Restriction Digest

Introduction

Protocol_Diagnostic Digest
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Diagnostic Digests are used to verify the g-block inserts are added to the backbone
Spe1 + EcoR1
GG_BBa_K2380044_His: 2047/1603bp
GG_BBa_K2380044_Strep: 2047/1600bp
GG_NC002505_His: 2047/1600bp
GG_NC002505_Strep: 2047/1597bp
PpChi_Li_pSB1C3: 2047/1759bp
rCutSmart NEBuffer (alternative is NEBuffer 4 + Albumin)
Ran out of SpeI
Redo
NC002505 VcCDA Sequences: SacI + NdeI w/ rCutSmart
BBa_K2380044 VcCDA Sequences: SacI + NdeI w/ rCutSmart
PpChi Sequence: SacI + NheI w/ rCutSmart
SmChiA-RM: SacI + PsiI w/ rCutSmart

pSSB1C3-PpChi: 1515 + 2291
GG_BBa_K2380044_his VcCDA: 1316 + 8 + 2326
GG_BBa_K2380044_strep VcCDA: 1316 + 8 + 2323
GG_NC002505_his VcCDA: 1324 + 2323
GG_NC002505_strep VcCDA: 1324 + 2320
SmChiA-RM-pSB1C3: 1171 + 306 + 2566
SmChiA-Wu-pSB1C3: 2872 + 1171

Materials

For each Plasmid

- Plasmid to be tested (5uL)
- enzyme 1 (0.5uL) (Spe1)
- enzyme 2 (0.5uL) (EcoR1)
- enzyme buffer (1uL) (rCutSmart NEBuffer [or NEBuffer 4 + rAlbumin)
- MilliQ water (3uL)
- 1 X TAE Agarose (2g)
- Ladder (7uL)
- Loading Dye (2uL)
- Gel red (20uL)

Protocol: Diagnostic Digest

1. To prepare the sample:
    a. add in plasmids, MilliQ water, enzyme buffer and enzymes in the stated order
    b. once all added place the mixtures in a 37C water bath for 15mins
    c. add another 0.5 uL of each enzyme into the mixture
    d. incubate in 37C water bath for 45 minutes
    e. give the mixture a short centrifuge to remove condensation
    f. add in 2uL of 6x loading dye
    g. (skip if gel is ready) if agarose gel has not been prepared, store samples at -20C
    h. load all samples (12uL) into the wells
2. To prepare a large gel:
    a. add 2g of agarose and 200 mL of 1XTAE into a bottle
    b. loose the lid of the bottle; use microwave to heat 1 min, shake and heat for another 1 min
    c. the agarose should have dissolved after heating. Use insulated gloves when handling hot bottles. Wait for the solution to cool down
    d. when it is cold enough to be held by hand, add 20 uL of gel red (1 : 10,000)
    e. mix the gel red dye by shaking the bottle slightly and pour the solution on to a mold
    f. wait for the gel to solidify; transfer the gel to the gel tank and pour sufficient amount of 1XTAE to submerge the gel
    g. load 7 uL ladder; added 2 uL purple loading dye into each plasmid sample
    h. after loading, set to 180V, 400mA to run for 40min.