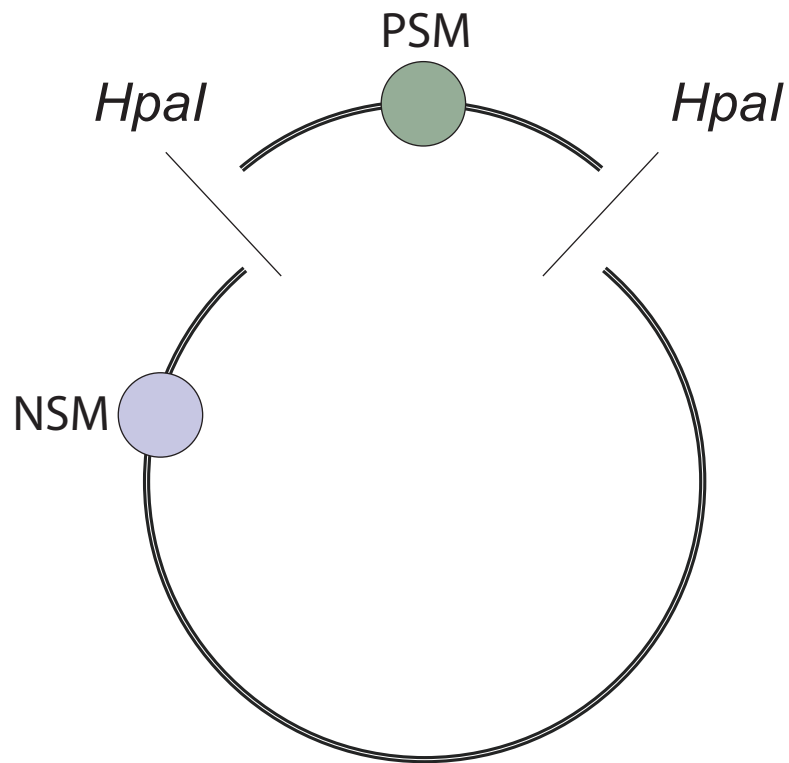
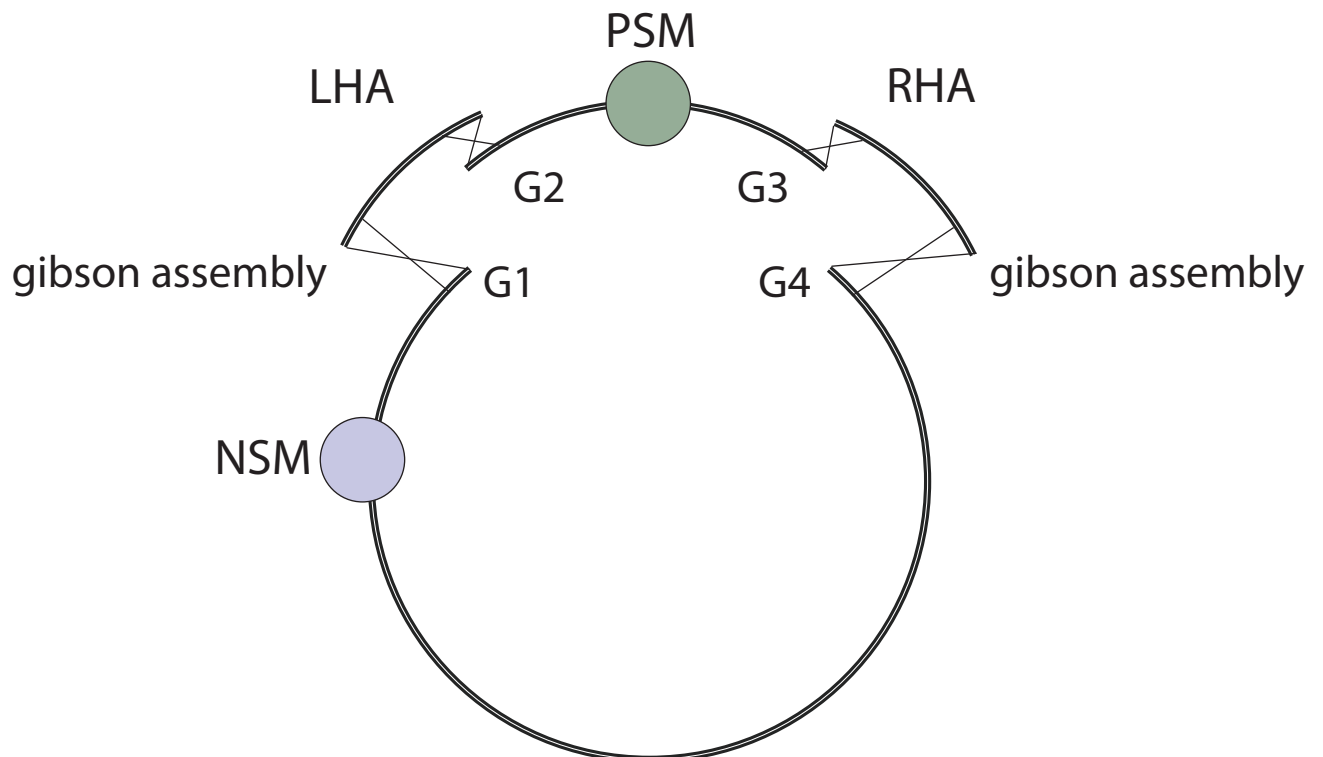


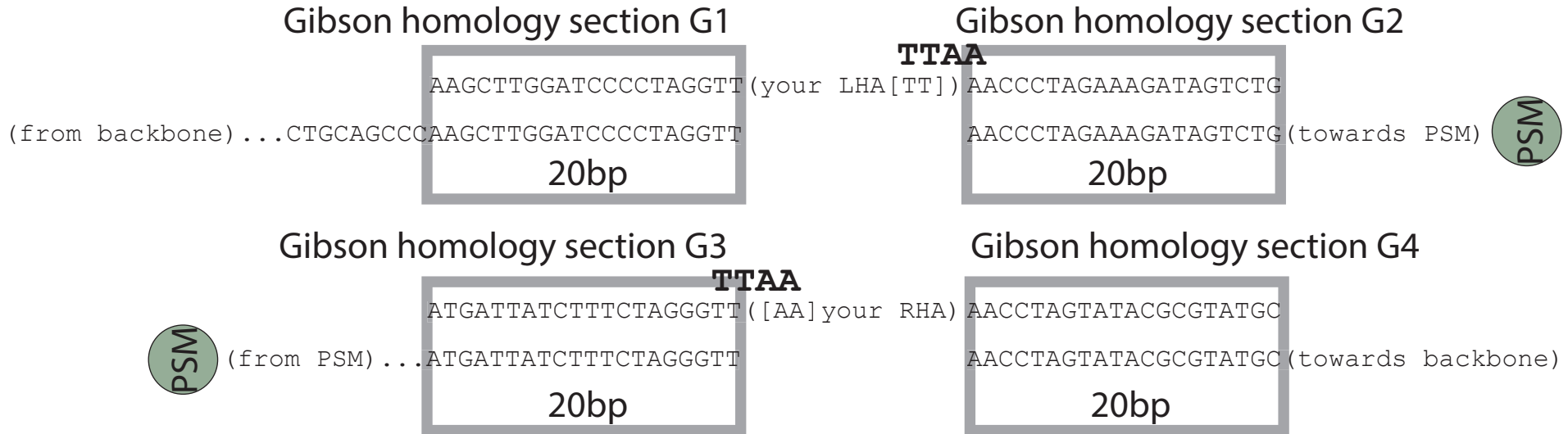
1. Digest with *HpaI*, column purify and nanodrop



2. one-step Gibson assembly with homology arms, molar ratio 1:1:1



in detail Gibson assembly sections



in detail homology arms

Left homology arm

20bp 20bp

AAGCTTGGATCCCCTAGGTT (your primer sequence [Ta~60C])

5' -AAGCTTGGATCCCCTAGGTT (.....your LHA.....) AACCTAGAAAGATAGTCTG-3'
 3' -TTCGAACCTAGGGGATCCAA (.....your LHA.....) TTGGGATCTTTCTATCAGAC-5'

(your primer sequence [Ta~60C] [AA]) TTGGGATCTTTCTATCAGAC

Right homology arm

20bp **TTAA** 20bp

AAGCTTGGATCCCCTAGGTT ([AA] your primer sequence [Ta~60C])

5' -ATGATTATCTTTCTAGGGTT (.....your RHA.....) AACCTAGTATACGCGTATGC-3'
 3' -TACTAATAGAAAGATCCCAA (.....your RHA.....) TTGGATCATATGCGCATAACG-5'

(your primer sequence [Ta~60C]) TTGGATCATATGCGCATAACG

AATT