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## Preparation of qPCR standards for AAV titering

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## Abstract

**qPCR standards for AAV titting**

**document annex to protocol "AAV Purification Protocol with Iodixanol gradient"**

1 ug of 1 kb DNA =  $9.1 \times 10^{11}$  molecules

Use any plasmid with known size with WPRE (so you can use WPRE primers)

Digestion 100ul reaction – do not use any enzyme with star activity and make sure to use a single restriction site enzyme.

| # of reactions      | volume      |
|---------------------|-------------|
| 1                   |             |
| DNA (10ug)          | x           |
| 10x CutSmart buffer | 10          |
| XbaI                | 5           |
| H2O                 | Up to 95 ul |
| V/reaction          | 100         |
|                     |             |

Mix digestion and incubate (37°C) for minimum 2 hours up to O/N.

PCR purification and elute in 30 ul EB.

Spec very well and perform multiple readings. Spec values (ug/ml)

### Calculations:

If using our pAAV.CBA.mcherry.WPRE = 6132 bases=6.132 kb

1 ug  $\Rightarrow$  for 1000 bp dsDNA =  $9.1 \times 10^{11}$  molecules

1 ug  $\Rightarrow$  for pAAV.CBA.mcherry.WPRE =  $9.1 \times 10^{11} / 6.132 = 1.484 \times 10^{11}$  molecules

**Standard  $10^8 = 2 \times 10^7$  molecules**

$2 \times 10^7$  molecules weight 0.135 ng =  $2 \times 10^7$  molecules /  $1.484 \times 10^{11}$  molecules

$2 \times 10^7$  molecules / ul = 0.135 ng / ul = 135 ng/ml

When we use 5 ul in qPCR  $\Rightarrow$  0.675 ng in 5 ul @  $2 \times 10^7$  / ul

**In 1 ml TE – 135 ng**

**Calculate the DNA volume to dilute in 1 ml of TE based on spec'ed concentration**

