**Plasmid information**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **plasmid** | **enzyme I** | **Bufffer** | **enzyme II** | **Bufffer** | **enzyme III** | **Bufffer** |
| **AKT1-PT3EF1αH****AKT2-PT3EF1αH****β-cat-PT3EF1αH****β-cat-S45Y-1αH****C-myc-PT3EF1αH****hMet-PT3EF1αH****Mmcl1-PT3EF1αH****NICD--PT3EF1αH** | BamH1-HF | 4 | EcoRV | 3+BSA |  |  |
| **sgAxin****sgPTEN** | SacI | 1+BSA | SphI | 2 |  |  |
| **AKT-shraptor****dnTEAD2-PT3EF5α****PIK3 E545K PT3EF5α** | BglII | 3 | EcoRI-HF | 4 | HindIII-HF | 4 |
| **TAZS89A-PT3EF5αH****AKT-shluc- PT3EF5α** | BamH1-HF | 4 | EcoRI-HF | 4 | HindIII-HF | 4 |
| **PT3EF1αH** | BglII/NotI | 3+BSA |  |  |  |  |
| **PBS185 CMV Cre** | Xbal | 4+BSA | EcoRV/PstI | 4+BSA |  |  |
| **PBS-185** | BamH1-HF | 4 | Xbal | 4+BSA |  |  |
| **Nras v12-caggs** | EcoRI-HF | 4 | HindIII-HF | 4 |  |  |
| **PT2-shP53** | EcoRI-HF | 4 | KpnI | 1+BSA |  |  |
| **PT3Gluc** | EcoRI-HF | 4 | HindIII-HF | 4 |  |  |
| **YAPS127A-PT3EF1αH** | BamH1-HF | 4 | EcoRV | 3+BSA |  |  |
| **SB** | SacI | 1+BSA |  |  |  |  |

Note: All these plasmids are selected by Ampicillin**;** Growth temperature is 37°C and Growth strain is top10. The ladder is <https://www.thermofisher.com/order/catalog/product/10787026>.

**1. AKT1-PT3EF1αH (**<https://www.addgene.org/31790/>)

**Digestive enzyme**: BamH1; EcoRV

**Digest map:**

1 2 3

1 uncut

2 BamHI

3 EcoRV

**2. TAZS89A-PT3EF5α**

**Digestive enzyme**: BamH1-HF,EcoRI-HF,HindIII-HF

**Digest map**



1. **Mmcl1-PT3EF1αH**

**Digestive enzyme**: BamH1-HF,EcoRV

**Digest map** 1 2 3 1 2 3

 

1 EcoRI

2 Hind 3

3 Bgl 2

**4. SB**

**Digestive enzyme**: sacI

**Digest map**  1 2 1 2 1 2

 

1 uncut

2 sacI

4 BglII

**5. sgPTEN/PX330**

**Digestive enzyme**: sacI,sphI

**Digest map**

 

**6. AKT-shraptor**

**Digestive enzyme**: BglII,EcoRI-HF,HindIII-HF

**Digest map**



**7. h-Met-PT3EF1 αH(**[**https://www.addgene.org/86498/**](https://www.addgene.org/86498/)**)**

**Digestive enzyme**: BamH1-HF; EcoRV

**Digest map**

 1 1 2 2 3 3

1 uncut

2 BamHI

3 EcoRV

**8. PBS185**

Vector: **pBS185 (from pBS185 CMV-Cre)**

Insert: **Cre (delete 420bp by Bamh1 compare to pBS185 CMV-Cre)**

Insert origin**: (cloning ID:**  **Plasmid #11916** **, Addgene)**

Insert size**: 680bp**

**Digestive enzyme**: BamH1-HF,Xbal

**Digest map**

****

**9.** **β-cat- PT3EF1 αH(**[**https://www.addgene.org/86499/**](https://www.addgene.org/86499/)**)**

**Digestive enzyme**: BamH1-HF; EcoRV

**Digest map**

**10.**  **Nras v12-caggs**

**Digestive enzyme**: HindIII-HF; EcoRI-HF

**Digest map**

1.  1 2 2 3 3

1 uncut

2 EcoR 1

3 Hind 3

4 BglII

**11. PT2shP53**

**Digestive enzyme**: EcoRI-HF,KpnI

**Digest map:**

****

**12. sgAxin/PX330**

**Digestive enzyme**: SacI,SphI

**Digest map:**



**13. PT3EF1αH**

**Digestive enzyme**: BglII/NotI

**Digest map:**

 1 1 2 2

 

1 uncut

2 BglII/ NotI

4 BglII

**14. β-cat-S45Y-PT3EF1αH**

**Digestive enzyme**: BamH1-HF, EcoRV

**Digest map:**

**15. YAPS127A-PT3EF1αH (**[**https://www.addgene.org/86497/**](https://www.addgene.org/86497/)**)**

**Digestive enzyme**: BamH1-HF, EcoRV

**Digest map:**

 1 1 2 2 3 3

1 uncut

2 BamHI

3 EcoRV

**16. PT3EF5 α dataF-PI3KCA-H1047R**

**Digestive enzyme**: BglII,EcoRI-HF,HindIII-HF

**Digest map:**

****

**17. AKT-shluc-5****αH**

**Digestive enzyme**: BamH1-HF, EcoRI-HF, HindIII-HF

**Digest map:**

****

**18.dnTEAD2-PT3EF5a**

**Digestive enzyme**: BamH1-HF, EcoRI-HF, HindIII-HF

**Digest map:**

****

**19. pBS185 CMV-Cre(Addgene** [**https://www.addgene.org/11916/**](https://www.addgene.org/11916/)**)**

**Digestive enzyme**: Xbal, EcoRV/PstI

**Digest map:**



**20. C-myc-PT3EF1αH(Addgene: #**92046**)**

**Digestive enzyme**: BamH1-HF, EcoRV

**Digest map:**

1 2 3

1 uncut

2 BamHI

3 EcoRV

**21. PI3KE545K-PT3EF5α**

**Digestive enzyme**: BglII, HindIII-HF, EcoRI-HF

**Digest map:**

****

**22. myr-AKT2-PT3EF1aH**

**Digestive enzyme**: BamH1-HF, EcoRV

**Digest map:**

****

**23. NICD-PT3EF1αH (**[**https://www.addgene.org/46047/**](https://www.addgene.org/46047/)**)**

**Digestive enzyme**: BamH1-HF, EcoRV

**Digest map:**

 1 2 3



1 uncut

2 BamHI

3 EcoRV