Messing about with vectors, using PCR and NEB HiFi assembly

The General Idea

**DNA Preparation**

From:
- PCR
- Restriction enzyme digestion
- Synthetic DNA (e.g., gBlocks)

**NEBuilder HiFi DNA Assembly Master Mix**

**Single-tube reaction**
- Exonuclease chews back 5’ ends to create single-stranded 3’ overhangs
- DNA polymerase fills in gaps within each annealed fragment
- DNA ligase seals nicks in the assembled DNA

**Transformation**

**DNA Analysis**

RE Digest OR Colony PCR OR Sequencing

From the NEB HiFi Manual, page 6:
1. **Design:**
   a. Piece together your sequences on the computer, as you intend them to be.
   b. Identify where there are junctions between different existing sequences.
   c. Design PCR primers that will amplify your sequences with ~25bp overlap. For applications like gRNA generation, primers for the vector should lack homology to allow re-use for different inserts, in this case the homology lies only on the insert primers (see above figure, seq in blue).

2. **PCR the parts:**
   a. Amplify the products at the appropriate temperature for each. For linearizing vectors NEB recommends ~10-100pg of template.

3. **Digest with DpnI:**
   a. Combine 1/10th volume 10x CutSmart buffer and 1/10th volume DpnI in ~10-20ul of reaction.
   b. Incubate at 37C for 30min-1hour.
   c. Heatkill at 80C for 20min.
   d. Proceed directly to HiFi assembly, without cleanup.

4. **Use HiFi assembly**