tube racks with open tubes in the cold room. (Put some paper towels over them to prevent dust or anything else from getting into them). 15m

Also leave the centrifuge bottles, a box of p200 pipette tips, and several 10mL and 25mL serological pipettes in the cold room to chill.

Also turn on the large centrifuge next to the 2nd floor cold room and set it to 4 °C, 4000 rpm, 00:10:00 so it can pre-cool.

7 Also while the cells are shaking, make fresh TfbI and TfbII. Ideally, these should be made fresh, but if necessary, they can be made the day before as well. 1h 30m

	Component	TfbI (150 mL)	TfbII (20 mL)
	Rubidium chloride	1.8 g	0.024 g
	Manganese (II) chloride tetra	2.30 g	none

	hydrate		
	Calcium chloride	0.17g	0.17g
	Potassium chloride	0.44 g	none
	MOPS	none	0.042g
	Glycerol	22.4 mL (28.4g)	3.0 mL (3.78g)

Add all dry reagents to the bottle, add glycerol (usually easier just to weigh it) ~3/4 of the autoclaved MilliQ water (in the past, we just used DI, but autoclaved MilliQ is best) needed to QS.

Then adjust pH:

- For TfbI adjust to pH 5.8 with 0.2M acetic acid.

> Note
>
> You may need to let the TfbI stir a long while to get the pH up to 5.8 or higher before you pH adjust back down!

Add slowly! If you add too much then the solution will turn yellow-green and you will have to remake it.

- For TfbII adjust to pH 6.5 with 1M KOH.

Then transfer to graduated cylinder to QS to final volume with autoclaved MilliQ water.

For TfbI and TfbII, filter sterilize with a 0.4 µm filter (not sure about the pore size, but it's what we used).

8 Chill cell culture in ice-water bath for 10-15 minutes. 00:10:00 10m

Note

All steps from here on should be done in the cold room. Make sure to keep the cells as cold as possible for the remainder of the protocol - so all containers, buffers, tips, etc should be cold as well!

9 Separate culture evenly between two chilled 250 mL centrifuge bottles. 10m

Note

Make sure that the cap of the centrifuge bottle screws on correctly and tightly! There are some dud caps that could cause your cell culture to spill out into the centrifuge chamber if used. Also check if there might be any autoclaved O-rings that go with the centrifuge bottles, because that would also help prevent spills.

Centrifuge at 4000 rpm (about 3000x g) at 4 °C for 00:10:00 .

10 In the cold room, decant medium and drain on a paper towel to allow all traces of medium to drain out. 5m

Tap the bottles on paper towels but be careful not to lose any cells (the pellet may start to slide down the side).

11 Gently resuspend each cell pellet in 4-8 mL ice-cold TfbI (use serological pipette). 2h 10m

"Gently" means lightly swirling the flask until all cells are resuspended. Don't shake, poke, or pipet.
DO NOT disturb the cell pellet by pipetting back and forth or shaking vigorously.

This may take a while, but make sure the pellets are fully resuspended (uniform solution). Pour one of the resuspended pellets into the bottle with the other resuspended pellet and bring the total volume to 120 mL (use serological pipette).

Incubate on ice in the cold room for 02:00:00 .

Note

This is usually a good time to go get lunch :)

12 Centrifuge again at 4000 rpm (about 3000x g) at 4 °C for 00:10:00 . 10m

Make sure to label the tube containing the cells. Then, fill another centrifuge bottle with water as a balance (and label that differently).

13 In the cold room, decant the buffer and discard. Gently resuspend cells in 16 mL ice cold TfbII (this could take 5-10 minutes, similar to before). Aliquot cells into chilled 1.5mL tubes. 25m

Aliquots should be 50-200µL (usually 100 µL). Chill your pipette tips in the cold room before aliquotting and swirl cells regularly during aliquotting. Try to work quickly!

14 Close the caps of all the 1.5mL tubes in the foam rack. Fill a plastic ice bucket with liquid nitrogen (ask for a PI or grad student's help here). Then place the foam rack into the liquid nitrogen for snap freezing. You will know the tubes are frozen when most of the popping/crackling sounds subside. 15m

Snap freezing ideally should be done in liquid nitrogen (as detailed above). However, you can also use dry ice if necessary.

Make sure you have some (pre-labeled!) empty freezer boxes to transfer the 1.5mL tubes to after snap freezing. Then put the boxes in the -80 °C freezer for storage.

If necessary, transfer more of the 1.5mL tubes to the emptied foam rack and repeat until all cell aliquots are snap frozen.

Testing Competency

15 Make sure to test your cells for transformation efficiency using the iGEM Comptent Cell Test Kit or similar. (BBa_J04450).

Refer to the RbCl Cells Transformation protocol for more details.