GATEWAY 96 well reactions (15-08-08)

(Before attempting these protocols refer to COSHH procedure and chemical assessments)

3 way Gateway reactions

Clonase mix	
Clonase LRII	1.0 µl/reaction (vortex for 10sec before use)
L1L2:ßactin:neo	100ng/reaction
L3L4:DTA:spec	1000ng/reaction
TE to a total of 2µl	

Calculate the number of reactions you require and add the relevant amounts of constituents and make up the volume with TE. Mix together and vortex for 15sec.

DNA	8µl
Clonase Mix	2µl

Centrifuge for 1min at 4000rpm.

Seal and run on a thermocycler at 25 C for 48hrs (lid temp at 30 C)

Add 1µl PK to each well
Centrifuge for 1min at 4000rpm

Incubate for 10min at 55 C.
Centrifuge for 1min at 4000rpm

The DNA can now be stored at 4°C

Transform the GATEWAY reactions in DH10B

Thaw Library Efficiency DH10B Competent Cells on ice. 1.5 Tubes per Gateway

Transfer 4µl of the Gateway reaction to a new 96 well plate.
Place the new Gateway reaction plate on ice and add 20µl of Library Efficiency DH10B Competent Cells (Mix cells before transfer to the plate)

Vortex for 10sec using the Multidrop combi shake function

Incubate on ice for 30 min

-place the plate in a 42°C water bath for 45s and transfer immediately on ice and leave for 2 min
-Add 50µl SOC medium to each well
-transfer all into a sterile 96well round bottom plate containing 150µl SOC
-transfer plate to the 37°C shaking incubator. Incubate 2hrs at 37°C
-plate 150 µl on well dried YEG-C1 plates (50 µg/ml Spec)
-spread with disposable spreaders or balls
-incubate O/N at 37°C