Protocol for sequencing biobricks

First it was done a plasmid extraction of the transformed bacteria with BSLA-AWD, BSLA-RACI, BSLA-WNT, BSLA-SOD, BSLA CNT, SOD, CNT AND TERT7. The following images, shows the gels of that extractions run at 100 volts in a 1% agarose gel for 60 minutes.

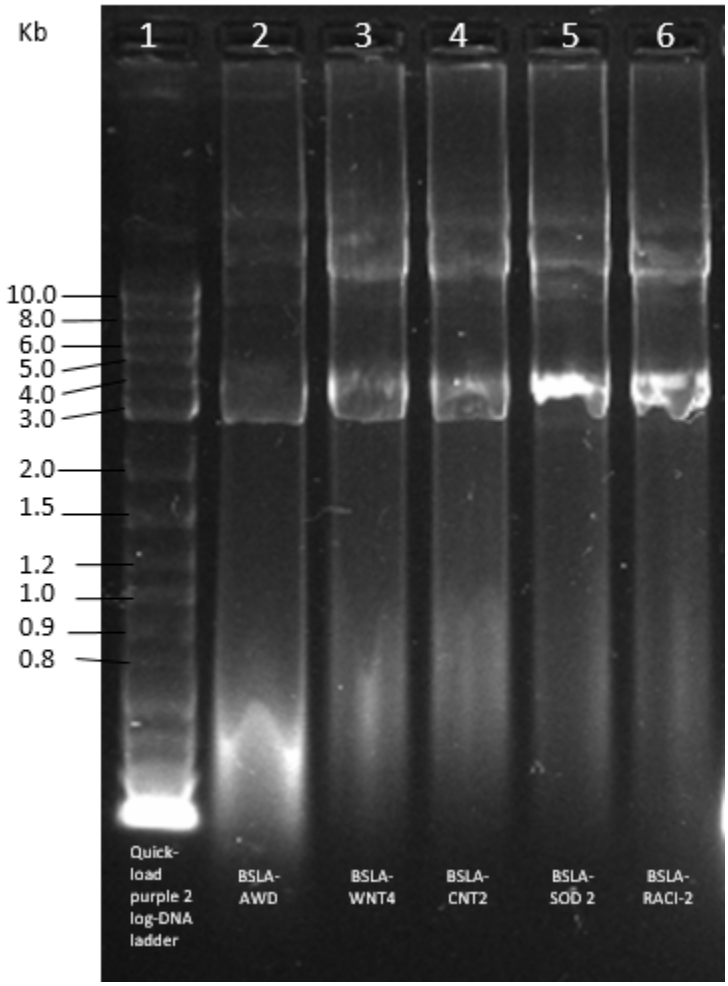

Figure 1. BSLA plasmid extractions, it was tested 5 ul of sample in a 1% agarose gel at 100 Volts for 60 minutes. It was used TBE at 1X.

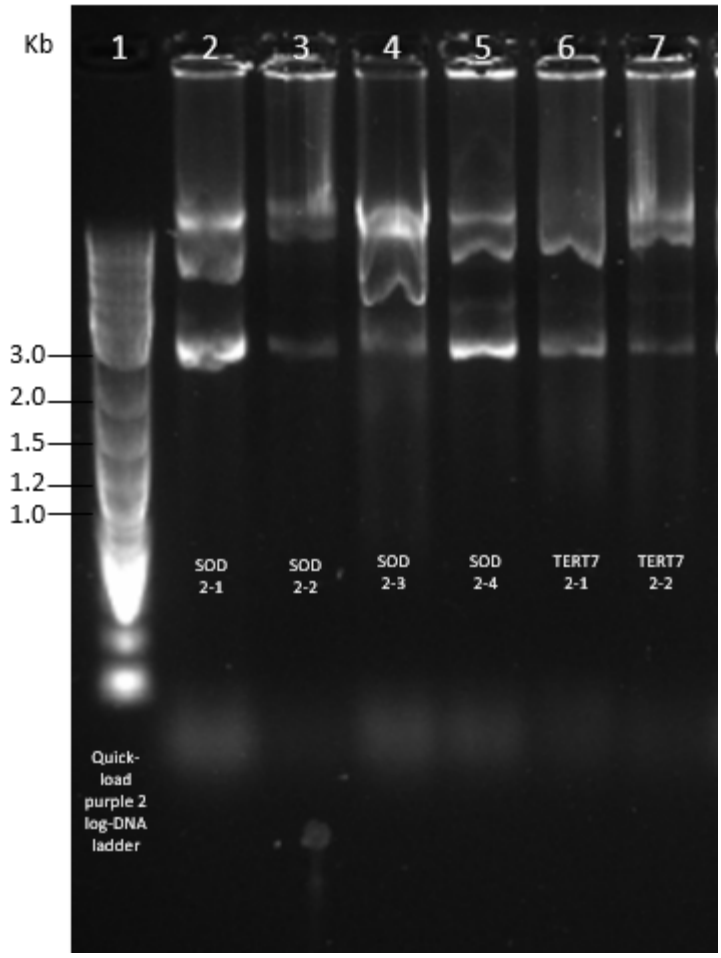

Figure 2. SOD and TERT7 plasmid extractions, it was tested 10 ul of sample in a 1% agarose gel at 100 Volts for 60 minutes. It was used TBE at 1X.

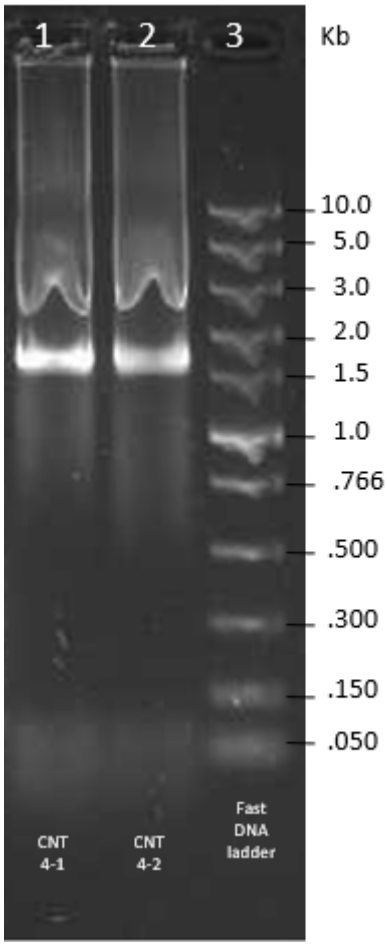

Figure 3. CNT plasmid extractions, it was tested 10 ul of sample in a 1% agarose gel at 100 Volts for 60 minutes. It was used TBE at 1X.

The samples chosen for the sequencing were the following*: BSLA AWD, BSLA RACI-2, BSLA WNT-4, BSLA-SOD2, BSLA CNT-2, SOD 2-4, CNT4*-1 *and TERT7 2-1*. The concentration of the samples were measured using a Synergy H4 plate reader. The following table shows the concentration of each sample.

Table 1. Concentration of the samples for the sequencing.

Sample	Concentration (ng/µL)
BSLA-AWD	295.33
BSLA-RACI-2	167.175
BSLA-WNT-4	161.17
BSLA-SOD-2	150
BSLA-CNT-2	197.97
SOD 2-4	199.35
CNT 4-1	236.05

TERT7 2-1	170

It was decided to use 280 ng of DNA for the PCR. The sequencing reaction was prepared using the following quantities of the reactants in the next table:

Table 2. Quantities of reactants for the sequencing reaction.

Reactant	**Stock Concentration**	**Final Concentration**	**BSLA-AWD**	**BSLA-RACI-2**	**BSLA-WNT-4**	**BSLA-SOD-2**	**BSLA-CNT**	**SOD 2-4**	**CNT 4-1**	**TERT7 2-1**	**Master Mix**
Ready Reaction Premix	2.5X	0.5X	4 µL	4 µL	4 µL	4 µL	4 µL	4 µL	4 µL	4 µL	32 µL
BigDye sequencing buffer	5X	0.5X	2 µL	2 µL	2 µL	2 µL	2 µL	2 µL	2 µL	2 µL	16 µL
DNA sample			0.95 µL	1.67 µL	1.74 µL	1.87 µL	1.41 µL	1.40 µL	1.19 µL	1.65 µL	-----
Deionized water			12.05 µL	11.33 µL	11.26 µL	11.13 µL	11.59 µL	11.6 µL	11.81 µL	11.35 µL	-----
Primer (VF2)	10 µM	1 µM	1 µL	1 µL	1 µL	1 µL	1 µL	1 µL	1 µL	1 µL	8 µL
Final volume	------	------	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL	20 µL	56 µL

Then it was done the sequencing reaction in the thermocycler using the following program:

Table 3. Thermocycler program for the sequencing reaction.

Temperature	Time	Cycles
96°C	30 s	25
50°C	15 s	25
60°C	4 min	25
4°C	Hold	----

Once the reaction was finished, the sample was purified to eliminate the marked nucleotides that weren´t incorporated during the reaction. The purification process was done with columns (Centri-Sep spin columns, Applied Biosystems). The columns were re-hydrated 2 hours before they were used with 0.8 ml of deionized water and mixing by inversion. At the beginning of the rehydration bubbles were eliminated (tapping the lower part of column).

Then the tops of the column were removed and was retired as much as possible water by gravity (it was necessary to apply a little pressure at the top of the column). After that the column was placed in a collector microtube and was centrifugated at 2500 RPM for 5 min.

The column was placed in a new microtube and the volume of the samples was placed (20 µL) in the center of it without touching it, then it was centrifugated at 2500 RPM for 10 min (It is important to take care that the column in the same orientation that in the last centrifugation). After that the column is discarded and the sample was at the bottom of the tube.

The sample was dried for 40 minutes at medium temperature and then stored at -20°C until its sequencing in a ABI PRISM 310 equipment.