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ILT3

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We are still developing and optimizing this protocol

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Abstract

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Protocol materials

DraI enzyme (5 U/µl) **New England Biolabs** Step 14

MilliQ water Step 14

NEB 10X CutSmart Buffer **New England Biolabs Catalog #**B7204S Step 14

Safety warnings

BE SURE to discard all materials (mainly pipettte tips) in the biohazard waste bin you are working with GMOs. If you spill contact the supervisors directly: stop working (note your experiment is still safe time is not a huge issue here!).

Bacterial cell destruction

10s

1 Resuspend the bacterial pellet in 0.3 mL of Resuspension Buffer.

1.1 Mix vigorously using the vortex until no clumps remain.

The solution needs to be "clear" not "turbid" otherwise invert the tube a few times more and continue.

The lysate should appear viscous. Do not allow the lysis reaction to proceed for more than 5 min.

There may be little treads seen by opening the tube when you start step 3. That is the genomic DNA!

From now on, the bacteria are lysed so no GMO any more (lysed bacteria are no bacteria any more). However, you need to continue working under MLI conditions as the whole laboratory is MLI!

2 Add 0.3 mL of Lysis Buffer, mix thoroughly by vigorously inverting the sealed tube for 10 times.

3 Add 0.3 mL of chilled Neutralisation Buffer, mix immediately and thoroughly by vigorously shaking 00:00:10 .

10s

If the mixture still appears viscous, more mixing is required to completely neutralize the solution.

Plasmid recovery

12m 10s

4 Centrifuge at maximum speed in a centrifuge for 00:02:00 .

2m

Do not disturb the pellet. You can stick your pipette directly through the potentially small white top layer. The white stuff that may stick to the outside of the pipet is no problem as long as you do not transfer this into the new tube, only all the liquid is needed.

5 Collect 700 µL the supernatant in a 1.5 ml microcentrifuge tubes.

Do not disturb the pellet. You can stick your pipet directly through the potentially small white top layer. The white stuff that may stick to the outside of the pipet is no problem as long as you do not transfer this into the new tube, only all the liquid is needed.

6 Precipitate DNA by adding 700 µL isopropanol to the plasmid DNA containing supernatant. Mix vigorously using the vortex.

7 Centrifuge at maximum speed in a centrifuge for 00:07:00 .

7m

Always place the tubes with the hinge of the lit pointed outwards so you know where the pellet should be if you cannot see it in the next steps.

8 Pipet away all the fluid. take care NOT to touch or suck up the pellet (your plasmids).
Be care full not to touch your pellet or suck it up. (don't scratch the bottom with your pipet tip) Leave the lowest/last 10 microliters there. no need to remove them now.

9 Add 1 mL [M] 70 % (v/v) ethanol to the tube with the plasmid pellet, close the tube and vortex shortly (00:00:10). 10s

10 Centrifuge at maximal speed for 00:03:00 . 3m

11 Remove the 70% ethanol by pipetting.
Use a 200 microliter pipet tip instead of the blue 1000 microliter tips. Pipet the last remaining microliters qualitatively. Do this really carefully to not disturb the plasmid DNA pellet.

12 Air dry for 00:05:00 5m
If the pellet just turns from white "opaque" it's already ok! Please set a timer to not over dry the pellet 5 minutes should be ok to evaporate the ethanol the remaining little droplets are water.

13 Add 50 µL sterile water and vortex.
If the vortexing results in scattering all the water though out the tube shorty centrifugate to collect the water with plasmids again. The plasmids are now in solution and will stay in solution regardless the centrifugation.

Restriction enzyme digestion (D2) 30m

14 Make the mixtures for the restriction analysis, a total of 20 µL :

- DNA sample 5 µL
- NEB 10X CutSmart Buffer **New England Biolabs Catalog #**B7204S 2 µL
- DraI enzyme (5 U/µl) **New England Biolabs** 1 µL
- MilliQ water **Contributed by users** 12 µL

14.1 Mix components by pipetting the reaction mixture up and down, or by "flicking" the reaction tube. Follow with a quick ("touch") spin-down in a microcentrifuge.
Do not vortex the reaction too vigorously -this may kill the restriction enzyme).
Do not discard the remaining plasmid product! You need to analyze this as well!
Do not forget to add the enzyme as last component.

15 Label a reaction tubes and place them in the heating block. Digestion will take place at 37 °C for 00:30:00 . 30m

16 Add DNA gel loading dye to each sample to stop the reaction.

Agarose gel preparation

17 Prepare the casting tray by putting the sides together.

18 The silicon coated site should point towards the inside.

19 Apply the elastic band around the extruding parts in the middle of the sides to hold everything in place.

20 Flip the assembled tray in the correct position and press it lightly on the table to ensure proper alignment of the both sides.

21 Add the comb and put the tray in the box to catch any potential spills if not correctly assembled.

22 Prepare a 1% agarose gel in 1x TAE buffer. The total volume depends on the size of the casting tray; we will make 100 mL gels. Calculate and weigh the amount of agarose required for your volume. Deposit the agarose in a glass Erlenmeyer flask and add the indicated volume of 1x TAE buffer.

23 Pour the mixture in the casting tray with combs. Remove any air bubbles with a plastic filter tip.

24 Allow the gel to solidify (00:10:00).

24.1 Remove the comb.

25 Insert the casting tray into the electrophoresis equipment; the side of the slots in the direction of the cathode (black).
Do not add the metal casting system into the electrophorese system, just the gel.

26 Prepare and add 1x TAE Buffer to the system until the gel is completely submerged.

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