VPS13D DNA plasmid generation

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Due to low expression of big DNA constructs (>10kb), we decided to generate a codon-optimized cDNA encoding for human VPS13D, including an mScarlet fluorescent protein after aminoacid 1576 flanked by BamHI restriction enzyme sites. The construct was generated by and purchased from Genscript. From this initial construct, we generated several VPS13D constructs. The specific primers and enzymes used for each construct are included in Table 1 of our manuscript: https://doi.org/10.1083/jcb.202010004

For most of our cloning procedures, Infusion cloning (Takara) was used. The steps for these cloning procedures are:

1. Linearize the backbone with restriction enzymes or by PCR amplification.
2. Amplify the insert by PCR with primers including 15nt of overlapping sequence with the target backbone.

Suggestion: Takara has an online tool to help with primer design at: https://go.shr.lc/3daJf0E

3. Run both products in an agarose gel to confirm expected size and purify the DNA from gel using a NucleoSpin Gel and PCR Clean-up kit (Takara).
4. Ligate the linearized backbone and the insert with the Infusion enzyme mix (Takara).

Suggestion: We found that big DNA constructs accumulate mutations easily when amplified in bacteria, this can be minimized by growing bacteria at 30ºC.