# USER linearization

To linearize vectors by opening the USER casette, we use the following digestions.
The first digestion cuts the casette, the second creates the overhang that will be used for insertion of the genes in the vector.

## Protocol

1.   Digestion 1

| Component | 100 µl reaction |
| --- | --- |
| pET102 iGEM | 42 µl |
| AsiSI enzyme | 2.5 µl |
| Thermofisher tango buffer | 10 µl |
| Nuclease free water | 45.5 µl |

2.   Mix and incubate for 3 hours at 37 °C
3.   Column purify (gel extraction protocol, but add 150 µl binding buffer no matter the amount of gel)
4.   Digestion 2

| Component | |
| --- | --- |
| Elution from purification | 50 µl |
| Nb.BsmI enzyme | 1 µl |
| NEBuffer 3.1 | 6 µl |
| Nuclease free water | 3 µl |