pNHIS Vector:

```
5′ ATGCACCATCATCATCATCATTCCGGTATTGAGGGTCGCTCTAACTCTCCTCTGGATATCGGATCCGAATTCGAGC 3′
3′ TACGTGGTAGTAGTAGTAGTAAGGCCATAACTCCCAGCGAGATTGAGAGGAGACCTATAGCCTAGGCTTAAGCTCG 5′
```

Step 1. Linearize (BseR I)

```
5′ ATGCACCATCATCATCATCATTCCGGTATTGAGGGTCG   CTCTAACTCTCCTCTGGATATCGGATCCGAATTCGAGC 3′
3′ TACGTGGTAGTAGTAGTAGTAAGGCCATAACTCCCA   GCGAGATTGAGAGGAGACCTATAGCCTAGGCTTAAGCTCG 5′
```

Step 2. Create blunt ends

```
5′ ATGCACCATCATCATCATCATTCCGGTATTGAGGGT     CTCTAACTCTCCTCTGGATATCGGATCCGAATTCGAGC 3′
3′ TACGTGGTAGTAGTAGTAGTAAGGCCATAACTCCCA     GAGATTGAGAGGAGACCTATAGCCTAGGCTTAAGCTCG 5′
```

Step 3. LIC exonuclease treatment (dCTP)

```
5′ ATGCACCATCATCATCATCATTCC                 CTCTAACTCTCCTCTGGATATCGGATCCGAATTCGAGC 3′
3′ TACGTGGTAGTAGTAGTAGTAAGGCCATAACTCCCA                    CCTATAGCCTAGGCTTAAGCTCG 5′
```

cDNA Insert:

Step 1. PCR amplify with LIC primers

```
5′GGTATTGAGGGTCGC cDNA TAA GGCTCTAACTCTCCTCT 3′
3′CCATAACTCCCAGCG cDNA ATT CCGAGATTGAGAGGAGA 5′
```

Step 2. LIC exonuclease treatment (cGTP)

```
5′GGTATTGAGGGTCGC cDNA TAA GG 3′
3′            GCG cDNA ATT CCGAGATTGAGAGGAGA 5′
```

Final Product:

```
5′ ATGCACCATCATCATCATCATTCCGGTATTGAGGGTCGC cDNA TAA GGCTCTAACTCTCCTCTGGATATC 3′
3′ TACGTGGTAGTAGTAGTAGTAAGGCCATAACTCCCAGCG cDNA ATT CCGAGATTGAGAGGAGACCTATAG 5′
```