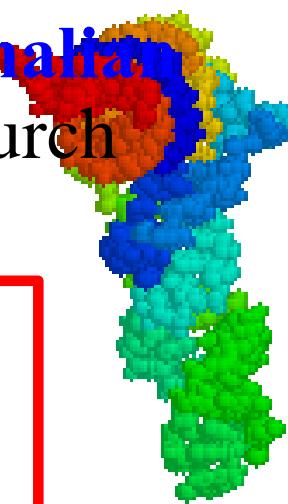


Accelerated evolution, proliferation & mammalian development.

14-Oct-2020, 10AM George Church
Cancer & Evolution Symposium



NATIONAL
CANCER
INSTITUTE



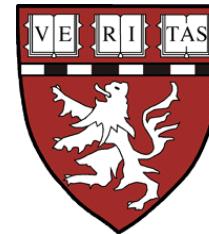
National Institutes
of Health



1987
2001

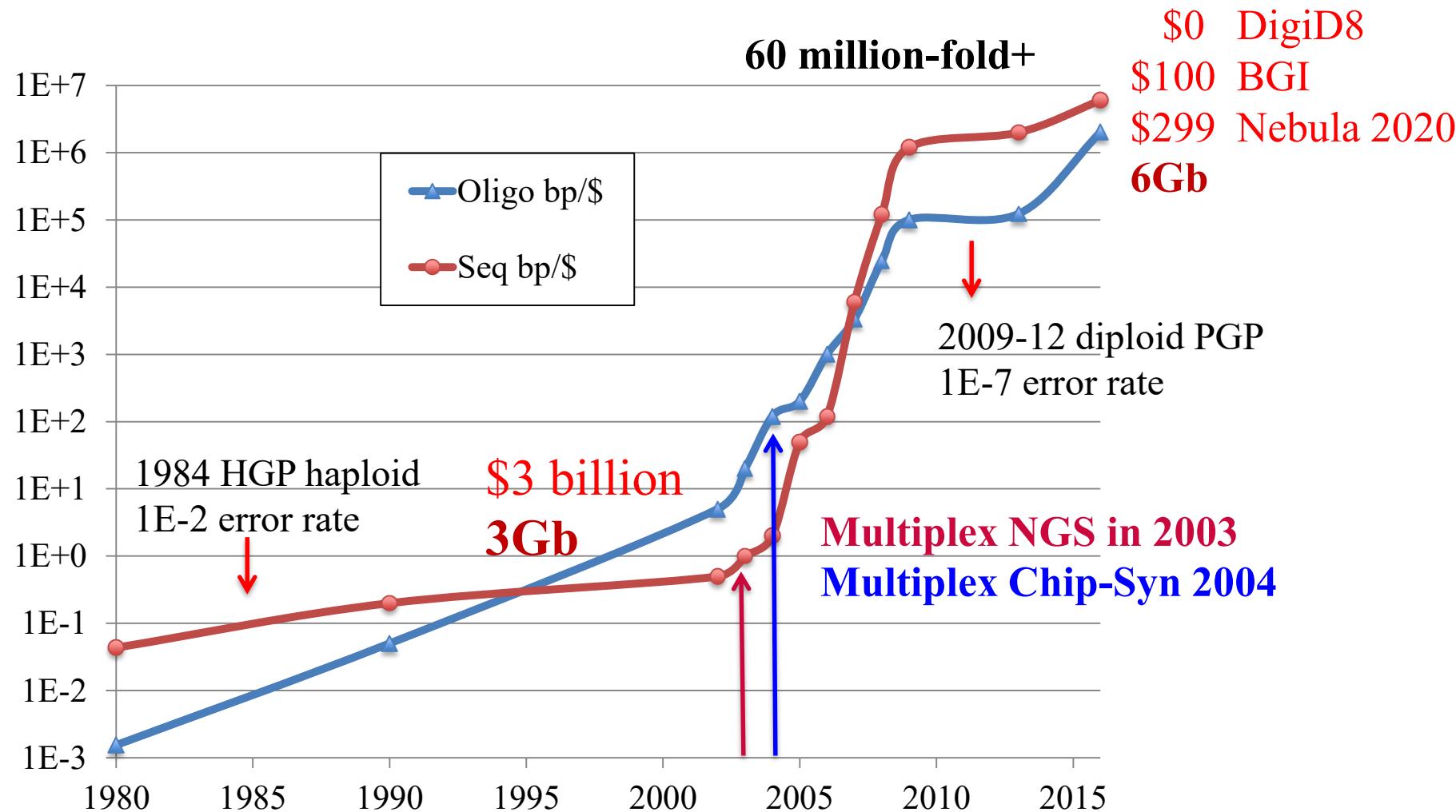


Lipper 1997
Foundation



George Church
Full COI list:
[v.ht/PHNc](http://vht/PHNc)

Nucleic Acid **Read** / Write cost & quality improving exponentially, faster than Moore's law



Therapies → Low cost alternatives

Therapy	Source	Genes	Disease	US/yr	\$
Luxturna	Spark	AAV2/RPE65	LCA-blindness	80	425k/eye
Imlygic	Amgen	HSV1/GM-CSF	Melanoma	7200	780k/6mo
Kymriah	Novartis	CAR(to CD19)	Leukemia	9600	475k
Yescarta	Kite Pharma	CAR(to CD19)	Lymphoma	8000	373k
Zolgensma	Novartis	AAV9/SMN1	SMA	500	2.1M
Spinraza	Biogen	ASO	SMA	500	4M/10yr
Multiplex	Rejuvenate	AAV/3genes	Multiplex	2,800,000	2k ?

- 
- 1) Rare genetic diseases: Prevention: dating/matchmaking
 - 2) Infectious diseases: Prevention: 24-7 monitoring
 - 3) Common diseases: (age-affected) spread R&D costs
 - 4) Less failure: ML+Libraries: delivery, action, testing

Preventing common diseases: Cancer

Pre-conception / pre-implantation genetics

Adult genetics: Pre-symptomatic surgeries (BRCA1/2, CDH1)

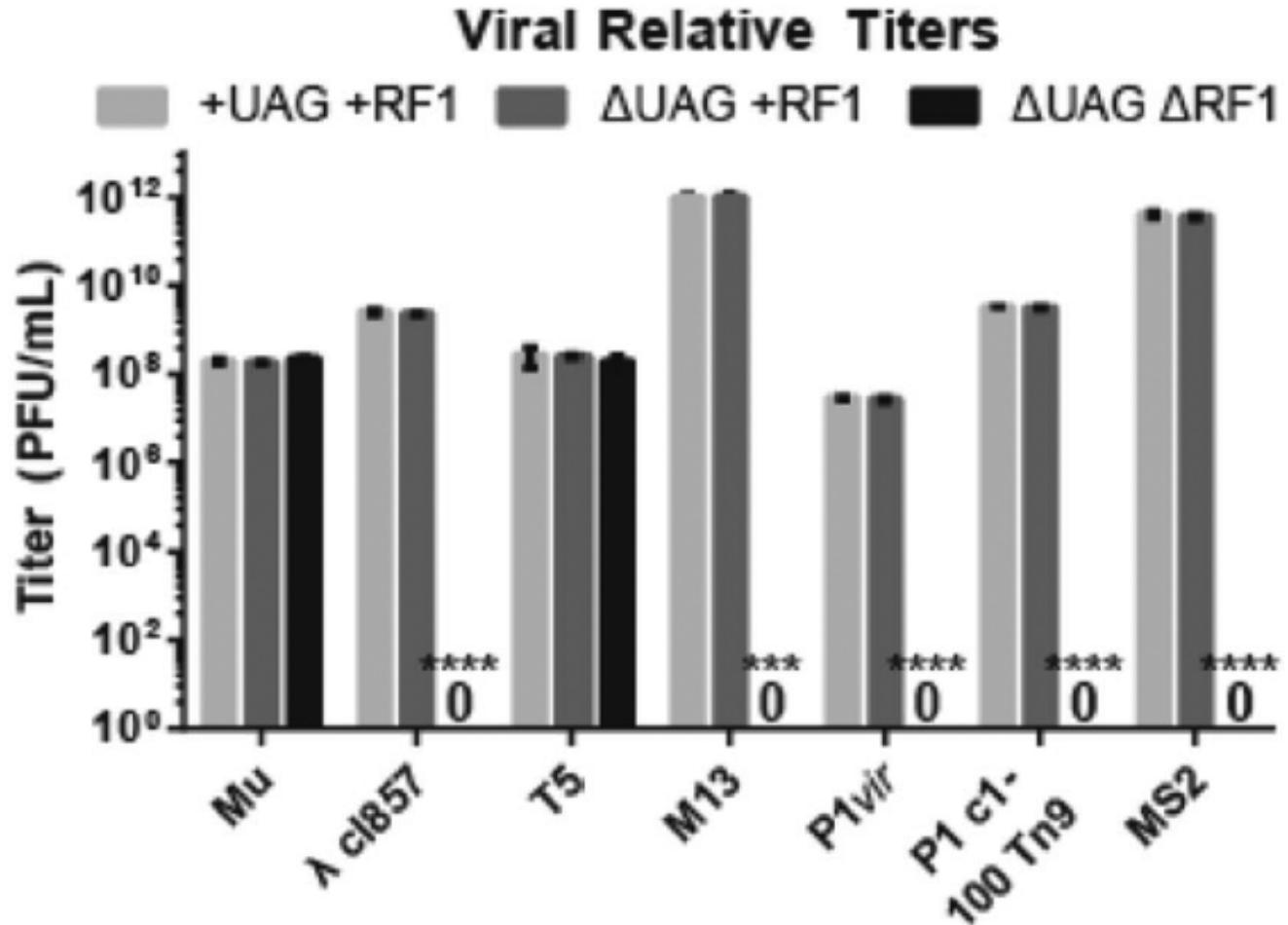
Environmental: Smoke, UV, other carcinogens

8 of 11 infectious cancer causes are viral.

(v) vaccine, (e) editing, (s) small molecules, (h)hygiene

MCPyV		Skin
EBV		Lymphoma, stomach, nasopharynx
HBV	(v,e)	Hepatic
HCV		Hepatic
HHV8	(h)	Sarcoma
HIV	(e, h, s)	Sarcoma
HTLV-1	(h)	Leukemia
HPV	(v)	Cervical, vaginal, vulvar, anal, penile, oropharyngeal
<i>Helicobacter pylori</i>	(s)	Stomach, Lymphoma
<i>Opisthorchis viverrini</i>	(h)	Cholangiocarcinoma
<i>Schistosoma hematobium</i>	(h)	Bladder

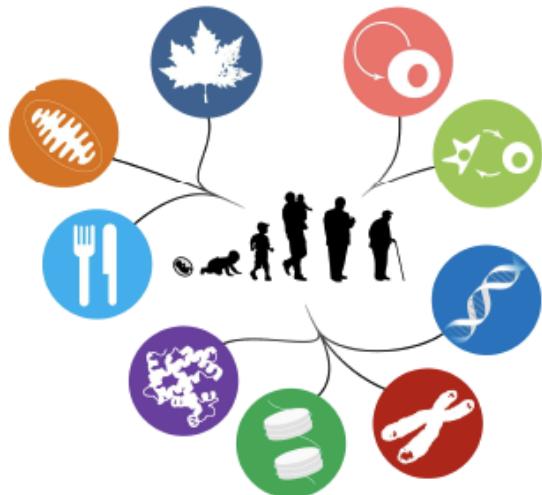
1/64 Recoding → resistance to all viruses



Cells Systems 2016 Ma, Isaacs, et al

Science 2016 Ostrov et al *Science* 2019 Ostrov et al
Nature 2015 Mandell et al *Science* 2013 Lajoie et al

Combinatorial gene therapies for multiple age-related diseases



FGF21: glucose handling,
sTGF β R2: Soluble form
 α Klotho: intracellular calcium, serum mineral-ion homeostasis

→ 5 aging diseases via combinatorial gene therapy
AAV →

GenAge The Ageing Gene Database

João Pedro de Magalhães



1. High fat diet obesity
2. Type 2 Diabetes
3. Osteoarthritis
4. Cardiac damage recovery
5. Kidney disease

Davidsohn et al. (2019) PNAS
Martinez-Redondo et al. (2020) Protein Cell.
Zullo, et al.(2019) Nature



Accelerated Evolution via multiplex-editing, ML, libraries

Addition, subtraction, precise-editing, epigenetics

Transgenics + cisgenics

Nucleases (error-prone)

De-aminases C→T, A→G

Recombinases & Primer-Editor (precise & flexible)

Methylation, Activation, Repression/Silencing

Accelerated Basic Science → Translation



CARIBOU
BIOSCIENCES

Intellia
THERAPEUTICS

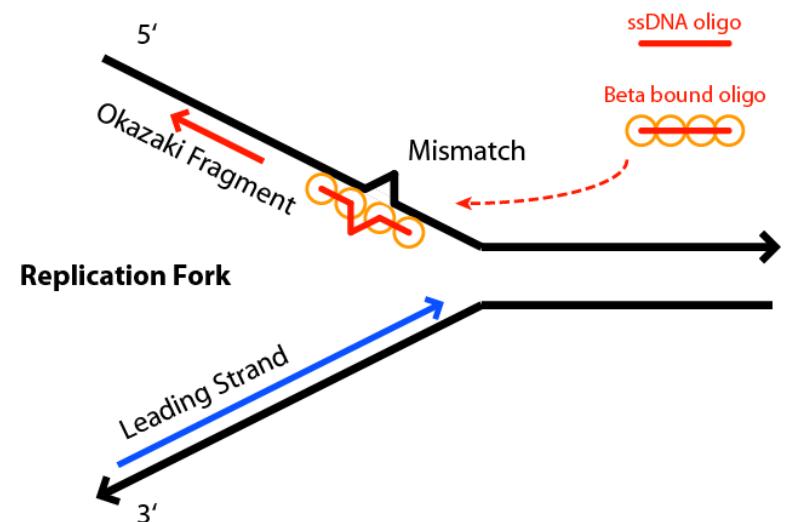
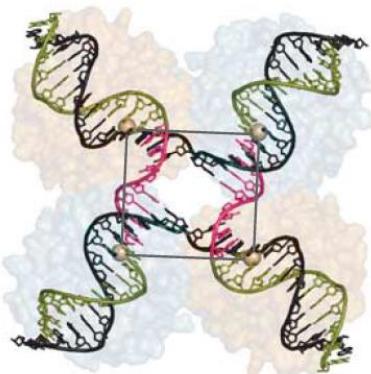
TESSERA
THERAPEUTICS

INARI

Recombinases & Integrases

- λ Red recombinase: Gabe Filsinger & Tim Wannier found interaction with Single-strand binding proteins (SSB) No custom protein or RNA (just donor DNA)

Small S/Y integrases: Ben Weinberg. ML-Syn
Custom protein (no RNA)

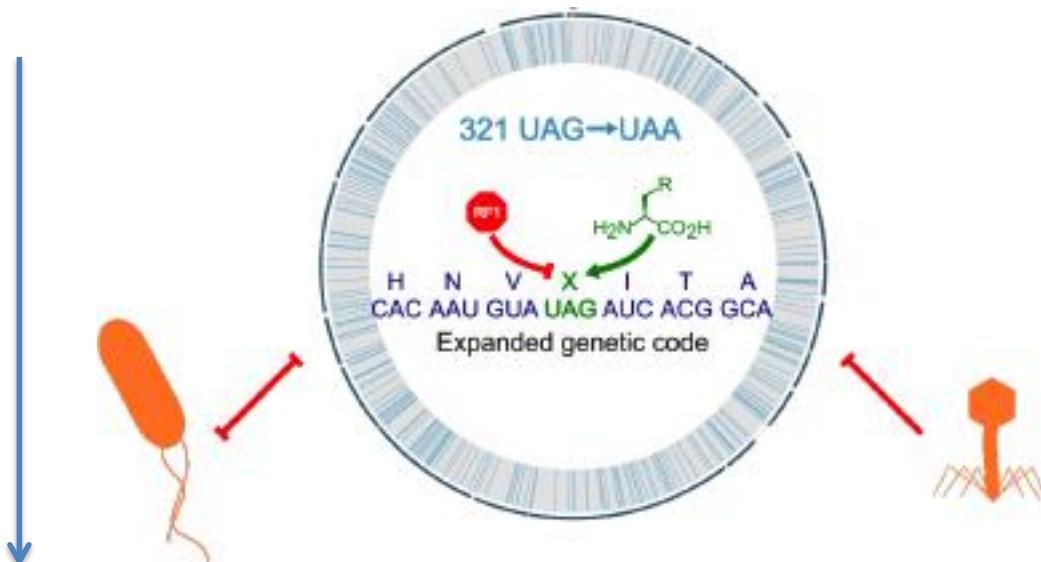


GP-Write: precise hyperediting (MAGE)

4 Million bp Genome Recoding: 63 & 57 codon types

321 (2013-5) → 62,214 codons (2016-9)

1. Non-standard amino acids (NSAA)



2. Genetic & Metabolic Isolation



3. Multi-Virus resistance

Science 2016 Ostrov, et al

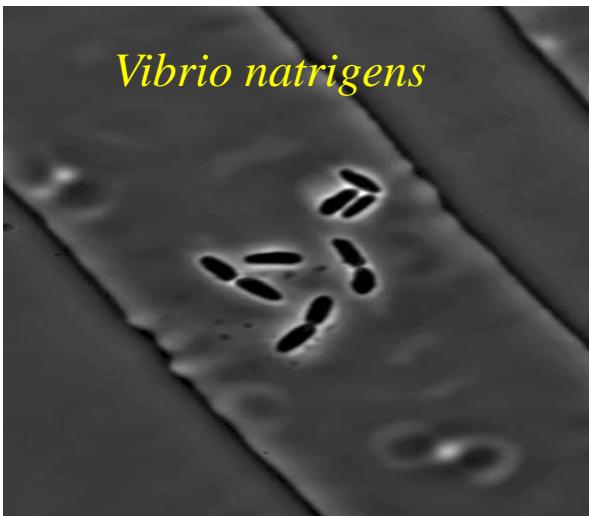
Nature 2015 Mandell et al

Science 2013 Lajoie et al

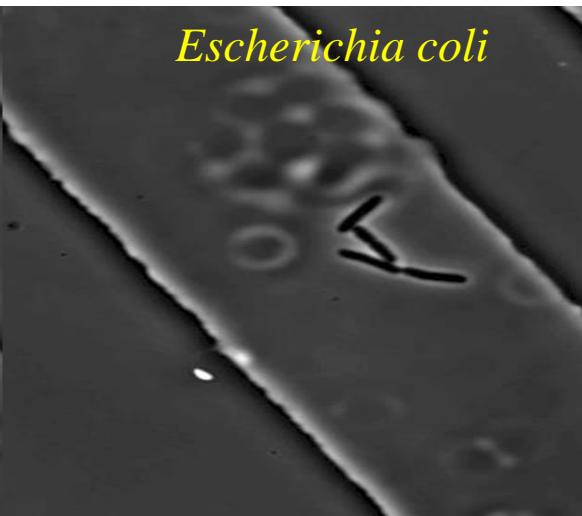


Species	Protein	Comment	Double hr
<i>Vibrio natrigens</i>	-	Heterotroph	0.16
<i>Synechococcus</i>	~3%	11801 in BG11 media .04% CO ₂	2.3
<i>Aphanothece sacrum</i>	-	Suizenji-nori cyanobac	15
<i>Arthrospira platensis</i>	60%	"Spirulina" no-N ₂ -fix cyanobac	24
<i>Wolffia arrhiza</i>	40%	duckweed-flower, watermeal, N ₂ -fix	30
<i>Azolla pinnata</i>	-	duckweed-fern, N ₂ -fix	46
<i>Chlorella pyrenoidosa</i>	-	no-N ₂ -fix	51
<i>Nostochopsis lobatus</i>	-	N ₂ -fix cyanobac	58
<i>Pyropia tenera</i>	39%	Red algae "Nori"	240
<i>Palmaria palmata</i>	-	Red algae	240
<i>Nostoc commune</i>	~3%	N ₂ -fix cyanobac	288

Vibrio natrigens

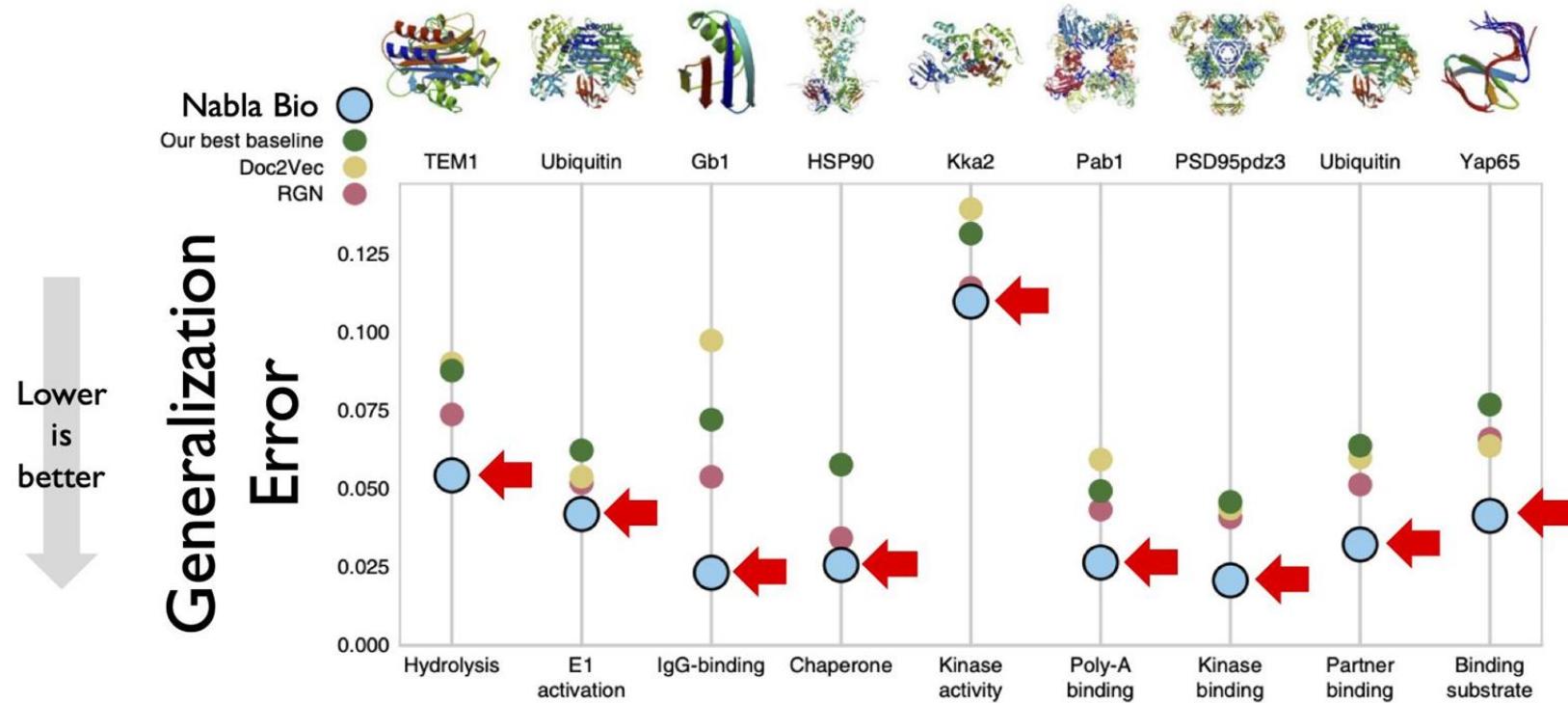


Escherichia coli



“Pre”-training on natural protein sequences enables learning of state-of-the-art virtual fitness landscapes

9 diverse proteins with diverse activities



nature methods

Alley, Khimulya, Biswas et al. 2019

Multiplex testing thousands of biologic designs in a single animal (via barcodes)



ManifoldBio



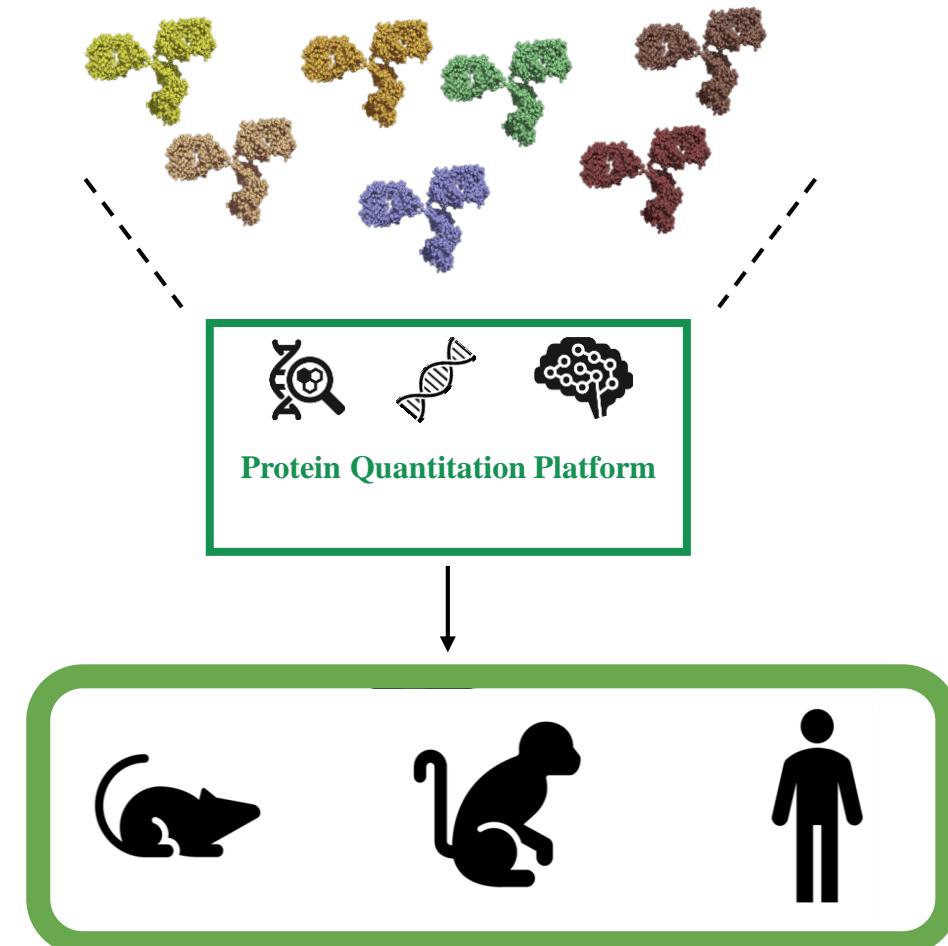
Pierce
Ogden



Gleb
Kuznetsov

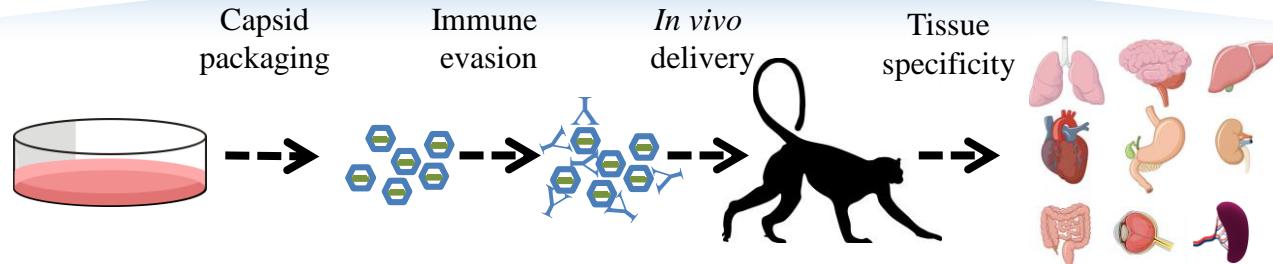
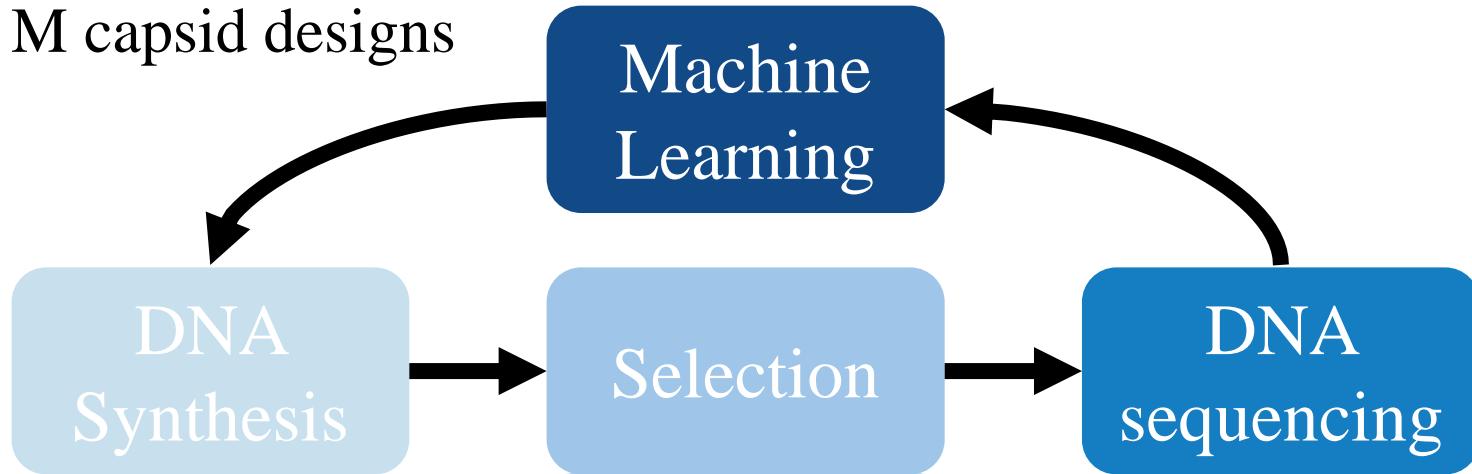


Shane
Lofgren



Protein Design (AAV & Ab): Multiplex deep sequence testing

1.2 M capsid designs



Pierce Ogden

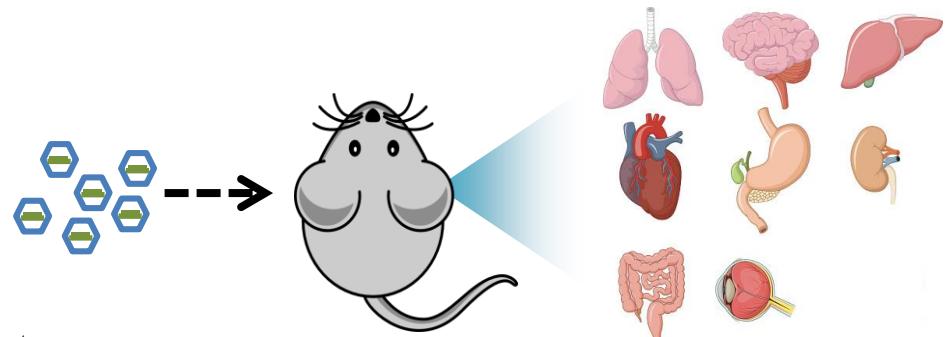


Eric Kelsic

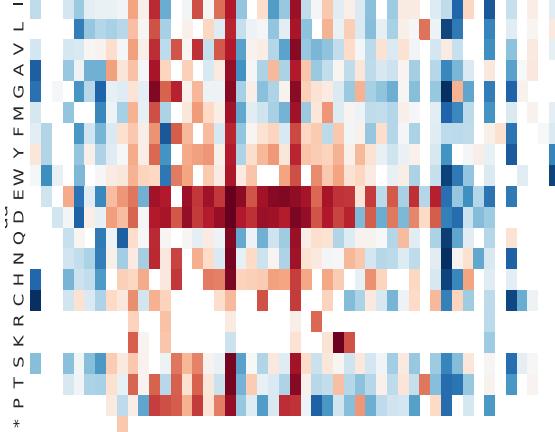
Science Nov 2019



Mapping the space of tissue tropism modifications



Blood

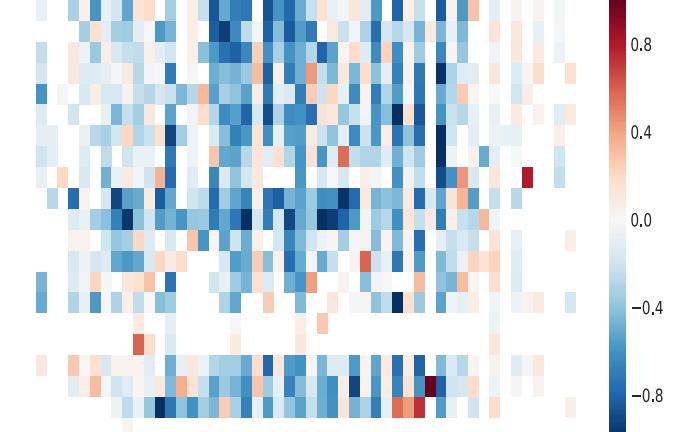


Heart

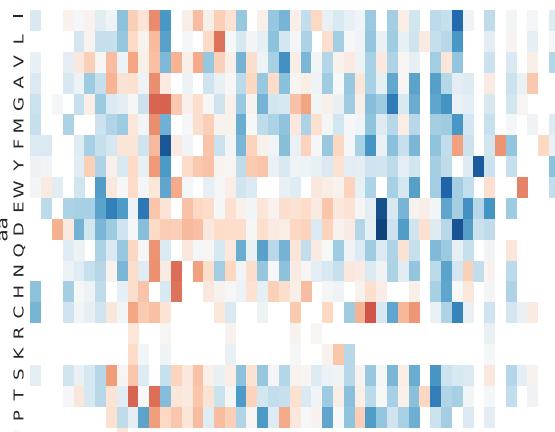
Ogden et al.
Science 2019



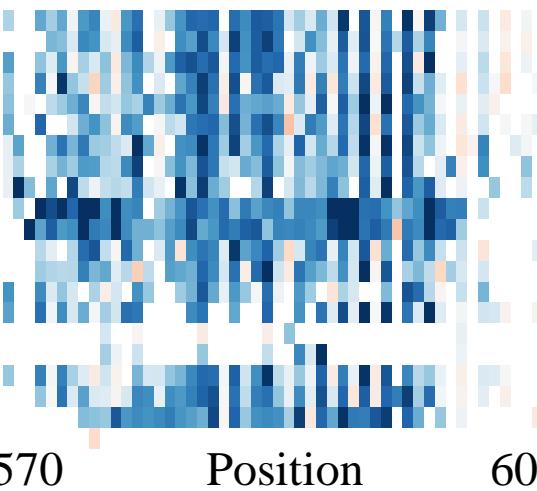
Liver



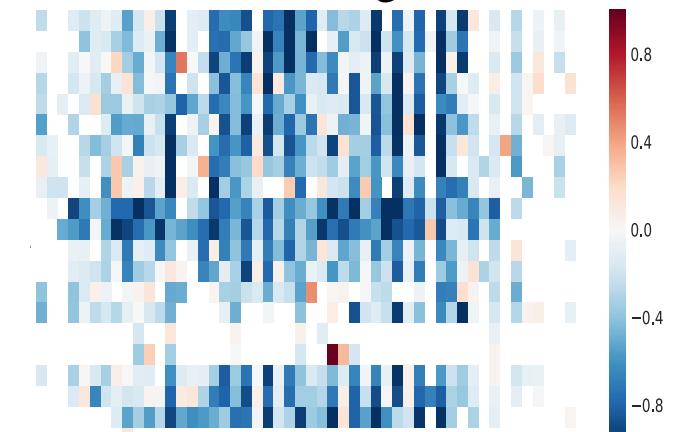
Kidney



Spleen



Lung

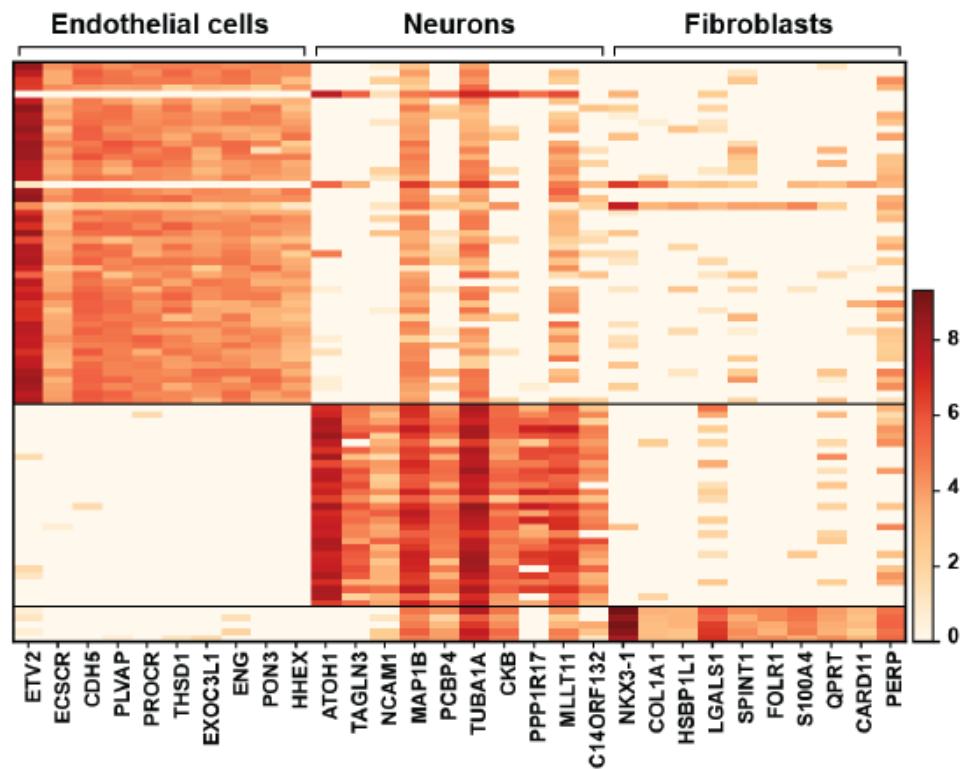


570

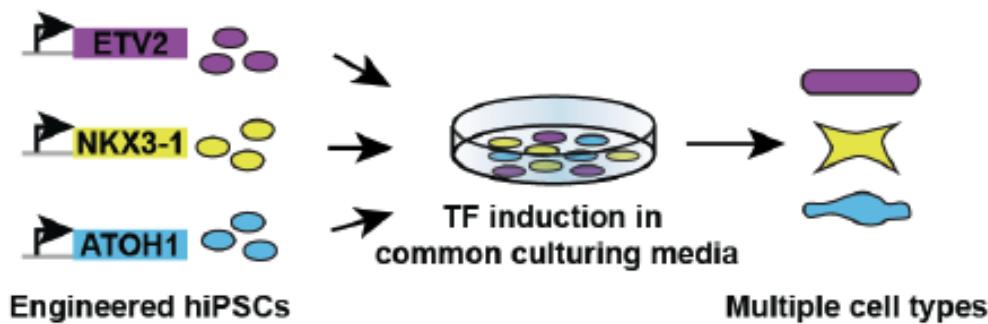
Position

605

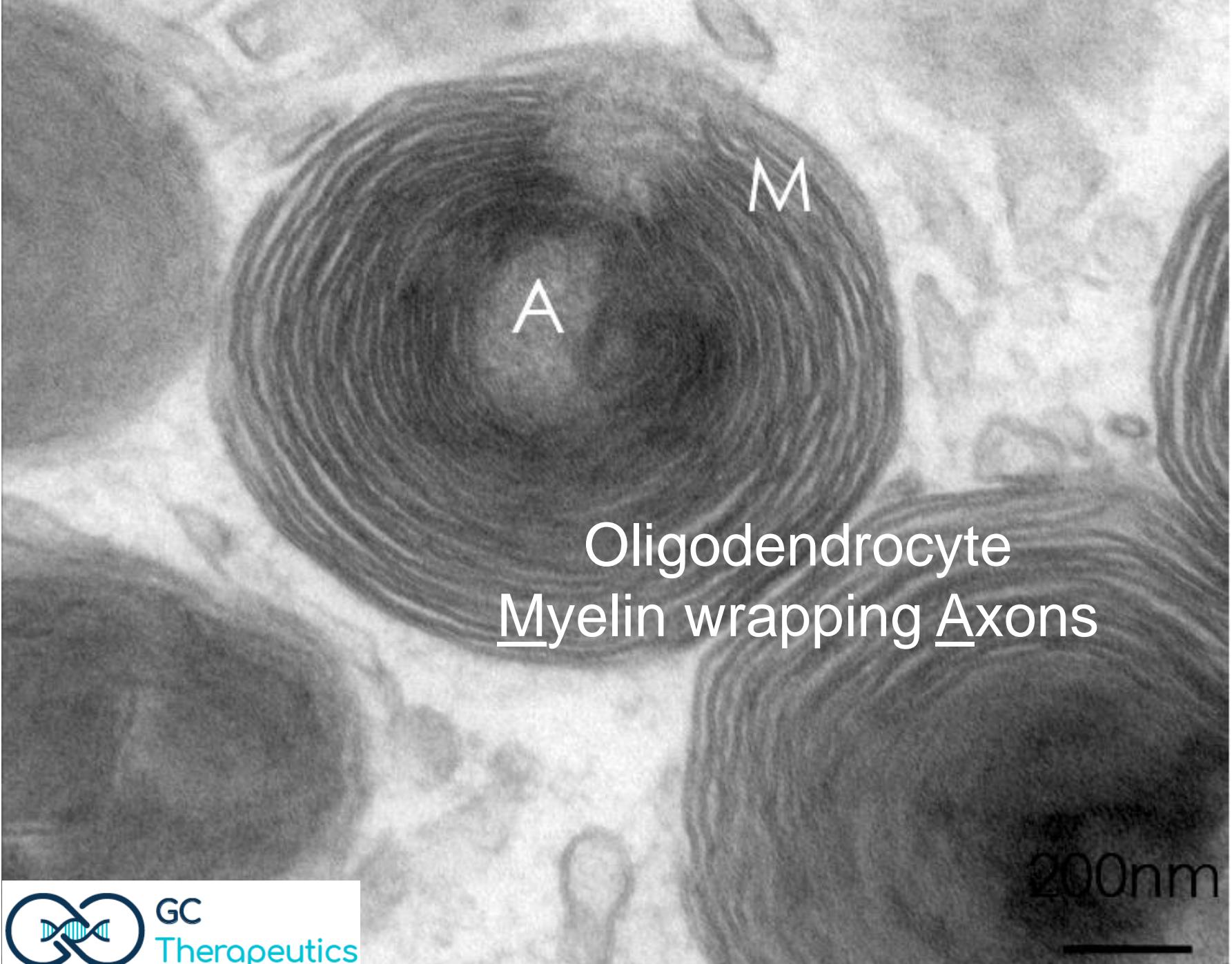
Multiplex cell & epigenetic engineering via complete human transcription factor expression library



1,748 human TF ORFs → 290 cell type recipes so far



Ng,
Khoshakhlagh, et
al. (2020) Nature
Biotech.



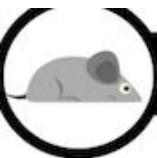
A

M

Oligodendrocyte
Myelin wrapping Axons

200nm

Human organoid transplant reversal & enhanced relative to original in resistance to demyelination



We observed enhanced motor function after OPC injection

Healthy mouse



Hind limbs far apart:
Score 4

Shiverer: OPC Injection



Hind limbs far apart:
Score 4

Shiverer: Mock Injection



Hind limbs close together:
Score 3

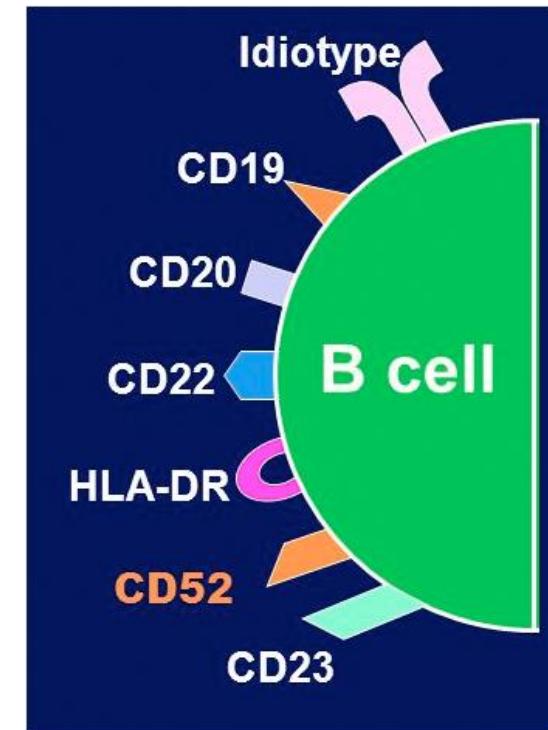


Ng, Khoshakhlagh, Shipman, Swiersy, Appleton, Huang, Saylor, Trono, Taipale, Hill, Vidal, Busskamp, Church (2020) in review

Universal Chimeric Antigen Receptor T-cells (UCART) (& Macrophages CAR-M)

Most B-cell malignancies express CD19, so target with CAR.

Multiplex editing (ZFN, TALEN, CRISPR):
TRAC-, B2M-, PDCD1-, CD52-, DCK-



ZFN: Torikai...Cooper Blood 2013 (U. TX)

TALEN: Valton...Poirot L. Molec Therap 2015 (Cellectis)

CRISPR: Liu...Wang Cell Research 2-Dec-2016 (Beijing)

Enhanced transplants & cell therapies. Multiplex (42-plex) germline editing

Sugars: GGTA1, CMAH, β 4GalNT2

Clotting: human TFPI, TBM, EPCR, vWF

MHC: HLA/SLA class I, II

Immune functions: human CTLA4-Ig, HLA-E/G/Cw3 inhibit NK cells

Complement: regulatory genes CD46, CD55, CD59

Porcine ERVs: 25 in normal fibroblasts



MGH:
Jim Markmann



Luhan Yang, et al 2015 Science
Dong Niu, et al. 2017 Science
Yanan Yue et al. 2020 Nat BME



Multiplex editing to reduce somatic mutations

Category	#/ hum cell	Length	Total bp	Previous mutation attempts
ERV	25	7500	2.4E+7	Cas9 Yang .. Science 2015, 2017
Telomeres	46	8000	3.7E+5	TERT Ramunas .. FASEB J 2015
rDNA repeats	300	43000	1.3E+7	I-CreI Paredes .. Genetics 2009
UCE	855	200	1.7E+5	HDR
→ LINEs	26,000	7000	1.8E+8	dABE Smith .. Biorxiv 2019
Centromeres	1.0E+6	171	1.7E+8	Cas9 Adikusuma .. Mol Ther 2017
SINEs (Alu)	1.5E+6	280	4.2E+8	Natural variants
SSR	3.0E+6	16	4.8E+7	Cas9 [14] Monteys..Mol Ther 2017
Triplex sites	1.7E+7	20	3.4E+8	

Repeats involved in senescence, neurogenesis, cancer, inflammation

Wang et al. Cell Cycle 2011

DeCecco et al. Nature 2019

Singer et al. Trends Neurosci. 2010

Enabling large-scale genome editing by reducing DNA nicking

Deamination

C → T

A → G

5' - ATTCTACCAGAGGTACAAGGAGG - 3'

5' - ATT~~T~~TATTAGAGGTACAAGGAGG - 3'

5' - ATTCTGCCAGAGGTACAAGGAGG - 3'

1. No nick. dCas9
2. No MMR
3. No U-glycosylase
4. Anti-apoptotic molecules
5. Growth factors (bFGF)

Smith CJ, Castanon O, Said K, Volf V,
Khoshakhlagh P, Hornick A, Ferreira R, Wu
CT, Güell M, Myllykallio H, Church
GM (2019) Biorxiv

Smith CJ, Castanon O, Khoshakhlagh P,
Güell M, Wang S, Said K, Yildiz R, Dysart
M, Thompson D, Myllykallio H, Church
GM (2019) Biocontainment...

Cory
Smith



Oscar
Castanon



Khaled
Said



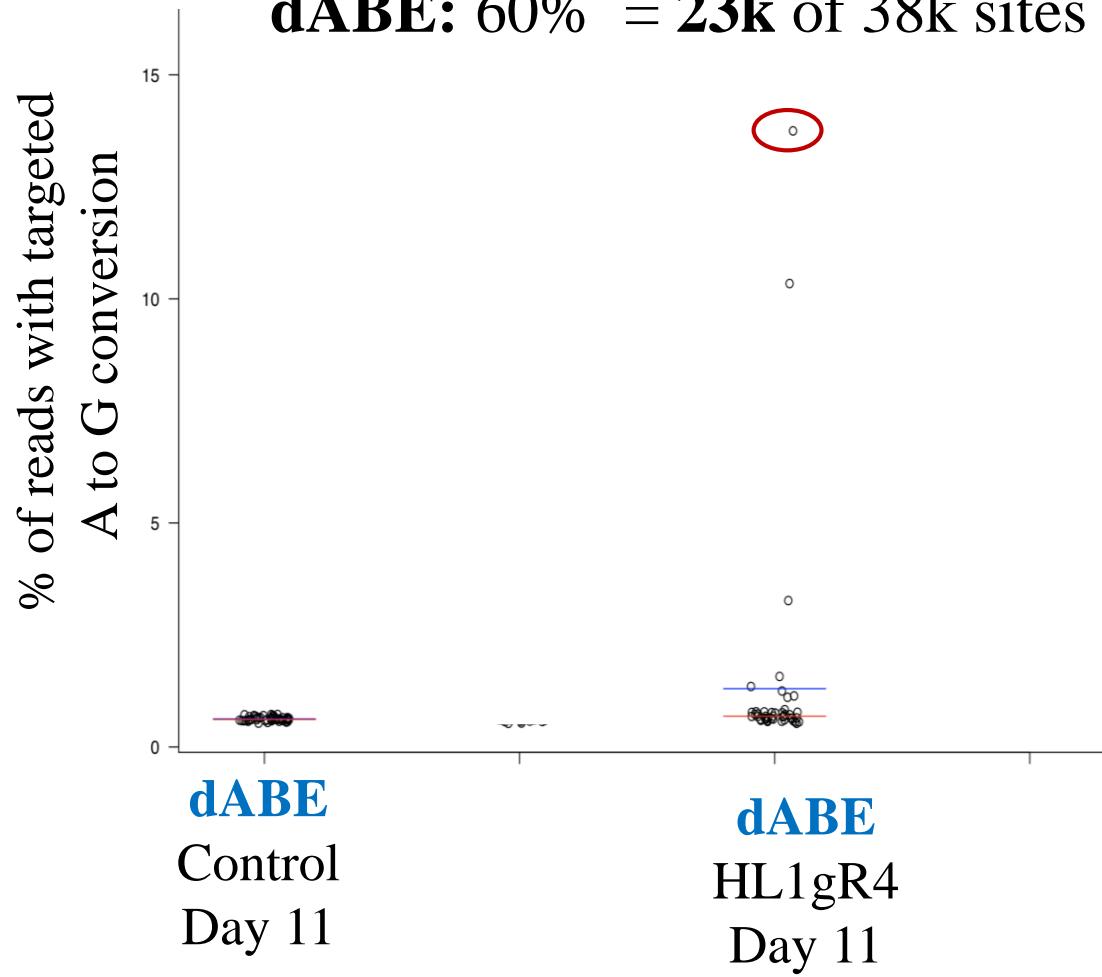
Verena
Volf



Nick-less dABE targeting of LINE-1 in PGP1 hiPSCs

Single cells - Day 11

dABE: 60% = 23k of 38k sites (diploid)





Accelerated evolution, proliferation & mammalian development.

Abstract: We have developed methods using human transcription factor expression libraries to accelerate some developmental processes from 300 days to 4 days and have used these semi-synthetic tissues to accurately evaluate late onset (70year) diseases like Alzheimer's. We have easily engineerable proliferative systems with doubling times of only 15 minutes that we would like to adapt to display analogous developmental processes. We have accelerated evolution way of a combination of gigabase-scale computer-controlled DNA synthesis plus machine learning. We have used this to design and test millions of viral capsids for improved AAV gene therapies.