1. **Principe**

This a biological molecular technic which allow to digest a DNA fragment or an entire gene with restriction enzymes cutting on specific sequences. To insert DNA in a vector, it is necessary to also digest the vector with the same enzymes and then to do a ligation.

1. **Material**

* Restrictions enzymes
* Buffer specific to the enzymes
* Water nuclease free
* PCR tubes 0,2 mL
* A pipette 20 or 200 µl
* PCR products or DNA

1. **Method**

*Protocol of digestion adapted to digestion – ligation for the insertion of biobricks in pSB1C3*

**INSERT :**

* In a PCR tube, **annotated with the name of the biobrick**, introduce :
* 1 µl of EcoR1 & Pst1
* 5 µl of NEB Buffer 2.1
* 500 ng of the insert or 24 µl of the PCR product (after Clean up)
* Water to 50 µl
* Incubate at 37°C during 30min to 2h
* Incubate at 65°C (for EcoRI & PstI) during 30min to inactivate the enzymes (temperature indicate by the supplier)

**PLASMIDE :**

* In a PCR tube, **annotated with the name of the plasmid**, introduce :
* 1 µl of EcoR1 & Pst1
* 1 µl of Alkaline Phosphatase
* 5 µl of NEB Buffer 2.1
* 1 to 2 µg of the plasmid (after Clean up)
* Water to 50 µl
* Incubate to 37°C during 30min to 2h
* Incubate to 65°C (for EcoRI & PstI) during 30min to inactivate the enzymes
* Realise a **clean up** on the digestion products in order to clean it for a final volum of 50µl.
* **Mesure** the concentration of the samples**, note it & preserve** the products on the rack digestion at 4°C
* Realise a **migration by electrophoresis** of 3µl of each sample on a 1% agarose gel to control the success of the digestion.