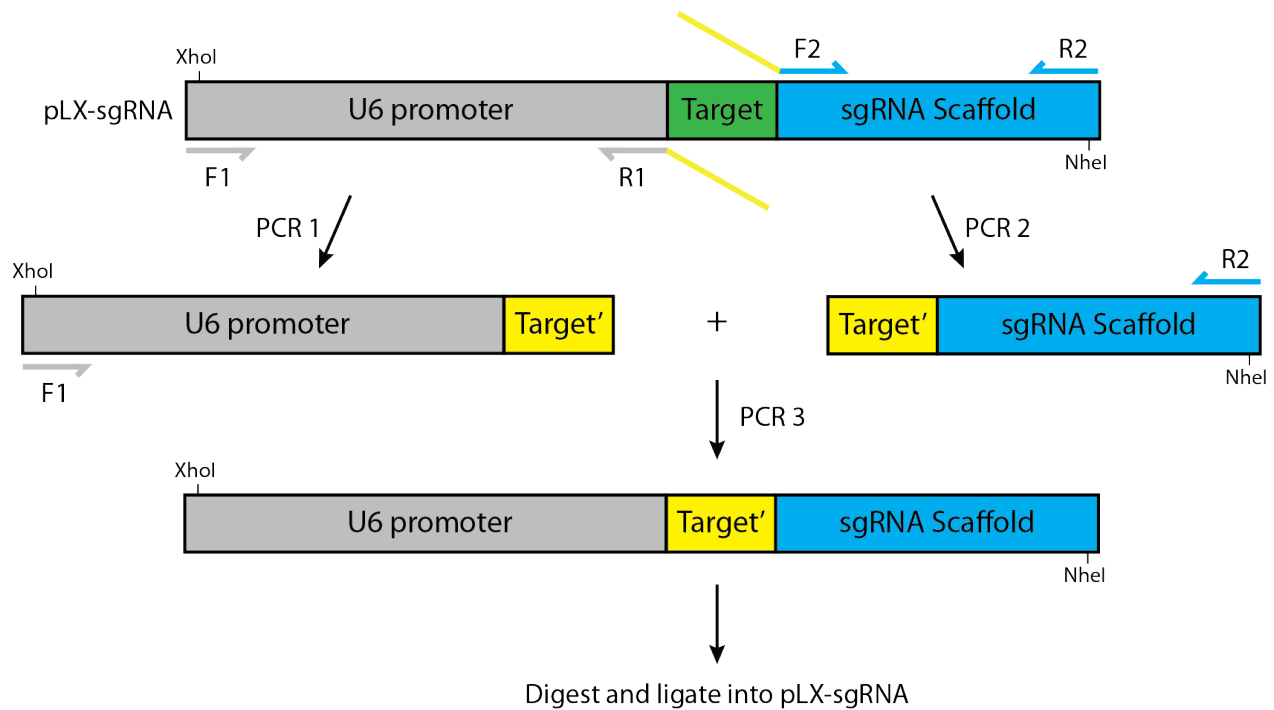


sgRNA Cloning



New target sequences can be cloned into pLX-sgRNA between the XhoI and NheI sites using overlap-extension PCR (see figure) followed by restriction/ligation via the following steps:

Step 1: Find 20-bp target sequence. If the 20-bp sequence does not start with a 'G', a single 'G' nucleotide must be prepended to allow efficient transcription from the RNA Polymerase III U6 promoter. Note that target sequences should NOT contain XhoI or NheI recognition sites.

Step 2: Order primers. To clone a sgRNA into pLX-sgRNA a total of 4 primers are needed, 2 of which are sgRNA-specific (R1 and F2, see table). For a sgRNA sequence which starts with a G (ie, GN₁₉), the primers are as follows:

F1: **AAACTCGAGTGTACAAAAAAGCAGGCTTTAAAG**

R1: **rc(GN₁₉)GGTGTTCGTCCTTCC**

F2: **GN₁₉GTTTTAGAGCTAGAAATAGCAA**

R2: **AAAGCTAGCTAATGCCAACTTTGTACAAGAAAGCTG**

Where GN₁₉ = new target sequence and rc(GN₁₉) = reverse complement of the new target sequence

Step 3: Amplify pLX-sgRNA using F1 + R1 in PCR 1 and F2 + R2 in PCR 2.

Step 4: Gel purify products from PCR 1 and PCR 2 and combine with F1 + R2 for PCR 3. Note that gel purification is necessary to remove the original pLX-sgRNA template.

Step 5: Digest PCR 3 along with pLX-sgRNA using NheI and XhoI.

Step 6: Ligate and transform.