Salk Gene Transfer, Targeting & Therapeutics Facility (GT3)

Core Director: Leszek Lisowski, Ph.D.
Faculty Advisor: Ed Callaway, Ph.D.
Website: http://vectorcore.salk.edu

OVERVIEW

The Gene Transfer, Targeting and Therapeutics (GT3) facility offers a wide range of viral and non-viral vectors and a number of other related services to support the work of the Salk Institute Cancer Center as well as researchers from other institutions (external users). In addition to the standard research-quality preparations, the facility is in the final stages of optimizing the production of high-purity rAAV vectors and will soon begin similar protocol optimizations for other vectors. These high-purity and high-titer vector preparations will facilitate the transition from pure research to pre-clinical studies.

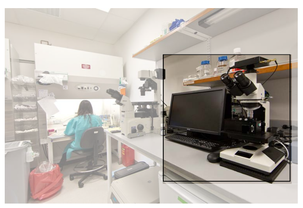
SERVICES

The GT3 core offers convenient ready-to-use stocks, as well as custom preparations of lentiviral vectors (LV), retroviral vectors, adeno-viral vectors (Ad), adeno-associated viral vectors (AAV), vesicular stomatitis viral vectors (VSV) and rabies vectors (RV). The core is starting to offer non-viral delivery technologies based on mini-circle(Chen Z.Y. et al. 2005) and mini-intronic plasmid (MIP)(Lu et al. 2012). In addition to viral/non-viral delivery systems, GT3 offers vector titration services, vector purification services, Replication Competent Lentivirus (RCL) testing, and limited cloning services, as well as consultation services.

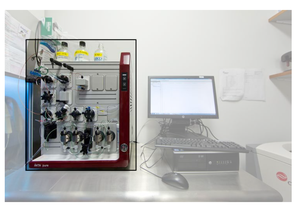
INSTRUMENTATION

The GT3 facility uses a designated working area for the production of standard research vectors (BSL2) as well as rabies/VSV vectors and vectors expressing known human oncogenes (BSL2+).

In addition to standard lab equipment used for vector cloning, production and titration (designated PCR space, QPCR machines, TC hoods and incubators, ultracentrifuges) the facility is fully equipped in state-of-the-art instruments to allow for production of high-titer and high-purity vector preparations:



The **NanoSight LM10-HSB/GFT14** system equipped with high-sensitivity sCMOS camera, blue (405nm) and green (532nm) lasers allows for direct visualization, sizing and counting of viral particles in liquid suspension. Direct measurements of vector aggregates allows for the validation of vector prep quality.



The **GE ÄKTA pure chromatography** system allows for fast purification of proteins (vectors) and nucleic acids from microgram to gram levels of target product. The system supports a wide range of chromatography techniques.
1. HiTrap AVB Sepharose High Performance column for one step purification of AAV1, 2, 3, 5 and DJ.
2. HiScreen Capto Core 700 column for intermediate purification of viruses in flow through mode.

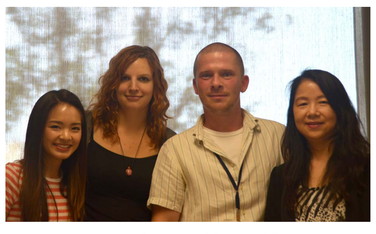
The **Eppendorf epMotion 5075LH** advanced liquid handling system is an excellent tool for demanding, small-volume applications such as RT-PCR set-up (96 and 384-well), serial dilutions, reformatting of plates, and total and viral DNA/RNA purification. The in TC hood setup allows for automated preparation of sterile vector aliquot as small as 1ul/tube.

CORE STAFF

Helen Fang
Research Assistant III

Christina Ly
Research Assistant II

Anne Beal
Research Assistant I



Leszek Lisowski
Core Director

RECHARGE RATES

Retroviral iPS reprogramming Kit	C3 / SCRM	External
Small OSKM Kit	$200	$350
Large OSKM Kit	$350	$600

Lentiviral and Retroviral Vectors	C3 / SCRM	External
Standard Prep with titration	$1,200	$2,000
Consultation [/hr]	$80	$130
Stock reporter virus (10 uL)	$80	$140

rAAV vectors	C3 / SCRM	External
Standard Prep with titration	$1,200	$2,000
Consultation [/hr]	$80	$130
Stock rAAV2 reporter virus (50ul)	$80	$130

G-deleted rabies	C3 / SCRM	External
Standard Prep with titration	$2,400	$4,000
Consultation [/hr]	$50	$130
stock G-deleted rabies (10ul)	$130	$200

Ad5 vectors	C3 / SCRM	External
Standard Prep with titration	$1,700	$2,800
Consultation [/hr]	$50	$130
Ad5 stocks	$100	$160

- GT3 services are available for users who are members of the UCSD and SBMRI Cancer Centers at the discounted rate plus a nominal 16% indirect cost rate.
- External user rates are subject to the additional Salk indirect cost rate of 94% unless otherwise negotiated through inter-institutional agreements.

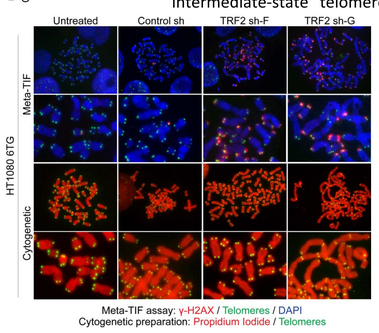
RESOURCE LOCATION

The GT3 facility is located on the ground floor of Sanford Consortium for Regenerative Medicine (SCRM) building, located just North from the main Salk campus. The Consortium was formed between The Sanford Burnham Medical Research Institute, The Salk Institute, The Scripps Research Institute and the University of California, San Diego, and was later joined by La Jolla Institute for Allergy and Immunology. The world-class facility was opened in December of 2011, with full occupancy reached in March of 2012.

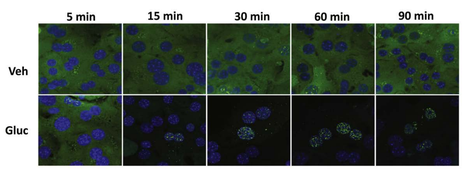
Salk Institute

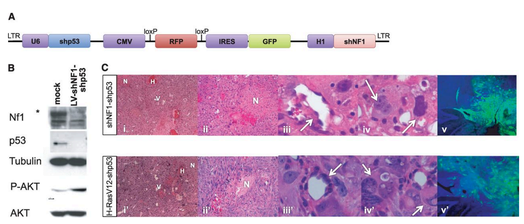
SALK CANCER CENTER DISCOVERIES

Lentiviral vector produced at GT3 facility was used by Dr. Karlseder's group from Salk Institute to express shRNA to knock down TRF2. shRNA-F resulted in an active DNA damage response at chromosome ends but no fusions (these are termed "intermediate-state" telomeres) whereas shRNA-G resulted in both intermediate-state telomeres and ends that were fusion sensitive (uncapped-state telomeres). These vectors led to the discovery that the telomere deprotection response to intermediate-state telomeres is functionally distinct from the genomic DNA damage response.



Collaborative study between Drs Evans, Montminy and Shaw demonstrated involvement of class IIa histone deacetylases (HDACs) in glucose homeostasis. Adenoviral vector expressing GFP-HDAC5 produced by GT3 core was used to infect primary human hepatocytes to demonstrate nuclear shuttling of class IIa HDACs.





Lentiviral vector shown in (**A**) produced at the GT3 core was used by researchers in Verma lab to show that that gliomas can originate from differentiated cells in the central nervous system (CNS), including cortical neurons. (**B**) Western blot analysis of the lentiviral vector function in MEF/Ikk2$^{-/-}$ cells. (**C**) histology and immunofluorescence of sections from either shNF1-shp53– or H-RasV12-shp53–induced glioblastomas in GFAP-Cre mice (HP injected). (i) and (i') show images of the tumors in which increased cellularity, vascularity (V), hemorrhage (H), and necrotic areas (N) can be observed. The classical GBM features: necrotic areas N in ii/ii', perivascular infiltration in iii/iii', and multinucleated giant cells in iv/iv'. (v) and (v') show the infiltrative characteristic of the tumor, crossing the midline and migrating to the other hemisphere (blue, DAPI; green, GFP).

NEW CORE SERVICES BEING DEVELPOED

The GT3 core is currently adding a number of new services:

1. Lentiviral vector system based on feline immunodeficiency virus (FIV) as developed by Dr. Eric M. Poeschla from the Mayo Clinic (Nature Medicine 4:354-7, 1998).
2. Two alternative 3rd generation High-Capacity vector systems (HC-Ad) (also known as: gutless, gutted, mini, fully-deleted, high-capacity, Δ, pseudo) based on vectors developed by Dr. Philip Ng (HC-Ad-Cre) and Dr. Pedro R. Lowenstein (HC-Ad-FLPe).
3. Novel AAV serotypes, including human specific AAV variants being currently evaluated in non-human primates as a future candidate for human trials.
4. Ion exchange chromatography (IEX) purification protocols for each individual AAV serotype further increasing the purity of our AAV preps.

GOVERNANCE

Leadership:	Leszek Lisowski, Ph.D.
Decision Making:	Leszek Lisowski, Ph.D.
Oversight:	Faculty Advisor: Ed Callaway, Ph.D. Senior Director, Scientific Core Facilities: James Fitzpatrick, Ph.D. Cancer Center Director: Tony Hunter, Ph.D. Cancer Center Administrator: Suzanne Simon
User Input:	Annual User Group Meeting Salk Core Facilities Review Direct User Feedback

SELECTED PUBLICATIONS

Dr. Lisowski's Selected Publications:

1. Lisowski L, Elazar M, Chu K, Glenn J.S., Kay M.A., The anti-genomic (negative) strand of Hepatitis C Virus is not targetable by shRNA. *Nucleic Acid Research*. 2013. PMCID: PMC3616702
2. Valdmanis PN, Lisowski L, Kay MA., rAAV-mediated tumorigenesis: still unresolved after an AAV assault. *Molecular Therapy*, 2012, 20(11): 2014-7. PMCID: PMC3498811
3. Lisowski L, Lau A, Wang Z, Zhang Y, Zhang F, Grompe M, Kay MA. Ribosomal DNA integrating rAAV-rDNA vectors allow for stable transgene expression. *Molecular Therapy*, 2012. 20(10):1912-23. PMCID: PMC3464642
4. Wang Z, Lisowski L, Finegold MJ, Kay MA, Grompe M. AAV vectors containing rDNA homology display increased chromosomal integration and transgene persistence. *Molecular Therapy*, 2012. 20(10): 1902-11. PMCID: PMC3464636
5. Wang Y, Zhang WY, Hu S, Lan F, Lee AS, Huber B, Lisowski L, Liang P, Huang M, de Almeida PE, Won JH, Sun N, Kay MA, Urnov FD, Wu JC. Human Embryonic and Induced Pluripotent Stem Cells Genome-Edited to Report on Their *In Vivo* Fate. *Circulation Research*, 2012. PMID:22967807
6. Sadelain M, Lisowski L, Chang A., Supplying therapeutic proteins from hematopoietic stem cell derived-erythroid and megakaryocytic lineage cells. *Molecular Therapy*, 2009; 17(12): 1994-9. PMCID: PMC2814379
7. Hayakawa J, Ueda T, Lisowski L, et al. Transient in vivo beta-globin production after lentiviral gene transfer to hematopoietic stem cells in the non-human primate. *Human Gene Therapy*, 2009 Feb 17. PMCID: PMC2828625
8. Chang AH, Stephan MT, Lisowski L, Sadelain M. Erythroid-specific human factor IX delivery from *in vivo* selected hematopoietic stem cells following nonmyeloablative conditioning in hemophilia B mice. *Molecular Therapy*. 2008; 16(10): 1745-52. PMCID: PMC2658893
9. Lisowski L, Sadelain M. Locus control region elements HS1 and HS4 enhance the therapeutic efficacy of globin gene transfer in β-thalassemic mice. *Blood*. 2007; 110(13): 4175-8. PMCID: PMC2234778
10. Samakoglu S, Lisowski L, Budak-Alpdogan T, Usachenko Y, Acuto S, Di Marzo R, Maggio A, Zhu P, Tisdale JF, Rivière I, Sadelain M. A genetic strategy to treat sickle cell anemia by co-regulating globin transgene expression and RNA interference. *Nat Biotechnol*. 2006; 24(1): 89-94. PMID: 16378095

Selected Publications of GT3 Core Supported Research:

1. Friedmann-Morvinski, D., Bushong, E.A., Ke, E., Soda, Y., Marumoto, T., Singer, O., Ellisman, M.H., Verma, I.M. (2012). Dedifferentiation of neurons and astrocytes by oncogenes can induce gliomas in mice. *Science* 338:1080-1084. PMCID: PMC3595315.
2. Spike, B.T., Engle, D., Lin, J., Cheung, S., and Wahl, G.M. (2012). A mammary stem cell population identified and characterized in late embryogenesis reveals similarities to human breast cancer. *Cell Stem Cell* 10:183-197. PMCID: PMC3277444.
3. Li, M-D., Ruan, H.B., Singh, J.P., Zhao, L., Zhao, T., Azarhoush, S., Wu, J., Evans, R.M., Yang, X. (2012). O-GlcNAc transferase is involved in glucocorticoid receptor-mediated transrepression. *J Biol Chem* 287:12904-12912. PMCID: PMC3339970.
4. Macfarlan, T.S., Gifford, W.D., Driscoll, S., Lettieri, K., Rowe, H.M., Bonanomi, D., Firth, A., Singer, O., Trono, D., Pfaff, S.L. (2012). Embryonic stem cell potency fluctuates with endogenous retrovirus activity. *Nature* 487:57-63. PMCID: PMC3395470.
5. Mihaylova, M.M., Vasquez, D.S., Ravnskjaer, K., Denechaud, P.D., Yu. R.T., Alvarez, J.G., Downes, M., Evans. R.M., Montminy, M., and Shaw, R.J. (2011). Class IIa histone deacetylases are hormone-activated regulators of FOXO and mammalian glucose homeostasis. *Cell* 145:607-621. PMCID: PMC3117637.
6. O'Sullivan, R.J., Kubicek, S. Schreiber, S.L., Karlseder, J. (2010). Reduced histone biosynthesis and chromatin changes arising from a damage signal at telomeres. *Nat Struct Mol Biol* 17:1218-1226. PMCID: PMC2951278.

CONTACT INFORMATION

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