Materials

JumpStart™ Taq ReadyMix™ - Sigma-Aldrich SKU P2893-400RXN

Forward Primer (Modified Scaffold, Wager et. Al.):

5’-gaaattaatacgactcactatagg-NNNNNNNNNNNNNNNNNNNNN-gtttaagtactctgugcuggaaacagc-3’

Reverse Primer (Modified Scaffold, Wager et. Al.):

5’-AAAAAAATCTCGCCAACAAGTTGACGAGATAAACACGGCATTTTGCCTTGTTTAAGTAGATTCTGTgctgtttccagcacagagtacttaaac -3’

Qiagen QIAquick PCR Purification Kit

MEGAscript® T7 Transcription Kit – Thermo Fisher

Zymo Research RNA Clean & Concentrator™-5

Procedure

PCR IVT Template:

Make Reaction Mix

 50 µL JumpStart

 5 µL F Primer (10 µM)

 5 µL R Primer (10 µM)

 40 µL dd H₂O

|  |  |  |  |
| --- | --- | --- | --- |
| Cycling Parameters: |  |  |  |
| Initial denaturation | 94° C | 2 min |  |
|  |  |  |  |
| Denaturation | 94° C | 30 sec |  |
| Annealing | 60° C | 30 sec | 30-35 cycles |
| Extension | 72° C | 2 min |  |
|  |  |  |  |
| Final Extension | 72° C | 5 min |  |
| Hold | 4°C |  |  |

IVT Template QC - Run products out on a gel:

* Combine 1 µL of 6x loading dye and 5 µL PCR product
* PCR purify using Qiagen QIAquick PCR Purification Kit (see protocol in kit).
* NanoDrop

Transcribe gRNA using MEGAscript T7 kit

* Obtain concentration from NanoDrop performed in previous step.
* Incubate the reaction at 37°C overnight

Purify IVT reaction with Zymo Research RNA Clean & Concentrator™-5 Kit

NanoDrop for concentration and run out 1 ul on a gel for QC