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 Materials

- Sequence(s) of interest (FASTA format)
- Prefered T_m for polymerase

3 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 prefered T_m.
2. Ensure that the ΔG° for this oligonucleotide's homodimer is ≥ -10 kcal/mol.
3. It is prefered 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 (e.g., 5'-$(\text{N})_n$(G/C)-3' rather than 5'-(G/C)$(\text{N})_n$-3).
4. Repeat steps 1–3 for the 3'-end of the sequence of interest.
5. Ensure that the ΔG° for these oligonucleotides' heterodimer is ≥ -10 kcal/mol.
6. Repeat steps 1–5 for each sequence of interest.

4 Tips and Troubleshooting

- You can use Benchling for all T_m and ΔG° calculations.
- Attempt to obtain a similar T_m for both primers.
- The order of importance for each parameter is as follows: ΔG° (homodimerization) $\geq T_m >$ ΔG° (heterodimerization) > G/C-cap > length. **Note: All parameters are still important. Only reference this hierarchy if you have tried everything to optimize all parameters.**
- All calculations should be done before appending 5'-homology if amplicons are to be used in homology-mediated recombination (e.g., Gibson cloning).
- Ensure that concentrations of PCR components are accurate when predicting values for T_m and ΔG° (i.e., [Mg^{2+}], [primers], [template DNA]).