



# Sanger Samples Preparations

Use only PCR tubes, strips or plates.

## Plasmid samples:

- Take **300 ng-600 ng** of plasmid in a maximum volume of 14  $\mu\text{L}$ .

Example: When the plasmid concentration is **40 ng/ $\mu\text{L}$** , calculate the required volume using this formula:

$$\frac{W[\text{ng}]}{C[\frac{\text{ng}}{\mu\text{L}}]} = V[\mu\text{L}] \text{ e.g., } \frac{450 \text{ ng}}{40 \frac{\text{ng}}{\mu\text{L}}} = 11.25 \mu\text{L}.$$

Add the calculated volume of the purified plasmid to a PCR tube.

- If the volume required exceeds 14  $\mu\text{L}$ , add 14  $\mu\text{L}$ .  
(Note: This situation might lead to low-quality sequencing results).
- Add 1  $\mu\text{L}$  primer (10 $\mu\text{M}$  stock)
- Complete the volume to 15 $\mu\text{L}$  with PCR-grade water.
- After mixing well, take 7.5 $\mu\text{L}$  to a new PCR tube/strip/plate well.
- Clearly write the reaction name on the tube cap and sides.

## PCR products:

- Calculate the required amount of PCR product ng using the formula below.

Example: For a PCR product of size **450 bp** at a concentration of **50 ng/ $\mu\text{L}$** , the

$$\text{calculation is: } \frac{0.03 \cdot \text{length of PCR product [bp]}}{\text{sample concentration } [\frac{\text{ng}}{\mu\text{L}}]} = V[\mu\text{L}] \text{ e.g., } \frac{0.03 \cdot 450 [\text{bp}]}{50 [\frac{\text{ng}}{\mu\text{L}}]} = 0.27 \mu\text{L}$$

If the required volume is too small to pipette, dilute the samples 1:10 and take 2.7  $\mu\text{L}$ .

\* If you use Exozap or any other enzymatic cleanup kit, use 4-8  $\mu\text{L}$  of the PCR product.

- Add 1  $\mu\text{L}$  of primer (10 $\mu\text{M}$  stock)
- Complete the volume to 15  $\mu\text{L}$  with PCR-grade water.
- After mixing well, take 7.5 $\mu\text{L}$  to a new PCR tube/strip/plate well.
- Clearly write the reaction name on the tube cap and sides.



## Calculate primers Tm:

\* *If multiple samples have Tm values that fall within the same temperature*

*range in the table, group them in strips or plates instead of single tubes.*

For each primer you use, calculate the melting temperature (Tm) using the following calculator in the link:

- Go to <http://insilico.ehu.es/tm.php>
  - Add your primer sequence into the top line.
  - Select the second option: **Base-Stacking Tm**
  - Adjust the following parameters as shown in the screenshot below.
  - Click on **Compute Tm** button at the top of the page.
  - Your Tm value will appear at the bottom of the page in red.
- Repeat this process for each primer you are using.

40 - 44.9 °C

45 - 49.9 °C

50 - 54.9 °C

55 - 60 °C

Above 60 °C

## Melting Temperature (Tm) Calculation

1 — **Primer (6-50 bases):**  
GTATGTGTATATATATGT — 4

LENGTH 20  
C+G% 25  
Molecular weight: 6272.715

[Basic Tm](#)  
Degenerated nucleotides are allowed

2 —  [Base-Stacking Tm](#)  
Degenerated nucleotides are NOT allowed

Primer concentration: 500 nM  
Salt concentration: 50 mM — 3  
Mg<sup>2+</sup> concentration: 1.5 mM

Tm: 50.7 °C — 5  
Enthalpy: -145.6  
Entropy: -419.42

Source code is freely downloadable at [biophp.org](http://biophp.org)





## Submission Instructions:

- Download the submission form from: <https://iki-labs.bgu.ac.il/genomic-analysis-2/> .
- Fill out the form (manual or automatic) and attach it to the samples when submitting.
- Alternatively, you can email the completed form to [avishagc@post.bgu.ac.il](mailto:avishagc@post.bgu.ac.il) and [shirandr@bgu.ac.il](mailto:shirandr@bgu.ac.il), and submit the samples with a note containing your name, phone number and lab.
- Submit the samples to **building 39, room 205 2<sup>nd</sup> floor**. In the fridge on the 2<sup>nd</sup> shelf from the bottom there is a tray with PCR tube stands for your use.
- Outside of working hours, you can submit the samples to the mailbox at the entrance to the unit. When submitting via mailbox, samples must be placed in a closed Ziplock bag with your form\note. Make sure your tubes are sealed!
- Samples are collected from the mailbox at 10:00 (AM) every day from Sunday to Thursday.

Thank you for your cooperation,

BGU Genomics

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