Ligation

Information:
- Add vector + insert to ligate them together (insert gotten from PCR), afterwards transformation into bacteria
- Before: PCR, Gel, Nanodrop, Restriction digestion of Plasmid and Insert with same enzymes
- After: Transformation (DH5alpha: Heatshock)
- Time: 30 mins

What we need:
- DNA (=Insert)
- Vector (=Plasmid)
- ddH2O (MilliQ / deionized and distilled)
- T4 DNA Ligase Buffer
- T4 DNA Ligase

Procedure:
- We want to add the vector and the insert in a 1:1 up to 1:5 ratio. We have the concentration from the Nanodrop and the size from the Gel (compare to our knowledge), which we can use to calculate the number of insert-molecules and then the volume of the insert we need to add.
- Important: Ligase Buffer needs to be kept on ice at all times, Ligase needs to be kept at **-20°C AT ALL TIMES**! There's a frozen rack in freezer that can be used for this, ask Arpan to show you how to do this!
- Ligation mixture:
Bold is fixed, *Italics* need to be calculated. Split up into 5µl and 15µl so that we can prepare some for all together, makes pipetting easier and faster. Doesn't have to be perfectly accurate, just want to achieve a 1:1 / 1:5 ratio V:I

			Example for 7x
Vector	**1.5µl**	**5µl** do this in 7 separate tubes	**1.5µl**
Insert	*0.9*	*0.9*	
H2O (MilliQ)	*2.6*	*2.6*	
Ligase Buffer	**2µl**	**15µl**	**14µl**
Ligase	**0.2µl**	**1.5µl**	
H2O (MilliQ)	**12.8µl**	**89.6µl**	
Total:	**20µl**		**~140µl**

- Calculations example:
  Size I from Gel / Knowledge
  V from Knowledge
  Size.V from Knowledge
  Conc. I from Nanodrop

Size Insert	SizeI * (conc.V / Size V) =	Amount I	Conc.I/AmountI =	Vol I
4.5kb	4.5 * (40/6) =	30 ng	25ng / 30 ng =	~0.9

- Mix everything together (tube for each insert+vector, and a control tube with just the vector) on ice
- leave at RT for 10-30 mins (prepare Transformation things: thaw bacteria on ice, warm up LB)
- After 30mins, put on ice to stop reaction
- Proceed with Transformation