Protocol for annealing oligos

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1. Mix 200 ng of each oligo

2. Incubate the following reaction for 3 min at 100 °C.

    Primer 200 ng
    Buffer H 5 μl
    Water up to 50 μl

3. Incubate for 5 min at 65-70 °C.

4. Cool down the reaction at RT.

* In ligation reaction, vector:oligo ratio should be 1:9.

Primer (5 μM)	1 μl each
Buffer H*	5 μl
Water	43 μl

→ 3 min at 100 °C.

→ 5 min at 70 °C.

→ Cool down the reaction at RT

→ Primer concentration: 100 nM

20 ng/μl of vector (6000 bp) = 5 nM

*H buffer: from Roche; restriction enzyme buffer
    50 mM Tris HCl
    10 mM $MgCl_2$
    100 mM NaCl
    1 mM Dithioerythritol (DTE)
    pH 7.5 at 37 °C

To convert μg to pmol:

$$\mu g \times \frac{10^6 pg}{1 \mu g} \times \frac{pmol}{330 pg} \times \frac{1}{N} = pmol$$

where N is the number of nucleotides and 330pg/pmol is the average MW of a nucleotide.