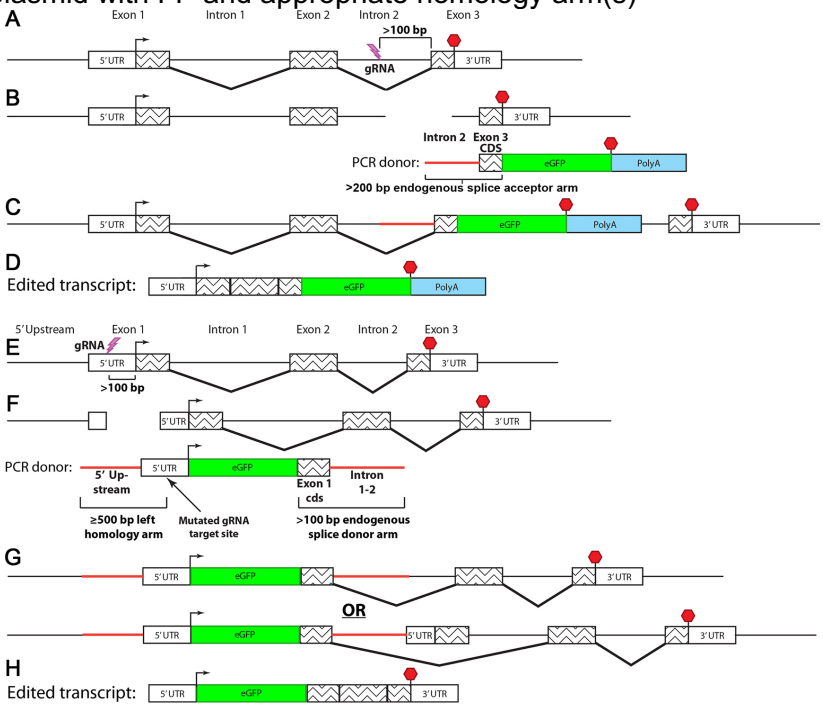
Synthetic gRNAs (adapted from Hoshijima et al, Developmental Cell, 2019):

1. Anneal crRNA (100 uM) and tracerRNA (100 uM) at a 1:1 ratio (both RNAs are in Duplex buffer from IDT)
    1) 4C for 3:00
    2) 95C for 5:00
    3) 25C ramping down 0.1C/sec for 1:00
    4) 4C for 0:00 ramping down 0.5C/sec and put on ice
2. Make injection cocktail (2 ul)
    - 0.2 ul of Cas9 mRNA (100 ng/ul)
    - 0.3 ul of donor plasmid with 30 bp homology arms (~30 ng/ul)
    - 0.5 ul of crRNA gene specific gRNA (12.5 uM)
    - 0.5 ul of ugRNA universal gRNA (100 ng/ul)
    - 0.5 ul of phenol red
3. Inject 2.3 nl into single cell fertilized embryos

Levic Method (adapted from Levic et al, Development, 2021):

1. Make plasmid with FP and appropriate homology arm(s)



2. In vitro transcribe gRNAs (both ugRNA and target-specific) the normal way (I use T7 hi-scribe)
3. Injection cocktail (5 ul total):
    - 0.875 ul of cas9 protein (7 uM)
    - 1.1 ul of gRNA (150 ng/ul)
    - 0.37 ul of ugRNA (150 ng/ul)
    - 1.67 ul of A2 plasmid (25 ng/ul)
    - 0.985 ul of phenol red
4. Inject 2.3 nl into single cell fertilized embryos