

#### iGEM TU/e 2014

Biomedical Engineering

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## Transformation of vector in NovaBlue



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#### 1 Transformation of vector in NB

- Switch on the water bath and set temperature at 42°C. Also turn on the heat/shakingblock and set up to 37°C
- Load a bucket with ice from the ice machine
- Thaw the cells (NovaBlue) on ice for ~5 min
- Add 1  $\mu$ L of ligation mixture to 20  $\mu$ L NB bacteria. (Leave on ice) . Mix well. Make sure you work near the Bunsen burner flame
  - In our Case COMPx, pEVOL & Pet29A
- Incubate on ice for 5 min
- Heat shock the solution (42°C for 30 s exactly)
- Return to ice for 2 min
- Add 80 µL of SOC medium to the bacteria
- Incubate for 60 min at 37°C and 300 rpm

### 2 Plating of the cells on an agar plate

- Take a dried agar plate out of the 37°C incubator
- Open an agar plate in close proximity of the Bunsen burner flame
- Pipette 100 μL of cells on the plate
- Spread the cells on the plate using the sterile Trigalski spatula
- Transfer the agar plate to the 37°C incubator
- Place the plate upside down, closed
- Let the cells grow on the plate overnight