Designing an ssODN template for HR-mediated repair

5′—(~60-nt 5′ homology) **CCNNNN(attP sequence)NNNNNGG** (~60-nt 3′ homology)— 3′

- **PAM**
- **NNN** corresponds to the 3-nt adjacent to the **PAM** site that remain following Cas9 cleavage. In this example, the 5′ PAM site is on the antisense strand and the 3′ PAM site is on the sense strand.
- **attP sequence (50-nt):**
  
  CTACGCCCCCAAACTGAGAGAActCAAAGGTACCACCAGTTGGGGCACTAC

Single-stranded oligonucleotides (ssODNs) of up to 200-nt can be ordered from Integrated DNA Technologies and other companies. Do not have the oligonucleotide PAGE-purified, as this can introduce trace chemical contaminates that reduce embryo viability.