Workflow plasmid/primer construction:

Plasmid:
--> Find MCS
--> Search for uniquie restriction sites (RS) in the plasmid, and search for absent restriction sites in the GOI -> write the RS/Enzymes down, select

GOI:
--> Identify start / stop: ATG and Stop already on gene usually.
--> Check if N-terminal of GFP or C-terminal of GFP (= before / after) (if combining with a fluorophore)
--> take the annealing temperature (60°C) considering the start/stop codons [1,2]
--> copy/paste these forward and reverse "pre-primers"
--> construct the primers

(1) Whether you keep the ATG of the GOI depends on how big the first gene is (if there is a gene before your GOI). If the gene before is small do not keep the ATG in your GOI! May mistreat the second ATG as the real ATG... Do not keep the Stop if you plan on having a gene after GOI

(2) Annealing temp should be around 60°C (measured over the part of the sequence that anneals), and the primer should stop with G or C

Construction of the primers

Forward

Copy "pre-primer" from beginning of GOI (including ATG) with annealing temperature of 60°C, end with G/C
check that it's unique

Add the restriction site to the 5' end

Add 3 random nucleotides to the 5' end (for pol to sit on)

Add FAN between RS and ATG

Reverse

Copy "pre-primer" from end of GOI (including Stop) with annealing temperature of 60°C, end with G/C
check that it's unique

-> **reverse complement** "pre-primer"

Add the restriction site to the 5' end (same if reverse-complemented^^)

Add 3 random nucleotides to the 5' end

5' - NNN - RS - FAN - ATG - "pre-primer" - 3' -(GOI) 5' - NNN - RS - TTATTA - "pre-primer" - 3' -(GOI)

FAN: Frame adjusting nucleotides. We have to consider that we want our gene to be in frame, so we may have to add a few nucleotides to the forward primer before the ATG (and if there already is an inserted gene after GOI: add FANs behind gene so that next gene also in frame)
To check if in frame: make sure you're looking at the plasmid where we introduced GOI already! Select region from ATG of gene before (GFP) until ATG of GOI, "Length" shows lenght of selected region + <#>, and the # is how many nucleotides we have from the last complete codom, ZB: ATG : 3 <0> , ATGT: 4 <1>
When adding FAN, watch out that you do not create a stop codon, and try to build a simple, non-harmful AA like glycine