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Preparation of qPCR standards for AAV titering

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Roberta Marongiu[1,2], Santiago Unda[1,2], Michael G. Kaplitt[1,2]

[1]Department of Neurological Surgery, Weill Cornell Medical College, New York, NY 10065;
[2]Aligning Science Across Parkinson's (ASAP) Collaborative Research Network, Chevy Chase, MD 20815, USA

Jacquelyn Haytayan
Weill Cornell Medicine

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Abstract

qPCR standards for AAV tittering

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

1 ug of I kb DNA = 9.1 × 1011 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 tp 95 ul
	V/reaction	100

Mix digestion and incubate (37^{o}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 ⇒ for 1000 bp dsDNA = 9.1×10^{11} molecules
1 ug ⇒ for pAAV.CBA.mcherry.WPRE =$9.1\times10^{11}/6.132=1.484\times10^{11}$molecules

Standard 10^{8} = 2×10^{7} molecules
2×10^{7} molecules weight 0.135 ng= 2×10^{7} molecules/1.484×10^{11}molecules
2×10^{7} molecules/ul = 0.135 ng /ul = 135 ng/ml
When we use 5 ulin qPCR ⇒ 0.675 ng in 5 ul @ 2×10^{7}/ul
In 1 ml TE – 135 ng
Calculate the DNA volume to dilute in 1 ml of TE based on spec'ed concentration