Oliver Hoeller, 2009-07-03

DICTY TRANSFORMATION (iGEM09)

DAY1:

1) **collect the cells you want to transform (e.g. Ax2 phdA-GFP): wash them off tissue culture plate or take them from flask in shaken suspension and count them in hemocytometer.**
   You will need 4x10 6 cells/ transformation- think how many you need in total and take the appropriate volume of cells in suspension.
   In practice you do not want to take less than 2x10 7 cells (of a solution of not less than 2x10 6 cells/ml) , else its hard to get a pellet in the next step.

2) **spin down cells (1400rpm 3minutes) in the tissue culture centrifuge**

3) **pour of supernatant and resuspend cell pellet in 30 ml ice cold H50 buffer**

4) **spin down again**

5) **resuspend cells in ice cold H50 buffer @ 4x10 7 cells/ml; keep on ice**

6) **label your electroporation cuvettes (BioRAD, brown, 0.1cm)**

7) **put the plasmid you want to transform into the cuvette (~ 2ug DNA = 10-15ul of a miniprep)**

8) **add 100 ul of your cell suspension; keep cuvettes on ice**

9) **prepare for each transformation a tissue culture plate with 11.5ml HL5 media in it.**

10) **Prepare a FALCON tube with HL5 media (1ml/ transformation)**

You and your cells are now ready for electroporation.

11) BIORAD GenePulserXcell
Switch it on.

Select EXPONENTIAL PROTOCOL:

Set	**Voltage (V):**	**750**
	Capacitance (uF):	**25**
	Resistance (Ohm):	**50**
	Cuvette (mm):	**1**

You will need to deliver 2 pulses to your samples. Here is how:

- **Dry cuvette, put it into the slot and deliver one puls.**
- **0.5ml of HL5 out of your FALCON tube and keep it in the pipette.**
- **Deliver a second puls, open the lid and gently add the 0.5ml of media and put the cuvette back on ice.**

Repeat steps with any additional samples.

12) wait 5 minutes and then pipette your sample into the pre-prepared tissue culture plate.

DAY2:

13) add the appropriate selection drug to your plates (hygromycin, final concentration: 50ug/ml)

DAY3- ~10:

Hygromycin kills non transformed cells after about 2-3 days. Check on your cells everyday – you should see clusters of resistant cells appear and grow over time until the plate is confluent.

Once your plate is confluent we freeze the cells.