JRC AAV Production-Iodixanol Alternate Protocol—Updated 12/06/16 (KR)

1. Process per normal protocol through clarification spin (Day 1, step 9).
2. During benzonase treatment, set up iodixanol (Optiprep) gradients in Optiseal centrifuge tubes (29.9 ml- #361625):
    a. See solutions section for gradient recipes. Start with the lightest concentration and layer each successive heavier concentration from the bottom with a cannula needle (load all tubes with 15%, then 25%, etc).
    b. 6.7 ml 15% + 1M NaCl
    c. 4.5 ml 25%
    d. 3.7 ml 40%
    e. 3.7 ml 58% + 1M NaCl
3. Bring sample to 10 ml with 1xGB.
4. Layer virus on top of iodixanol gradient. Balance tubes with 1xGB buffer.
5. Spin at 360,000 x G (70,000 rpm) for 1 hr, 18º C in Ti70 rotor (can do 2 hr).
6. Collect virus fraction:
    a. Puncture tube at 58/40 interface with 18G needle attached to 10ml syringe.
    b. Collect about 2 ml with bevel up and 2 ml with bevel down. **AVOID PROTEIN BAND AT 40/25 INTERFACE.**
    c. Note-there are actually 2 peaks of infectious virus within this 4 ml fraction separated by a fraction of high GC, low infectivity virus. These peaks can be independently collected by taking smaller fractions, but in practice this is more time consuming that it is worth as it does not significantly affect the quality of the virus produced.
7. Exchange into storage buffer over concentration columns (Millipore 30K MWCO 4ml #803024)
    a. Pre-wet with .01% pluronic for 1 hr.
    b. Spin at 4K rpm and replace volume with storage buffer to dilute out Iodixanol (as the concentration of iodixanol is reduced, the volume will decrease faster).
    c. Concentrate down to desired final volume and proceed with titration protocol.

Solutions:

Optiprep Gradients:

	15% + 1 M NaCl	*25%*	*40%*	*58%*
Optiprep 60% (ml)	40	46.67	64	96.67
10x GB	16	11.2	9.6	3.33
5M NaCl	32	0	0	0
ddH_2O	72	56	22.4	0
Phenol Red (5 mg/ml)	0	280 ul	0	240 ul

Mix for 1 minute
Store at 4º C