

Complete manual to get cDNA-probes

Everything you use should be autoclaved (except bacteria, RNA, DNA, plasmids, ...)!

Preparation of competent bacteria

EVERYTHING SHOULD BE STERILE

1. Plate competent bacteria (INV, DH5 α , ...) on a LB-Plate
2. Incubate over night on 37°C
3. Pick some colonies with a toothpick and transfer each into approx. 5ml LB-Medium (Starting culture)
4. Incubate with shaking (225rpm) at 37°C over night
5. Transfer the Starting culture into 500ml LB-Medium in an Erlenmeyer-vessel
6. Incubate with shaking (225rpm) at 37°C for 3-4 hours
7. Centrifugate at 4°C, 6000rpm for 10min.
8. Resuspend pellet in 125ml ice-cold 0,1M MgCl₂
9. Incubate for 30min. on ice
10. Centrifugate at 4°C, 6000rpm for 10min.
11. Resuspend pellet in 50ml ice-cold 0,1M CaCl₂
12. Incubate for minimum 1h, maximum overnight on ice
13. Add 7,5ml Glycerol
14. Aliquot in Eppendorf-tubes à 100 μ l (approx. 10-20 tubes, remaining bacteria à 1ml)
15. Store aliquots at -70°C

This is an original-protocol from Dr. Peter Hufnagl. It's scaleable.

Transformation

1. Thaw 100 μ l competent bacteria on ice
2. Add 10-50ng of plasmid
3. Incubate for 30min. on ice
4. Incubate for 2min. at 43°C (waterbath)
5. Add 1ml of LB-Medium
6. Incubate with shaking (225rpm) at 37°C, 1h
7. Centrifugate at 4°C, 6000rpm for 5min.
8. Remove supernatant (leave approx. 50 μ l in vial)
9. Resuspend bacteria
10. Plate on a LB-AMP-plate
11. Incubate over night at 37°C

Check for "positive" colonies (1)

1. Pick some colonies with a toothpick and transfer each into approx. 5ml LB-Medium (Startculture)
2. Incubate with shaking (225rpm) at 37°C over night

3. Pipett out 1ml of bacteria-suspension into an Eppendorf-tube (keep remaining bacteria-suspension at 4°C)
 4. Centrifugate at 4°C, 6000rpm for 10min.
 5. Remove supernatant
 6. Resuspend bacteria in 100µl STETL
 7. Incubate at 100°C (or 98°C) for 1min. (waterbath)
 8. Centrifugate at 4°C, 13000rpm for 15min.
 9. Remove pellet (with a toothpick)
 10. Add 100µl Isopropanol
 11. Centrifugate at 4°C, 13000rpm for 15-60min.
 12. Remove supernatant
 13. Wash with 500µl 70%-EtOH
 14. Centrifugate at 4°C, 13000rpm for 5min.
 15. Remove supernatant
 16. Centrifugate at 4°C, 13000rpm for 1min.
 17. Remove remaining supernatant with a 100µl-pipette
 18. Air-dry the pellet
 19. Dillute in 10-20µl water
 20. Store at -20°C or go on with:
- (Steps 16 and 17 are for faster drying of pellet, but not necessary)**

Check for “positive” colonies (2)

1. Digest above plasmid-samples with the suitable restriction-enzyme(s) at 37°C for 3h
2. Store at -20°C or go on with:

Check for “positive” colonies (3)

1. Prepare a 1%-agarose-gel
2. Prepare above DNA-samples for electrophoresis (add 1/6 volume of loading-buffer)
3. Load samples on gel (don't forget DNA-Marker)
4. Run electrophoresis at 100-140V for about 1h
5. Make a photo under UV-light
6. Check your samples for positive colonies (correct size of insert)

Preparation of larger amounts of plasmids

1. Transfer the remaining 4ml of positive bacteria (step 3 of “*Check for positive colonies (1)*”) into approx. 200-250ml LB-AMP-medium
2. Incubate with shaking (225rpm) over night at 37°C
3. Go on to step 3 of “*Check for positive colonies (1)*” until step 6 of “*Check for positive colonies (3)*”
4. Go on with plasmid-preparation (see **QIAGEN® Plasmid Handbook**), but keep approx. 1-2ml from the bacteria-suspension for freezing

Freezing bacteria

1. Centrifugate 1-2ml of bacteria (step 4 of "Preparation of large amounts of plasmid") at 4°C, 4000rpm for 5min.
2. Add 500µl of 10mM MgCl₂
3. Resuspend Bacteria (vortex)
4. Add 500µl of Glycerol
5. Mix (vortex)
6. Store at -70°C

Plasmidpreparation from frozen bacteria

1. Pick out a small amount with a toothpick
2. Plate bacteria on a LB-AMP-Plate
3. Incubate at 37°C over night
4. Continue with step 1 of "Check for positive colonies (1)", until here

Preparation of cDNA-probes from larger amounts of plasmids (1)

1. Digest plasmid with the suitable restriction-enzyme(s) (1 unit per 1µg plasmid) at 37°C for 3h
2. Store at -20°C or go on with:

Preparation of cDNA-probes from larger amounts of plasmids (2)

1. Prepare a 1%-agarose-gel
2. Prepare above cutted plasmid for electrophoresis (add 1/6 volume of loading-buffer)
3. Load sample on gel (don't forget DNA-Marker) (maybe you need more than one lane because of bigger volume)
4. Run electrophoresis at 100-140V for about 1h
5. Make a photo under UV-light
6. Cut out the gelpieces containing the insert with a scalpel
7. Store gel-pieces at -20°C or go on with:

Preparation of cDNA-probes from larger amounts of plasmids (3)

1. Extract probe from the gel-pieces (see **QIAGEN® QIAEX II Handbook**)
2. Store cDNA-probe at -20°C

Buffers and Media

H₂O

Should always be autoclaved

Ampicillin-Stock (50mg/ml)

dilute 500mg Ampicillin in 10ml H₂O, filter sterile, aliquot in Eppendorf-Tubes and store at -20°C

LB-Medium

dilute 10g Bacto-Tryptone, 10g NaCl, 5g Bacto-Yeast-Extract in 800ml H₂O, adjust pH to 7,5; adjust volume to 1000ml, autoclave and store at 4°C

LB-AMP-Medium

dilute 10g Bacto-Tryptone, 10g NaCl, 5g Bacto-Yeast-Extract in 800ml H₂O, adjust pH to 7,5; adjust volume to 1000ml, autoclave, let it cool to approx. 60°C, add 1ml of Ampicillin-Stock and store at 4°C

LB-Plates

dilute 10g Bacto-Tryptone, 10g NaCl, 5g Bacto-Yeast-Extract in 800ml H₂O, adjust pH to 7,5; add 7,5g Agar, adjust volume to 1000ml, autoclave, let it cool to approx. 60°C, pour into 90mm Petridishes (approx. 10-20ml per dish) and store at 4°C

LB-AMP-Plates

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STETL

- 1.) dilute 8g Saccharose, 0,5g Triton X-100, 1,861g EDTA (Titriplex III), 0,606g Tris in 80ml H₂O, adjust pH to 8,0; adjust volume to 100ml and store at 4°C (= STET)
- 2.) dilute 50mg Lysozyme in 10 ml of STET, aliquot in Eppendorf-tubes (à 50 or 100µl) and store at -20°C (= 10xSTETL)
- 3.) for use mix 1 volume of 10xSTETL with 9 volumes STET

50xTAE

dilute 242,2g Tris, 18,6g EDTA (Titriplex III) in 600ml H₂O, add 57,1ml Acetic-acid, adjust pH to 7,8; adjust volume to 1000ml and store at room-temperature or 4°C

Ethidiumbromide-stock

GibcoBRL Cat.No. 15585-011 (10ml; 10mg/ml)

1%-agarose-gel

mix 5ml 50xTAE, 2,5g Agarose and 250ml H₂O, heat it in the microwave until Agarose is diluted, let it cool to approx. 50°C, add 12,5µl Ethidiumbromide-stock and pour the gel. This recipe is scalable, depending on the size of the gel-tray.

Running-Buffer

1xTAE (dilute 50xTAE 1:50 with water)

Loading-Buffer

dilute 0,025g Bromphenol-blue, 0,025g Xylene Cyanol in 5ml H₂O and 5ml Glycerol, store at 4°C