Viral Core Facility
Production Sheet Lenti-virus

CHARITÉ UNIVERSITÄTSMEDIZIN BERLIN

VIRAL CORE FACILITY VCF CHARITÉ - UNIVERSITÄTSMEDIZIN BERLIN

VCF Order No.: 2147	Virus No.: BL-1908a

User information:

Name: Jeong-Eun Lee Group leader: Katja Nowick

Lab genetic engineering facility number: 404/20

Lab Bio Safety level of user: S1☐S2☒

Vector service:	Shuttle vector plasmid name: f(Tet-O)hNgn2-2A-hNGN1-IRES-GFP-2A-Puro
Shuttle vector properties (Promoter, GOI, reporter gene)	Tet-O promotor: expression of hNgn2-2A-hNGN1-IRES-GFP-2A-Puro
Shuttle vector provided by the user:	No ☒ Yes ☐
Shuttle vector cloned by VCF:	No ☐ Yes ☒, Virus number BL- 1907
Generation of new vector by VCF: Cloning description with dates:	No ☐ Yes ☒, cloning by: Katja Pötschke PCR am 15.05.2023, Verdau vom Vector am 16.05.2023, GC am 23.05.2023, Mini's am 26.05.2023,zur Seq.#17+18, check seq. am 1.6.23, #18 ist OK!, PCR GFP Puro am 5.6.23, Verdau von #18 am 6.6.23, Ligation am 07.06.2023, Mini's am 09.06.2023, zur Seq. am 14.06.2023 #4, check seq. am 16.6.23, bitte noch einmal #4 mit EGFP-N1-R seq. Übergang zur IRES ist unsauber., check seq. am 20.6.23, #4 ist OK!
Plasmid DNA preparation for transfection:	No☐ Yes☒, preparation by: Katja Pötschke Prep Date: 22.06.23

Virus request: No☐ Yes☒, done by: Bettina Brokowski

HEK cell seeding date:	22.06.2023	Transfection method: PEI		Transfection date:	23.06.2023
Shuttle vector:	Prep date: 22.06.2023	Transfection (%), evaluation HEK cells:	GFP: strong signal, 80%TE		
Packaging plasmid:	Prep date: 20.04.2023				
Envelope plasmid:	Prep date: 26.04.2023	Harvest date: 26.06.2023	Volume: 20 x 50µl		

Virus quantification / efficiency test:

Quantification by reporter gene expression in cell cultures: No☒ Yes☐, done by:

Cell-culture:	#Cell / date:	Viruses (µl):	Transducing date:	Counting date:	Titer (IU/ml):	µl 100% recom.:

Quantification by qPCR of viral genome: No☐ Yes☒, done by: Bettina Brokowski

qPCR date: 27.06.23 Titer: 1,49E+08 (attached are the Roche lightcycler sheets)

Efficiency test by qPCR of transcript of GOI or shRNA kd: No☒ Yes☐, done by:

Cell-culture:	#Cell / date:	Viruses (µl):	Transducing date:	Cell lysis date:	cDNA date:	qPCR date:

Roche UPL # % Expression from WT cells (per virus amount):

Primer seq. fw: 5'-3'

Primer seq. rev: 5'-3' (attached are the Roche lightcycler sheets)

Efficiency test by Western Blotting: No☒ Yes☐, done by:

Cell-culture:	#Cell / date:	Viruses (µl):	Transducing date:	Cell lysis date:	Protein (µg/µl):	Proteins / WBlot (date)

Prim. Antibody (Name/company): Dilution 1.AB: Incubation date: ECL date:

Sec. Antibody (Name/company): Dilution 2.AB: % Expression from WT cells.

Date project start: 17.02.2023	Date material released/shipped to user: 27.06.2023