Vibrio transformation journal

MONDAY, 7/1/2019

different methods of electroporation and chemical transformation have been tested.
the working protocol is a chemical transformation protocol

SUNDAY, 8/4/2019

Plan: check Transformation protocol in Vibrio, clear scheme on planned strains
3 Transformation samples: 1) no DNA, 2) plasmid with RFP coding device and AmpR, 3) plasmid with lacI-rbs-amilCP and KanR
procedure of transformation in Vibrio: 3.5 ul of overnight culture in LBv2 diluted with 350 ul of fresh LBv2 and 30 ng of DNA incubated statically for 4 hrs at 30 °C; add 1 ml of LBv2 and incubate for 1 more hour at 30 °C shaking; plated on plates to grow overnight at 37 °C (no DNA on plate w/o antibiotics, Kan, Amp; plasmid 2 on plate w/o antibiotics & Amp; plasmid 17 on plate w/o antibiotics & Kan)

MONDAY, 8/5/2019

transformation did not work, the transformation protocol used yesterday needs a gene (from V. cholerae) (for details checkout the link above)
found new protocol in https://www-nature-com.proxy-ub.rug.nl/articles/nmeth.3970; nuclease activity seems to be particularly high in Vibrio -> discuss ko of dns for chemical competency, try electroporation

TUESDAY, 8/6/2019

made electrocompetent cells: inoculated 100 ml of BHIv2 with 1 ml of o/n culture, grown at 37 °C to reach OD 0.5, pelleted at 4°C, 20 min, 6500 rpm, washed in electroporation buffer (680 mM sucrose in 7 mM KPi pH 7) 3 times (decant, resuspend, cetrifuge 4 °C, 20 min, 6750 rpm), resuspended to final OD 16 and aliquoted 400 μl into chilled tubes, frozen at -80 °C

WEDNESDAY, 8/7/2019

Electroporation trial: Electrocompetent cells were thawed on ice, combined with 3 μl of plasmid with Kanamycin resistance (3 from second isolated batch), transferred into 4 mm electoroporation cuvette (chilled) and elecroporated at 700 V, 25 μF, 200 ohm, recovered with 500 μl recovery medium (BHIv2 supplemented with 680 mM sucrose, filtered), cultured for 1 hr at 37 °C, 50 μl plated on small plate and grown o/n at 37 °C
unfortunately the cuvettes leaked in both cases, tried to get out what was there and washed them out with BHI once and combined for culturing in one tube. Medium not turbit after one hr of growth, still plated.

THURSDAY, 8/8/2019

from yesterday's electroporation: growth on standard plate, no growth on plate with kanamycin, left in the incubator for another 24 hr

Electroporation trial 2: same reagents as yesterday, 9 μl of plasmid with Amp resistance (plamid two from second batch of isolation), 1 mm cuvette, 1: 600 V, 2: 800 V, cuvettes didn't leek this time (transferred immidiately into culture tube)

FRIDAY, 8/9/2019

one colony on plate with Kanamycin (I've got hopes, don't let me down Vibrio), growth on plates with and without antibiotic, also in the negative control. FIGURE OUT AMPICILLIN RESISTANCE

TUESDAY, 8/20/2019

start of culture for chemically competent cells

WEDNESDAY, 8/28/2019

preparation of chemically competent cells and transformation

THURSDAY, 8/29/2019

colony PCR to confirm the plasmid in Vibrio after chemical transformation

wooop woop the transformation worked!

picked, and grew clones 2, 3 & 4

TUESDAY, 9/3/2019

1. isolate plasmid & try PCR again
2. try to get RFP by inducing with IPTG