Protocol: Designing DNA-Primers for PCR

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1 Purpose

This protocol is intended for those wanting to design DNA oligomers to be used as primers for PCR. This protocol will not describe how to identify the sequence you wish to amplify, since this is variable depending on what you wish to accomplish. Therefore, a prerequisite for this protocol is to already have a target sequence in mind.

2 Method

1. Identify the first 18–21 base pairs at the 5'-end of the sequence which has a T_m within 1°C of the preferred T_m.
2. Ensure that the $\Delta G°$ for this oligonucleotide's homodimer is ≥ -10 kcal.
3. It is preferred if the base pair at both the 5'-end and the 3'-end are G/C pairs (a G/C-cap), although, if this is not possible, then prioritize the 3'-end.
4. Repeat steps 1–3 for the 3'-end of the sequence.
5. Ensure that the $\Delta G°$ for these oligonucleotides' heterodimer is ≥ -10 kcal/mol.
6. Repeat steps 1–5 for each sequence of interest.

3 Tips and Troubleshooting

- You can use Benchling for all T_m and $\Delta G°$ calculations Figure 1.
  - Concentrations can be changed by selecting the wrench next to the T_m estimate under the header **Verify**.
  - Reaction temperature can be changed next to the button '`Check Secondary Structure`' next to the header **Verify**.
- The order of importance for each parameter is as follows: $\Delta G°$ (homodimerization) $\geq T_m >$ $\Delta G°$ (heterodimerization) $>$ G/C-cap $>$ length. **Only reference this hierarchy once you have tried to optimize all parameters.**
- Attempt to obtain a similar T_m for both primers (within 0.5–1°C).
- All calculations should be done before appending 5'-homology if amplicons are to be used in homology-mediated recombination (e.g., Gibson cloning).

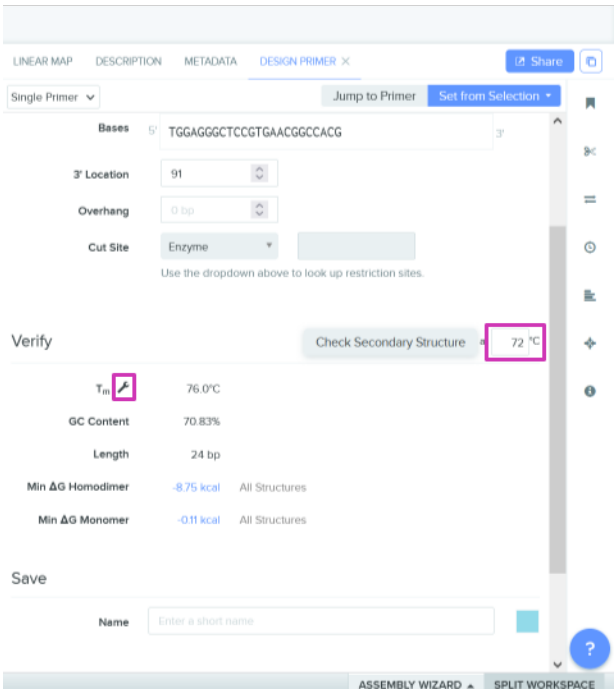

Figure 1: Screenshot from Benchling's primer editor. To open, **Right-click** DNA selection and select `Create Primer`.