

# 5' Phosphorylation of primers with T4 PNK

VENDREDI 08/10/2021

- Add the following components to a microcentrifuge tube:

Table1		
	A	B
1	Component	Volume
2	Oligonucleotide	300 pmol
3	T4 Polynucleotide Kinase Reaction Buffer 10X	5 µl
4	T4 Polynucleotide Kinase 10 U/µl (NEB# M0201S/L )	1 µl
5	ATP 10mM	5 µl
6	H2O	to a final volume of 50 µl

- Incubate the reaction at 37°C for 30 minutes.
- Inactivate the T4 Polynucleotide Kinase at 65°C for 20 minutes. Alternatively PNK reaction can be phenol-extracted and ethanol precipitated or purified with a commercial kit.
- The reaction products can be stored at -20°C or added directly to the mutagenesis reaction.