Cycle sequencing protocol

For each sample, the target gene will be sequenced in both forward and reverse directions. Therefore, we need to prepare **two separate cycle sequencing reactions per sample**.
Osea, dos placas.

1. Make BigDye mix.

	X1	X72	X81
Nuclease-free H_20	6.25µl	450 µl	506.25 µl
BigDye Buffer	1.75µl	126 µl	141.75 µl
10 µM Forward **OR** Reverse primer	0.50 µl	36 µl	40.5 µl
Primer BigDye	0.50µl	36 µl	40.5 µl
DNA Sample	1.00µl		
TOTAL	**10 µl**	**648 µl**	**729 µl**

** BigDye is light sensitive, so keep it in freezer until you need to add.

2. In a clean 96-well plate, add to each well:
9 µl BigDye mix + 1 µl cleaned PCR product (from SPRI bead clean-up)

3. Run in thermocycler:

95°C for 0:30 sec 50°C for 0:30 sec 60°C for 4:00 min	35x
10°C	forever

** Upon completion, BigDye plates must be stored in the refrigerator (4°C), **not in** the freezer.

Protocol

1. Prepare two (**2**) **BigDye mix** tubes, one for the forward reaction and one for the reverse reaction. Both of X81 volume.
2. Agregas 9 µl de BigDye mix a cada pocillo (72) y 1 µl del clean PCR product.
3. Corres en el termociclador con el programa guardado como BigDye.