Sanger Samples Preparations

Use only PCR tubes, strips or plates.

Plasmid samples:

- Take 150 ng-300 ng of plasmid in a maximum volume of 7 µL.

  Example: When the plasmid concentration is 70ng/µL, calculate the required volume using this formula:

  $\frac{150[ng]}{C[\frac{ng}{\mu L}]} = V[\mu L]$ e.g., $\frac{150\ ng}{70\ \frac{ng}{\mu L}} = 2.14\ \mu L.$

  Add 2.2 µL of the purified plasmid to a PCR tube.
- If the volume required exceeds 7 µL, add 7 µL.

  (Note: This situation might lead to low-quality sequencing results).
- Add 0.5 µL primer (10µM stock)
- Complete the volume to 7.5µL with PCR-grade water.
- Clearly write the reaction name on the tube cap sides.

PCR products:

- Calculate the required amount of PCR product ng using this formula:

  $\frac{0.015 \cdot length\ of\ PCR\ product\ [bp]}{sample\ concentration\ [\frac{ng}{\mu L}]} = 0.135$

  Example: For a PCR product of size **450** bp at a concentration of **50** ng/µL, the calculation is: $\frac{0.015 \cdot \mathbf{450}\ [bp]}{\mathbf{50}\ [\frac{ng}{\mu L}]} = 0.135$ µL.

If the required volume is too small to pipette, dilute the samples 1:10 and take 1.35 µL.

* If you use Exozap or any other enzymatic cleanup kit, use 1µL of the PCR product.

- Add 0.5 µL of primer (10µM stock)
- Complete the volume to 7.5µL with PCR-grade water.
- Clearly write the reaction name on the tube cap and sides.

Shiran Dror, Ph.D. | Advanced Genomics
Phone: +972-(0)8-6479085
P.O.B 653, Be'er-Sheva 84105, Israel
https://iki-labs.bgu.ac.il/
e-mail: shirandr@bgu.ac.il

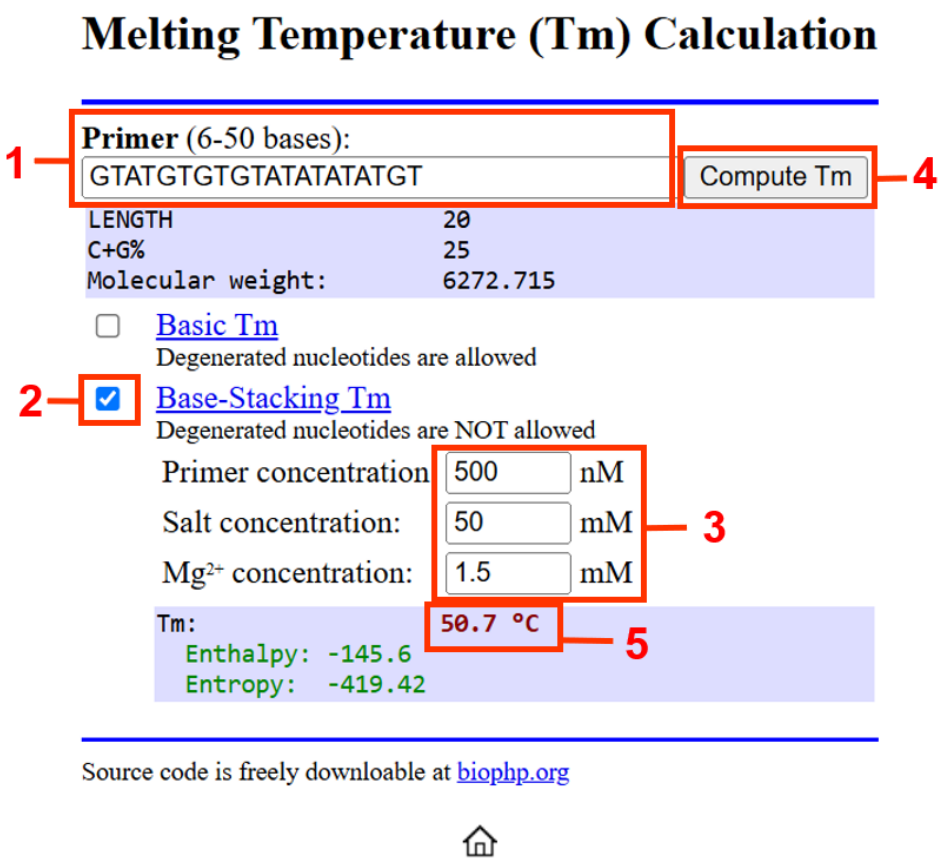
Calculate primers Tm:

40 - 44.9 °C
45 - 49.9 °C
50 -54.9 °C
55 – 60 °C
Above 60 °C

* 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, calculated the melting temperature (Tm) using the following calculator in the link:

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



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e-mail: shirandr@bgu.ac.il

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 and 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 2nd floor***. In the fridge on the 2nd 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. Whan submitting via mailbox, samples must be placed in a closed Ziplock bag a 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,

Avishag Cahana

avishagc@post.bgu.ac.il

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