

# Digestion

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July 21, 2010

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<b>Where</b>	On iGEM wetbench
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	<b>Quantity</b> <b>What</b>
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	1-5 $\mu$ g   DNA (volume depends on concentration!)
<b>Material needed</b>	<i>The volume of DNA should be 50% of the Total Volume(Tot.V)</i>
	10%Tot.V   Buffer 2 (10 $\times$ )
	1%Tot.V   BSA (100 $\times$ )
	5%Tot.V   Each restriction enzyme
	ddH <sub>2</sub> O   to reach the Tot.V
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<b>Steps</b>	
	1. Calculate the DNA prep volume
	2. by knowing that the total volume is the double of the DNA prep volume, calculate the volumes of Buffer 2, BSA, restriction enzymes, and ddH <sub>2</sub> O.
	3. Sometimes the volume of BSA is really small and will not be possible to pipett it. In those cases dilute the solution to obtain BSA(100 $\times$ ), and take 10%Tot.V instead 1%Tot.V. Adapt then the ddH <sub>2</sub> O volume.
	4. Mix everything together, the enzymes should be added last.
	5. keep the solution at 37° for 1-2 hours.
<b>Warnings</b>	
	<ul style="list-style-type: none"><li>• Enzymes has to be added as the last thing!</li></ul>

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