

PCR Optimization: Primer Concentration

- ▶ Primer concentration in a common PCR reaction is about 100-500 nM per primer.
- ▶ Increasing primer concentration may improve the outcome of the PCR reaction and should be considered as a way to optimize PCR reactions.
- ▶ Increasing primer concentrations too much may inhibit the reaction.
- ▶ Use the following conversion factors for primers in your PCR:
330 g mol⁻¹ x bp = molecular weight of ssDNA
660 g mol⁻¹ x bp = molecular weight of dsDNA
- ▶ Example of primer dilution: How do I make a 10 mM stock solution starting with 24 nmol of oligo?
Start by converting mM into nmol/ml: 10 mM = 10 mmol/ml. Then figure out what volume you need to resuspend your oligo in based on your starting amount: 10 mmol/ml = 24 nmol/X ml, solving for X = 2.4 ml or 2400 µl.