1. Linearize the pFN18a plasmid with BamHI

|  |  |
| --- | --- |
| H2O | 7.8 uL |
| Buffer E (10x) | 2 uL |
| BSA (10 ug/uL) | 0.2 uL |
| Plasmid (100 ng/uL) | 10 uL |
| BamHI | 1 uL |

Incubate at 37\*C for 1 hour, then inactivate at 65\*C for 15 minutes

1. Purify with a phenol/chloroform extraction

* Add phenol/chloroform mix, pH = 8 for DNA
* Save the aqueous (top) layer
* Add 1mL of cold 100% ethanol and 40 uL of 3M NaAc, pH 5.2
* Precipitate for 2 hours at -80\*C, or overnight -20\*C
* Centrifuge at 12500 rpm for 30 minutes at 4C
* Add 1mL of cold 70% ethanol
* Centrifuge at 12500 rpm for 25 minutes at 4C
* Allow pellet to dry on benchtop
* Resuspend in ddH2O

1. Digest with Mung Bean nuclease – 1 hour

|  |  |
| --- | --- |
| DNA | 1 ug (30 uL) |
| Buffer (10x) | 10 uL |
| Glycerol (100%) | 5 uL |
| H2O | 54 uL |
| Mung Bean Nuclease | 1 uL |

Incubate at 37\*C for 1 hour

1. Repeat phenol chloroform step
2. Confirm complete digestion in a gel
3. Run in vitro transcription kit
4. DNase removal
5. Phenol chloroform - use RNA adjusted pH phenol/chloroform mix instead
6. Qubit quantification + run in gel