Protocol for cloning antigen coding gene into mammalian cell expression plasmid

AV-01 Gateway cloning of the gene of interest into HuEV-A destination vector (LR reaction)

1.0 Introduction / Description

This method is intended for the sub-cloning of each TF gene into the HuEV-A vector (Human Expression Vector-A). The latter contains a Gateway cassette that enables the easy sub-cloning of the gene of interest from an "entry" to a "destination" vector. The TF genes are available as entry clones in our clone collection comprising the new ORF set, the Kinase set, DFCI, Genocopia and the new Invitrogen collection. The TF genes are cloned into HuEV-A destination vector through "Gateway LR reaction".

2.0 Materials

Glassware/Plasticware

96 well plate- Thermo cat.# AB600L
96 well filter plate- Nunc cat.# 278010
96 well receiver plate- Nunc cat.# 267245

Reagents

HuEV-A destination vector (Human Expression Vector-A) (150ng/ul)
Entry clone-TF
ddH_2O
70% ethanol
Isopropanol
Gateway LR Clonase II- Invitrogen cat.# 5648
DH5α competent cells (produced in the lab)
Carbenicillin LB plates
2xYT medium (Bacto Tryptone and Bacto Yeast extract medium)
Solution I: 50mM glucose
25mM Tris-HCl pH 8.0
10mM EDTA pH 8.0
0.1g/L RNAse A added
Store solution at 4°C

Solution II: 0.2M NaOH
1% SDS

Solution III: 3M Potassium acetate
11.5% glacial acetic acid

TE buffer:
Tris-HCl 10mM pH 8
EDTA 1mM

3.0 Gateway LR reaction

- ✓ Reaction mix =

HuEV-A vector	0.5ul
Clonase	0.25ul
H_2O	0.95ul
Entry clone-TF	0.8ul

- ✓ Incubate at room temperature for 1hr.
- ✓ Add 20ul of competent cells (DH5a) to the reaction mix

- ✓ Heat shock transformation at 42°C for 20''
- ✓ Plate cells in LB/carbenicillin plates (75mg/L of LB) at low (20µl) and high cell (cells spun down, resuspended in 15 µl of LB and plated) density cells
- ✓ Incubate plates overnight at 37°C.

4.0 Plasmid extraction

- ✓ With a pipette tip pick colony
- ✓ Grown picked colonies into 2xYT medium 1ml/well
- ✓ Incubate overnight at 37°C on a rotator at 230rpm speed
- ✓ Spin cells down at 3500 rpm for 5 min.
- ✓ Resuspend pellet in 100ul Solution I
- ✓ 100 µl Solution II added
- ✓ After 5 min add 100 µl Solution III
- ✓ Incubate on ice for 20 min.
- ✓ Spin at 4000 rpm for 20 min.
- ✓ Add 240 µl supernatant to filter plates attached to receiver plates.
- ✓ Spin @ 2000 rpm for 3 min.
- ✓ Add 180 µl isopropanol and incubate @-20°C for 20 min.
- ✓ Spin @4000 rpm for 25 min.
- ✓ Discard supernatant very carefully.
- ✓ Add 200 µl cold 70% ethanol and spin @4000 rpm for 25 min
- ✓ Discard supernatant and resuspend pellet in 50 µl TE buffer.
- ✓ Store at -20°C.