Gibson assembly

Using pre-mixed Gibson master mix (A-MM), the assembly is done in accordance with standard lab protocols:

1. PCR amplification and digested fragments are purified (see above protocol)
2. DNA concentrations are determined using the NanoDrop (duplex DNA)
3. Fragments should be used in equimolar masses (biggest fragment as reference ≤ 100 ng)
   Volumes for assembly are calculated using the following equation:

$$Insert\ quantity\ (ng) = Vector\ quantity\ (ng) \times \frac{Insert\ size\ (bp)}{Vector\ size\ (bp)}$$

   This is a 1:1 ratio, for better yield try a 3:1 or even a 10:1 ratio (insert : vector).
4. The calculated volumes are added to one 15 µL aliquot of A-MM
5. One control was prepared for each PCR product by adding 4 uL water instead of fragment.
6. Incubation at 50 °C for 1 h (Thermocycler)
7. Use 10 µL of the assembly mixture for transformation (see Heat shock transformation)