

## ssDNA Annealing

- ◆ Annealing with H<sub>2</sub>O
  - ◇ Dilute ssDNA in nuclease-free H<sub>2</sub>O.
  - ◇ Mix equimolar volumes of both ssDNA strands.
  - ◇ Incubate for 1 h at room temperature.
  - ◇ Store at 4 °C or -20 °C
- ◆ Annealing with HEPES buffer
  - ◇ Dilute ssDNA in nuclease-Free H<sub>2</sub>O (final concentration = 100 μM)
  - ◇ For each reaction (100 μL):
    - 5 μL of each ssDNA strand.
    - 90 μL HEPES buffer (30 mM)
  - ◇ Thermocycler conditions:

°C	m:s
95	5:00
Gradient to 4	~0.1 °C/minute

- ◇ Store at 4 °C or -20 °C

◆ Annealing with 1x Composite Buffer

◇ 1x Composite Buffer:

- 10 mM Tris-HCl
- 50 mM NaCl
- 1 mM EDTA

◇ Dilute ssDNA in 1x Composite Buffer.

◇ Mix equimolar volumes of both ssDNA strands.

◇ Thermocycler conditions:

°C	m:s
95	5:00
Gradient to 4	~0.1 °C/minute

◇ Store at 4 °C or -20 °C