VectorBuilder.com

From design to therapy:

Custom cloning and virus packaging services

Justin Mirus

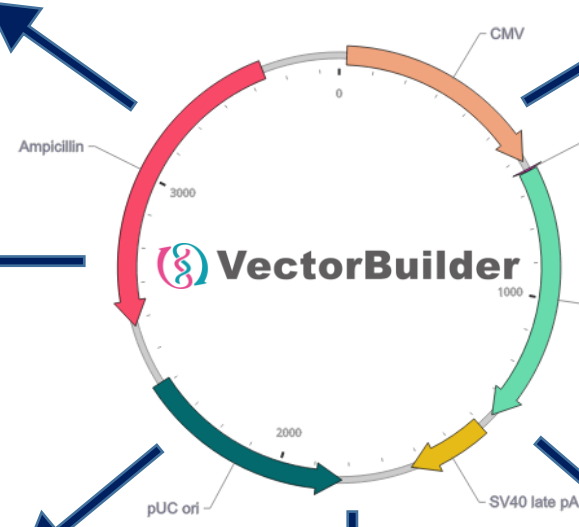
Senior Territory Manager
VectorBuilder GmbH

What is VectorBuilder?

VectorBuilder's online platform provides researchers with one-stop solutions to vector design, custom cloning, virus packaging and more…

Vector design and construction

Virus Packaging:
- Lenti, Adeno, AAV, MSCV, MMLV, Baculo



DNA and RNA preparation:
- Transfection/microinjection ready kits

Mutagenesis

BAC recombineering

shRNA/CRISPR, lncRNA and small peptide libraries

Custom Stable Cell Lines

1010 W 35th Street, Suite 515, Chicago, IL 60609, USA
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Focus of VectorBuilder's Development

Establishing a Niche

VectorBuilder.com website as a truly user-friendly design tool for comprehensive and concise custom cloning design

Validation, optimization and R&D

Continuous implementation of acquired knowledge to provide superior technologies/listening to client feedback

Future Development

Gearing up for GMP grade virus for therapeutic purposes

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How does VectorBuilder.com work?

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Why does it work?

➢ User-friendly & adaptable online designing and ordering

- Reducing back-and-forth consultation – design and order on the-go!
- Global access – online accessibility and ease of design process for researchers at all levels

➢ Achieving high quality standards with quick delivery times

- High success rate of project deliveries on schedule

➢ Consistent and reproducible cloning methods (Optimized pipelines)

- Standard SOPs for 100% sequence guarantees and adherence to SOPs
- Design tool compatible with modular cloning procedures

➢ Online and face-to-face communication

- Experienced territory managers/application scientists
- Skilled technical design team

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VectorBuilder Highlights

>100 Vector Systems

Expansive database components: Promoters, ORFs, Tags, Linkers, shRNA, Enhancers, gRNAs.

Rich annotation of vectors: Vector design viewed in a richly annotated manner wilth detailed description and application notes

Highly affordable prices: shRNA vectors start at $99.

Store credit option and parallel cloning of large projects: Massive parallel cloning possible (30+ vectors); tab system for budgetary reasons optional

Rapid turnaround: Within one week for basic designs

R&D: Optimizing vector systems, promoters, tags, virus packaging protocols, introducing new systems a constant priority

- Mammalian Gene Expression Vectors
- Mammalian Non-Coding RNA Expression Vectors
- Mammalian shRNA Knockdown Vectors
- Mammalian CRISPR Gene Editing Vectors
- Mammalian CRISPR Gene Regulation Vectors
- Enhancer/Promoter Testing Vectors
- Zebrafish Gene Expression Vectors
- Drosophila Transformation Vectors
- Plant Gene Expression Vectors
- Recombinant Protein Expression Vectors
- In Vitro Transcription Vectors

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Vector Systems

	Plasmid	Lentivirus	AAV	Adenovirus	MMLV, MSCV	piggyBac; Sleeping Beauty
Vector type	non-viral	viral	viral	viral	viral	transposon
Target cell type	broad but varying	broad	broad	broad	limited	broad but varying
Transient or stable expression	transient	stable	transient	transient	stable	stable
Copy number in host cell	very high	low	high	very high	low	moderate
Cargo limit	large (30 kb)	moderate (9.2 kb)	small (4.7 kb)	large (38.7 kb)	moderate (8 kb)	large (30 kb); moderate (10 kb)
Immune response	weak	weak	weak	strong	intermediate	weak
Promoter customization	yes	yes	yes	yes	no	yes
Delivery method	transfection, electroporation	transduction	transduction	transduction	transduction	transfection, electroporation

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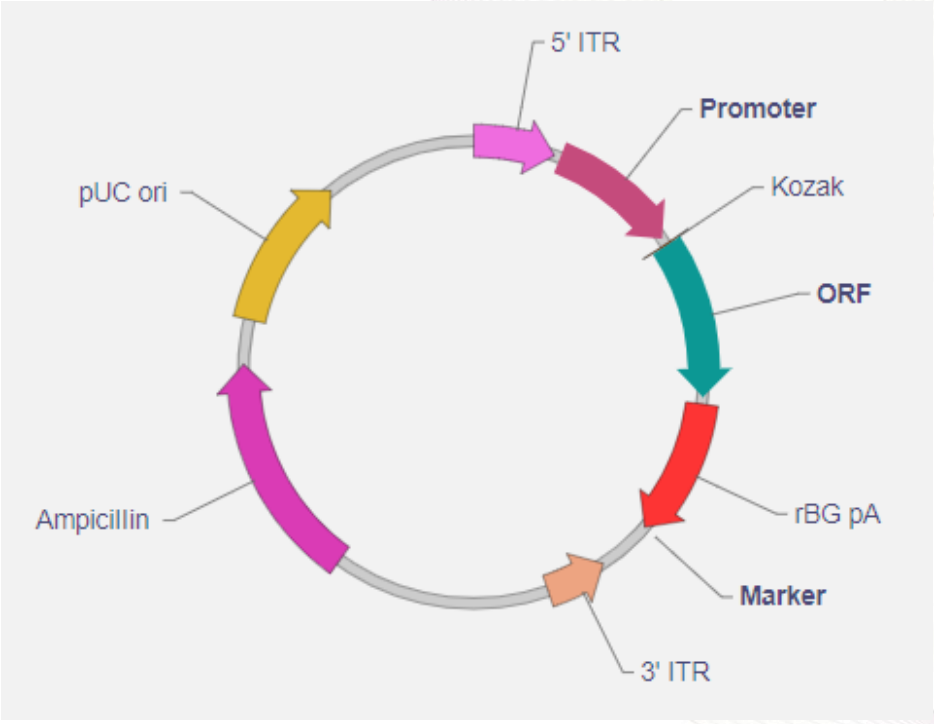
The PiggyBAC transposon/transposase system



- Transposase ID‘s ITRs and inserts DNA into host typically in TTAA sites
- Non-viral integration into host genome
- ~27 kb capacity for custom constructs
- Relies on transfection (primary cells and non-dividing cells more resistant)
- PiggyBAC Tet-On, FLEx, lncRNA, shRNA, CRISPR vectors available

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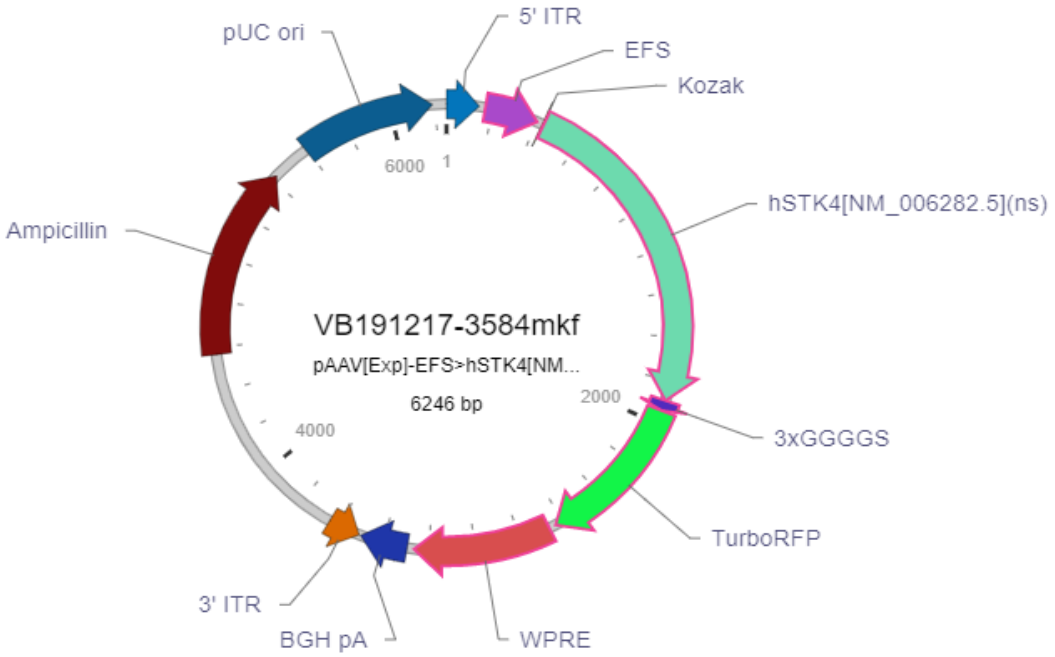
The AAV system



- No integration into host genome
- ~4.2 kb capacity for custom constructs
- Less immunogenic than Ad
- High viral titers can be achieved (>10^{13} GC/ml)
- qPCR used to determine physical titer

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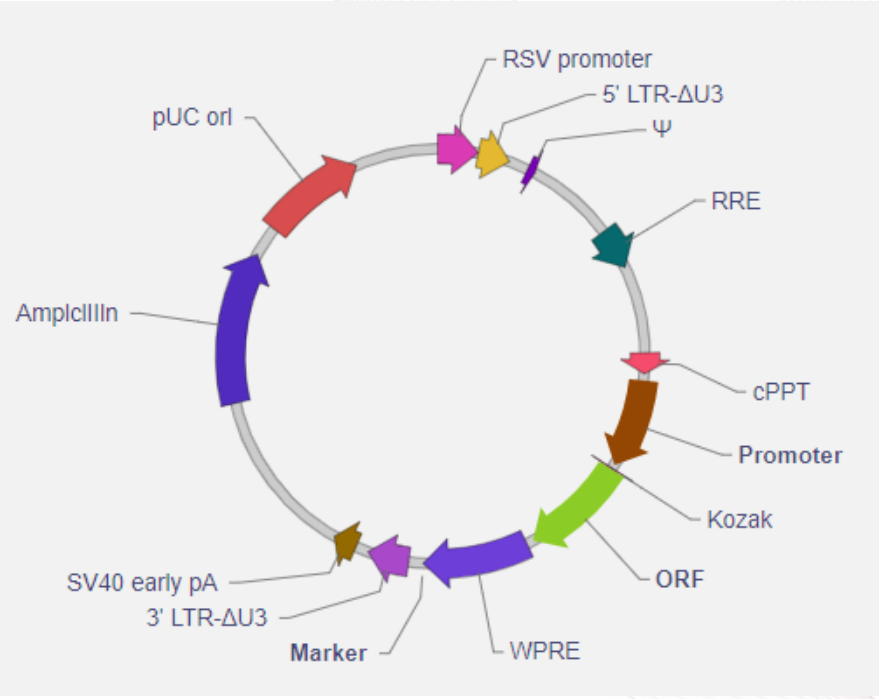
The lentiviral system



- Integration into host genome
- ~6.4 kb capacity for custom constructs
- Broad tropism
- High viral titers can be achieved (>10^8 TU/ml)
- p24 ELISA for titer determination

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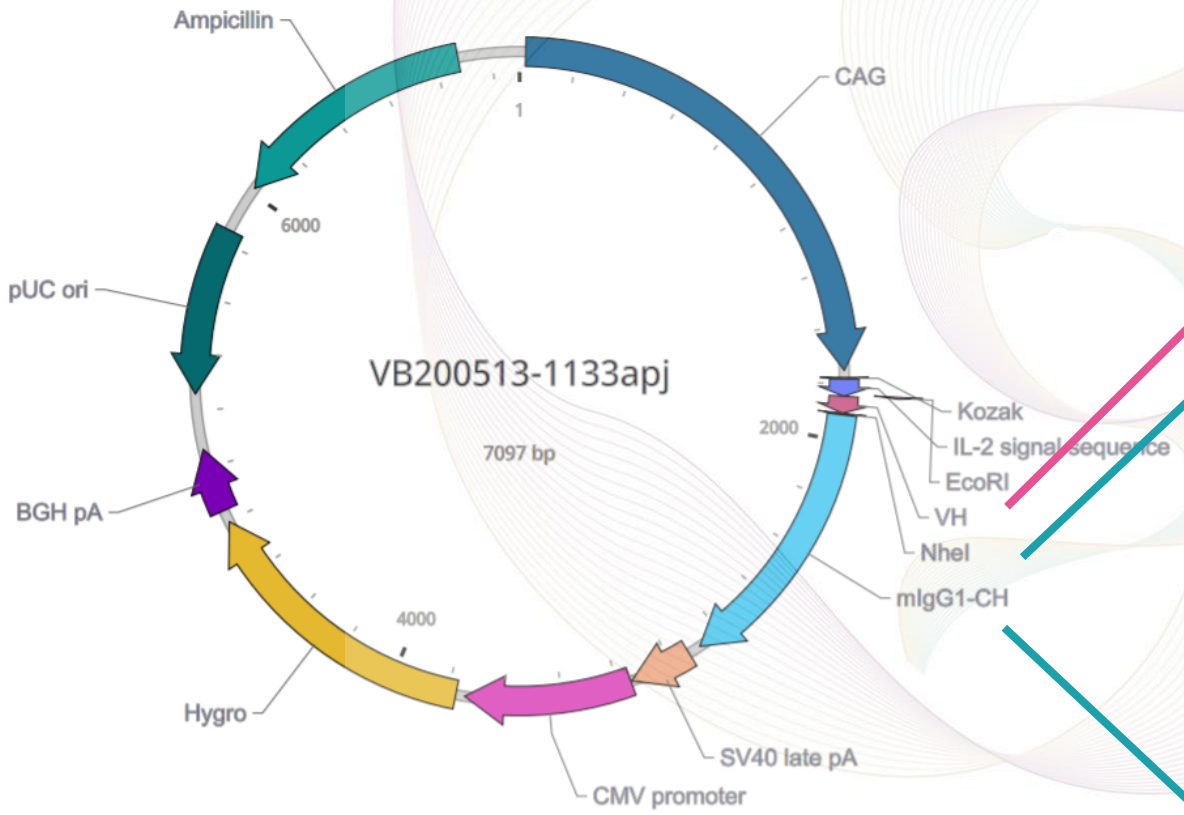
Sample antibody vectors



Vector Sequence

Full length: 7097 Residue: 1910-2884 (length: 975)

1751	ATTGCAAGTT	TGTACAAAAA	AGCAGGCTGC	CACCATGTAC	AGGATGCAAC
1801	TCCTGTCTTG	CATTGCACTA	AGTCTTGCAC	TTGTCACGAA	TTCGNNNNNN
1851	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN
1901	NNNGCTAGCG	CTAAAACGAC	ACCCCCATCT	GTCTATCCAC	TGGCCCCTGG
1951	ATCTGCTGCC	CAAACTAACT	CCATGGTGAC	CCTGGGATGC	CTGGTCAAGG
2001	GCTATTTCCC	TGAGCCAGTG	ACAGTGACCT	GGAACTCTGG	ATCCCTGTCC
2051	AGCGGTGTGC	ACACCTTCCC	AGCTGTCCTG	CAGTCTGACC	TCTACACTCT
2101	GAGCAGCTCA	GTGACTGTCC	CCTCCAGCAC	CTGGCCCAGC	GAGACCGTCA
2151	CCTGCAACGT	TGCCCACCCG	GCCAGCAGCA	CCAAGGTGGA	CAAGAAAATT
2201	GTGCCCAGGG	ATTGTGGTTG	TAAGCCTTGC	ATATGTACAG	TCCCAGAAGT
2251	ATCATCTGTC	TTCATCTTCC	CCCCAAAGCC	CAAGGATGTG	CTCACCATTA
2301	CTCTGACTCC	TAAGGTCACG	TGTGTTGTGG	TAGACATCAG	CAAGGATGAT
2351	CCCGAGGTCC	AGTTCAGCTG	GTTTGTAGAT	GATGTGGAGG	TGCACACAGC
2401	TCAGACGCAA	CCCCGGGAGG	AGCAGTTCAA	CAGCACTTTC	CGCTCAGTCA
2451	GTGAACTTCC	CATCATGCAC	CAGGACTGGC	TCAATGGCAA	GGAGTTCAAA
2501	TGCAGGGTCA	ACAGTGCAGC	TTTCCCTGCC	CCCATCGAGA	AAACCATCTC
2551	CAAAACCAAA	GGCAGACCGA	AGGCTCCGCA	GGTGTACACC	ATTCCACCTC
2601	CCAAGGAGCA	GATGGCCAAG	GATAAAGTCA	GTCTGACCTG	CATGATAACA
2651	GACTTCTTCC	CTGAAGACAT	TACTGTGGAG	TGGCAGTGGA	ATGGGCAGCC
2701	AGCGGAGAAC	TACAAGAACA	CTCAGCCCAT	CATGGACACA	GATGGCTCTT
2751	ACTTCGTCTA	CAGCAAGCTC	AATGTGCAGA	AGAGCAACTG	GGAGGCAGGA
2801	AATACTTTCA	CCTGCTCTGT	GTTACATGAG	GGCCTGCACA	ACCACCATAC
2851	TGAGAAGAGC	CTCTCCCACT	CTCCTGGTAA	ATGAACCCAG	CTTTCTTGTA

Pricing: ~$539; 25-36 days TAT

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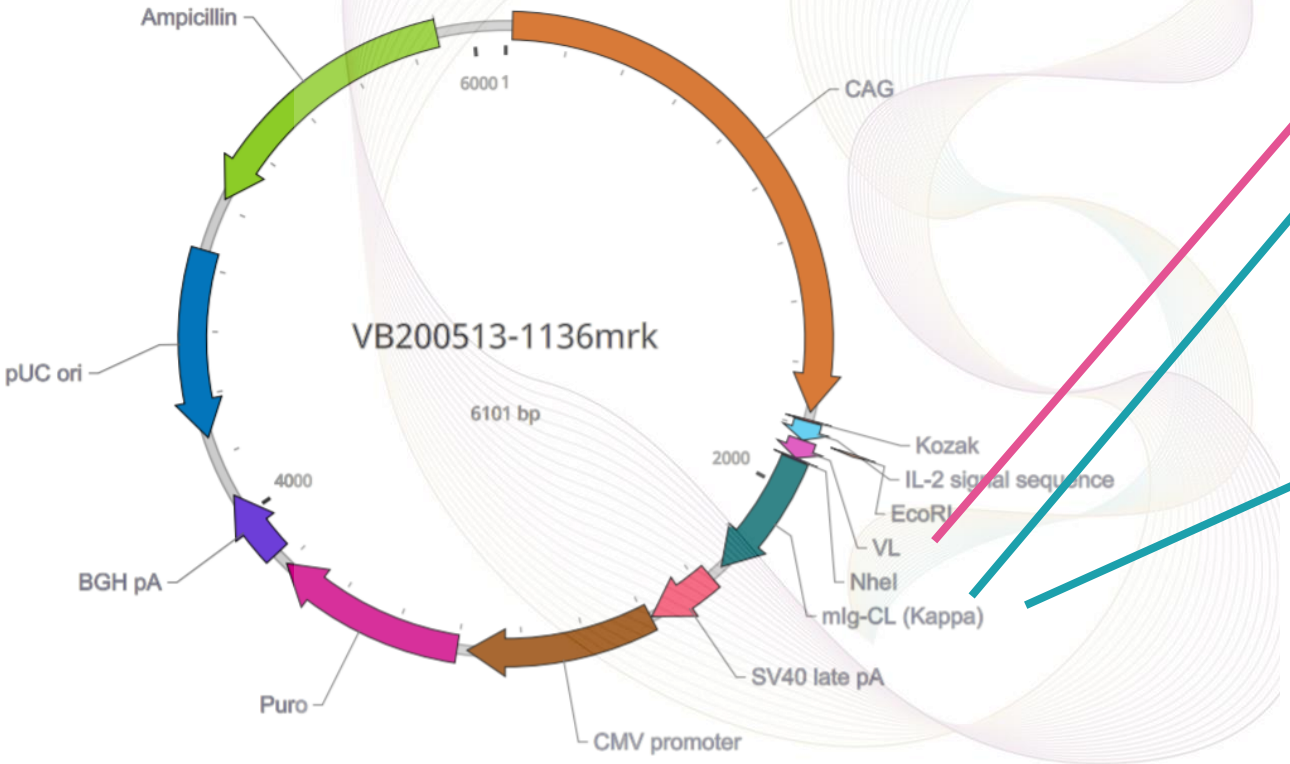
Sample antibody vectors



Full length: 6101 Residue: 1910-2314 (length: 405)

1751	ATTGCAAGTT	TGTACAAAAA	AGCAGGCTGC	CACCATGTAC	AGGATGCAAC	
1801	TCCTGTCTTG	CATTGCACTA	AGTCTTGCAC	TTGTCACGAA	TTCGNNNNNN	
1851	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	NNNNNNNNNN	
1901	NNNGCTAGCA	TGTACAGGAT	GCAACTCCTG	TCTTGCATTG	CACTAAGTCT	
1951	TGCACTTGTC	ACGAATTCAC	CGGTCACCCT	CGAGATCAAA	CGGGCAGATG	
2001	CTGCACCAAC	TGTATCCATC	TTCCCACCAT	CCAGTGAGCA	GTTAACATCT	
2051	GGAGGTGCCT	CAGTCGTGTG	CTTCTTGAAC	AACTTCTACC	CCAAAGACAT	
2101	CAATGTCAAG	TGGAAGATTG	ATGGCAGTGA	ACGACAAAAT	GGCGTCCTGA	
2151	ACAGTTGGAC	TGATCAGGAC	AGCAAAGACA	GCACCTACAG	CATGAGCAGC	
2201	ACCCTCACGT	TGACCAAGGA	CGAGTATGAA	CGACATAACA	GCTATACCTG	
2251	TGAGGCCACT	CACAAGACAT	CAACTTCACC	CATTGTCAAG	AGCTTCAACA	
2301	GGAATGAGTG	TTAGACCCAG	CTTTCTTGTA	CAAAGTGGTG	ATGGCCGGCC	
2351	GCTTCGAGCA	GACATGATAA	GATACATTGA	TGAGTTTGGA	CAAACCACAA	
2401	CTAGAATGCA	GTGAAAAAAA	TGCTTTATTT	GTGAAATTTG	TGATGCTATT	
2451	GCTTTATTTG	TAACCATTAT	AAGCTGCAAT	AAACAAGTTA	ACAACAACAA	
2501	TTGCATTCAT	TTTATGTTTC	AGGTTCAGGG	GGAGGTGTGG	GAGGTTTTTT	
2551	AAAGCAAGTA	AAACCTCTAC	AAATGTGGTA	CGCGTTGACA	TTGATTATTG	
2601	ACTAGTTATT	AATAGTAATC	AATTACGGGG	TCATTAGTTC	ATAGCCCATA	
2651	TATGGAGTTC	CGCGTTACAT	AACTTACGGT	AAATGGCCCG	CCTGGCTGAC	
2701	CGCCCAACGA	CCCCCGCCCA	TTGACGTCAA	TAATGACGTA	TGTTCCCATA	
2751	GTAACGCCAA	TAGGGACTTT	CCATTGACGT	CAATGGGTGG	AGTATTTACG	
2801	GTAAACTGCC	CACTTGGCAG	TACATCAAGT	GTATCATATG	CCAAGTACGC	
2851	CCCCTATTGA	CGTCAATGAC	GGTAAATGGC	CCGCCTGGCA	TTATGCCCAG	

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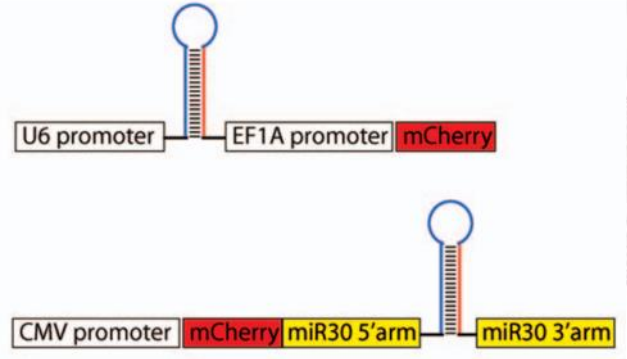
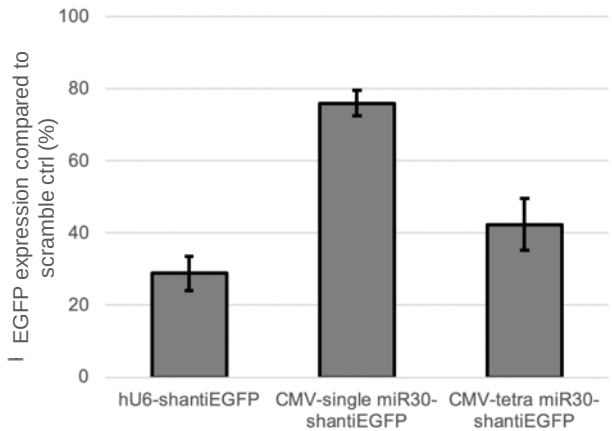
Vector Systems

- Small RNA transcription usually driven by RNA Pol III promoter (e.g. U6, H1).
- RNA Pol II promoter regulated shRNA transcription can be enabled via miRNA scaffold (e.g. miR30, miR155).
- However, U6-driven shRNA tends to have better knockdown efficiency than miR-mediated shRNA.





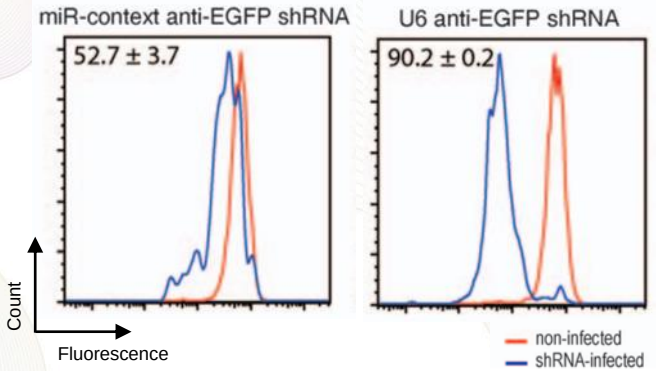


Table 1. Overview of the efficacy of shRNA-miRs-guided target knockdown in various human cell lines.

	Raji	Jurkat	THP1	293T	HeLa	HT29
U6	90.5±0.3	90.2±0.2	95.2±0.1	92.5±0.1	92.3±0.2	97.4±0.1
EF1A	80.0±2.2	86.3±0.2	90.5±0.2	90.2±0.1	90.7±0.1	95.8±0.1
CAGGS	53.4±0.9	70.7±1.8	84.7±0.7	91.8±0.1	89.9±4.3	86.8±0.8
CMV	50.0±1.6	52.7±3.7	61.5±3.0	88.0±0.4	91.9±0.2	77.7±0.3
PGK	44.3±1.2	52.9±0.7	71.8±0.8	71.9±3.3	65.3±3.8	80.6±0.3
UbiC	26.8±1.0	52.2±2.4	72.2±2.4	60.2±0.4	56.7±4.9	76.7±0.7

Vector Systems

Name	Max Excitation/Emission (nm)	Brightness (% of EGFP)	Application Notes
EGFP	484/507	100	Commonly used green fluorescent protein
EmGFP	487/509	116	Enhanced photostability and brightness compared to EGFP
TurboGFP	482/502	112	Very bright; fast maturation
hrGFP	500/506	100	Low cytotoxicity
d2EGFP	488/507	100	Fast turnover
NLS_EGFP	484/507	100	Nuclear localization
ZsGreen1	493/505	250	High solubility; bright emission; rapid chromophore maturation
EGFP_S65T	484/507	100	Higher expression in plant than EGFP
mNeonGreen	506/517	276	Brightest monomeric GFP; fast maturation; can be used for FRET
Venus	515/528	156	Fast maturation and high tolerance to acidosis and Cl-
EYFP	513/527	150	Excitaton and emission are sensitive to pH
YPet	517/530	238	Can be used in conjunction with CyPet for FRET
Cerulean	433/475	79	2.5-fold brighter than ECFP; can be used for FRET
CyPet	435/477	53	Can be used in conjunction with Ypet for FRET applications
EBFP	383/445	27	Low fluorescence and low photostability
TagBFP	402/457	99	Rank high in brightness, photostability and pH stability among BFP
TagBFP2	399/454	121	Rapid chromophore formation
dTomato	554/581	142	Fast maturation but lower brightness than DsRed
DsRed_Express2	554/591	72	High solubility; fast maturation
TurboRFP	553/574	187	Fast maturation; very bright
mRFP1	584/607	37	Fast maturation but lower brightness than DsRed
mCherry	587/610	47	Commonly used RFP
mApple	568/592	109	Brighter than mCherry; constant photostability
mKate2	588/633	74	A superior fluorescent tag for imaging in living tissues

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Vector Systems

Our recommendations:

- Single-color experiment

  EGFP, TurboGFP, mCherry, dTomato, or DsRed_Express2

- Two-color experiment

  EGFP+mCherry, or TurboGFP+mCherry

- Three-color experiment

  TagBFP+EGFP+mCherry, or CyPet+YPet+mCherry

  Note: The above single-color and multi-color options can be used with DAPI when proper filters are used.

- FRET

  CyPet - YPet

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Summary of VectorBuilder Legal Information

- Clients own designs and IP rights associated with vectors
- No sharing of client-specified sequences with third parties
- Customer identity, experimental design, order history confidential
- Use of elements under third-party IP at discretion and liability of customer
- **By purchasing vectors from VectorBuilder, customers acknowledge obtainment of all necessary IP licenses including being able to contract VectorBuilder to manufacture vectors
- License from TET Systems for rtTA and TET3G sequences – commercial/for-profit need license from TET to use such vectors

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Streamlining research

Viral vector services

Scale	Application	Titer & Volume
Pilot	Cell culture	>10^{11} GC/ml, 10x 25 ul, PBS buffer
Medium	Cell culture	>10^{11} GC/ml, 10x 100 ul, PBS buffer
Large	Cell culture	>10^{12} GC/ml, 10x 100 ul, PBS buffer
Ultra-purified pilot	Cell culture and in vivo	>10^{13} GC/ml, 4x 25 ul, PBS buffer
Ultra-purified medium	Cell culture and in vivo	>10^{13} GC/ml, 10x 50 ul, PBS buffer
Ultra-purified large	Cell culture and in vivo	>10^{13} GC/ml, 10x 100 ul, PBS buffer

	Lentivirus	Adenovirus	AAV	MMLV	MSCV
Target cell type	very broad	broad	depending on serotype	dividing cells	ESC, embryonal carcinoma cell, HSC
Transient or stable expression	stable	transient	transient	stable	stable
Cargo limit	**9.2 kb**	**38.7 kb**	**4.7 kb (ssAAV); 2.3 kb (scAAV)**	**8 kb**	**8 kb**
Promoter customization	yes	yes	yes	no	no
Effect of oversized genome	**low viral titer***	**genome rearrangement**	**truncated viral genome**	**low viral titer**	**low viral titer**

01 **Lentivirus + IDLV**

02 **AAV (Multiple serotypes)**

03 **Adenovirus**

04 **MMLV retrovirus**

05 **MSCV retrovirus**

06 **Baculovirus**

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Virus packaging

	Lentivirus	AAV	Adenovirus	MMLV	MSCV	Baculovirus
Virus type	VSV-G pseudotyped, 3rd-generation	rAAV based on AAV2 genome	HAd5	VSV-G pseudotyped	Developed from MMLV	Insect baculovirus strain AcMNPV
Virus subtype	Regular and Integrase-deficient lentivirus	ssAAV and scAAV in 18 serotypes	HAd5 and HAd5/F35 chimeric adenovirus	-	-	-
Titer scale	10^8 and 10^9 TU/ml	10^{11}, 10^{12}, 10^{13} GC/ml	10^{10}, 10^{11}, 10^{12} PFU/ml	10^7 and 10^8 TU/ml	10^7 and 10^8 TU/ml	10^6 and 10^7 PFU/ml
Purification method	Sucrose	CsCl	CsCl	Sucrose	Sucrose	-

Standard QC:

- Titration
- Bioburden test: bacteria, fungi, mycoplasma
- Purify assessment: SDS-PAGE
- Transduction test

Additional services upon request:

- ddPCR
- TCID50
- Endotoxin assay
- Full/empty particle analysis

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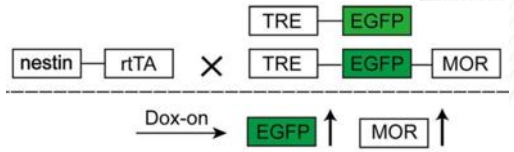
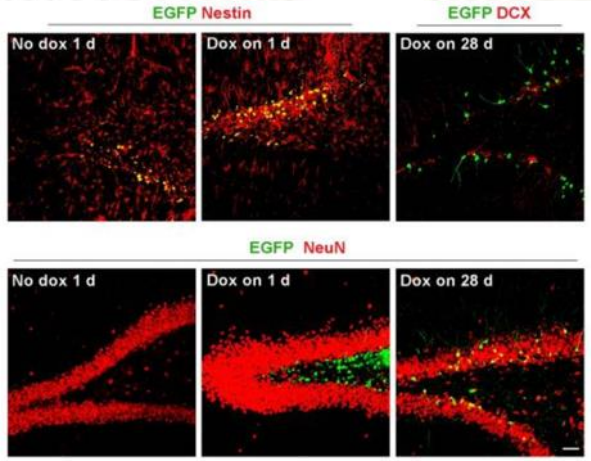
In vitro/in vivo Applications

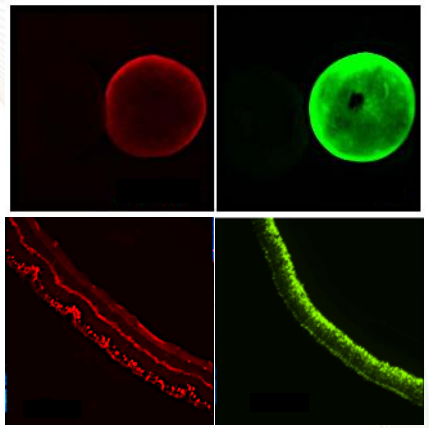


Lentivirus mediated Tet-inducible EGFP expression in neural stem cells



Sci Rep. 2019; 9: 1471.

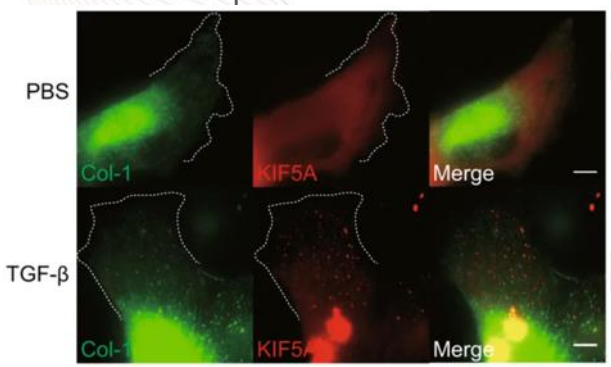
ProA1-mCherry RHO-EGFP



AAV8 mediated tissue-specific mCherry and EGFP expression in retina

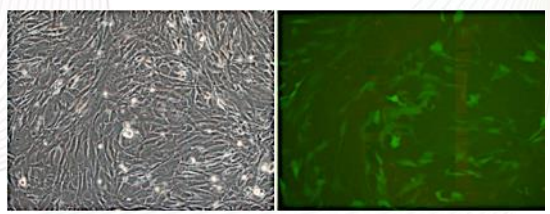
Hoang et al., unpublished

Adenovirus mediated gene expression in human pleural mesothelial cells



Sci Rep. 2017; 7:4556

MSCV mediated GFP expression in mouse mesenchymal stem cells



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Virus packaging of individual vectors and libraries

Virus Type	Scale	Application	Titer & Volume	Price (USD)
Lentivirus (including regular and non-integrating)	Pilot	Cell culture	>10^8 TU/ml, 250ul	$399
	Medium	Cell culture	>10^8 TU/ml, 1 ml	$599
	Large	Cell culture	>10^9 TU/ml, 1 ml	$999
	Ultra-purified medium	In vivo	>10^9 TU/ml, 500ul	$1199
	Ultra-purified large	In vivo	>10^9 TU/ml, 1 ml	$1499
Adenovirus	Pilot	Cell culture	>10^{10} PFU/ml, 250ul	$399
	Medium	Cell culture	>10^{10} PFU/ml, 1 ml	$599
	Large	Cell culture	>10^{11} PFU/ml, 1 ml	$999
	Ultra-purified	In vivo	>10^{12} VP/ml, 1 ml	$1499
AAV	Pilot	Cell culture	>10^{11} GC/ml, 250ul	$399
	Medium	Cell culture	>10^{11} GC/ml, 1 ml	$599
	Large	Cell culture	>10^{12} GC/ml, 1 ml	$999
	Ultra-purified pilot	In vivo	>10^{13} GC/ml, 100ul	$1099
	Ultra-purified medium	In vivo	>10^{13} GC/ml, 500ul	$1699
MMLV	Pilot	Cell culture	>10^7 TU/ml, 250ul	$399
	Medium	Cell culture	>10^7 TU/ml, 1 ml	$599
	Large	Cell culture	>10^8 TU/ml, 1 ml	$999
	Ultra-purified	In vivo	>10^8 TU/ml, 1 ml	$1499

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Shipment

Virus Packaging

QC

Shipment

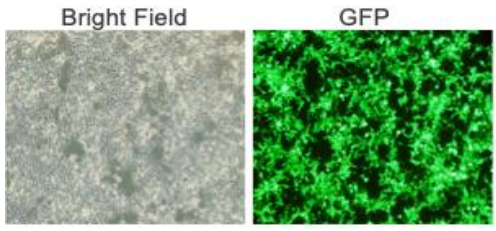
Lentiviral Packaging Pipeline

1.3G - Transfer plasmid carrying GOI co-transfected with our proprietary envelope plasmid encoding VSV-G and packaging plasmids encoding Gag/Pol and Rev - proprietary HEK293 packaging cell line

2. S/N collected and centrifuged to remove cell debris > filtered

3. Viral particles concentrated with PEG. For ultra-purification >further purified/concentrated by sucrose cushion centrifugation.

- Titer measured by p24 Elisa
- Bioburden tests for bacterial, fungal and mycoplasma contamination.
- in vitro functional tests - fluorescence or drug
- Endotoxin test by LAL.
- Can accommodate particle analysis CryoTEM



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Pipelines and technology

Shipment

↓

Virus Packaging

→

AAV

1. Triple transfection - proprietary HEK293 packaging cell line

↓

2. VP harvested/concentrated by PEG precipitation. Large Scale:
   S/N may also be collected and concentrated by TFF/PEG precipitation > yield

↓

3. Benzonase treatment to remove DNA/RNA contamination

↓

4. Ultra-purification is performed by CsCl centrifugation

↓

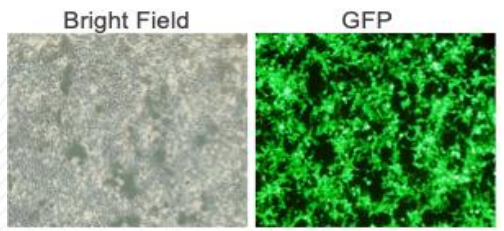
QC

→

- qPCR to measure vg number
- Bioburden tests for bacterial, fungal and mycoplasma contamination.
- SDS-PAGE validation of purity
- in vitro functional tests - fluorescence or drug
- Endotoxin test by LAL
- Can accommodate full capsid test by CryoTEM, total viral particle titration by ELISA, residual host cell DNA and plasmid DNA test by qPCR, residual chemical test by HPLC, residual protein test (e.g. benzonase) by ELISA, and viral particle aggregation test by Zetasizer particle analysis

↓

Shipment



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Streamlining research

CRISPR Genome Editing Solutions

01 Custom CRISPR Vectors

02 Pre-made CRISPR Vectors

03 CRISPR virus

04 RNA Preparation for Cas9 mRNA and gRNA

05 Donor DNA for Precise Genome Editing

06 Pooled CRISPR Libraries

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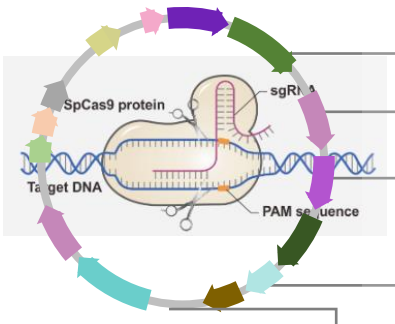
VectorBuilder's shRNA/CRISPR Libraries



Custom and premade shRNA libraries targeting human and mouse

- Whole Genome (~19,000 genes) and Elite Gene (~2,000 genes)
- Ready-to-use lentivirus with high functional titer (>10^8 TU/ml)
- Targeted by 5-6 different shRNAs, EGFP/Puro dual marker

CRISPR, CRISPRa or CRISPRi libraries – 1-vector or 2-vector systems

- Dual gRNA library: 5-6 gRNAs per gene
- ~20,000 human or mouse genes as ready-to-use high-titer lentivirus

Choose from various validated vector backbones

- lentivirus, adeno-associated virus (AAV), piggyBac and regular plasmid

Chip-based approach to synthesize high-quality shRNA oligo pools with a low error rate

Validated by NGS/NGS deconvolution of post-screening sample

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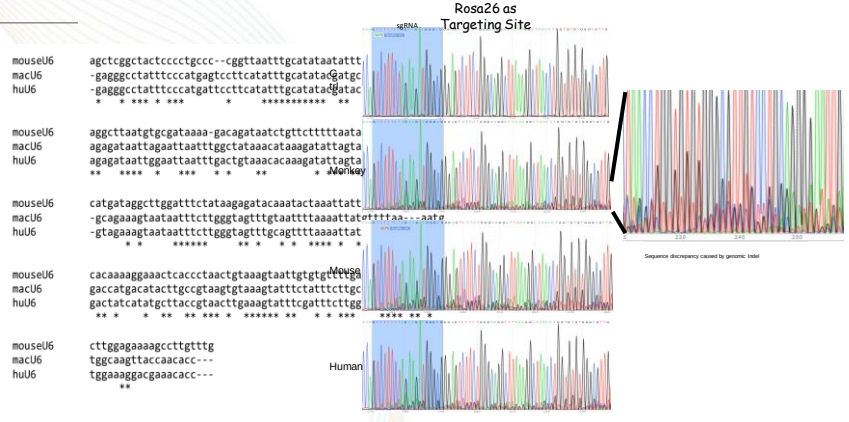
Pre-made dual gRNA Library

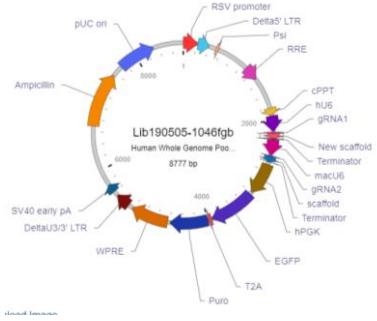
ONLY COMMERCIALLY AVAILABLE comprehensive dual gRNA library

Product name	No. of genes	No. of gRNA pairs	Scale*
Human Whole-Genome Dual-gRNA Lentivirus Library	20,048	91,926	Medium (>1.0x 10^8 TU/ml, 1 ml)
			Plus (>1.0x 10^8 TU/ml, 5 ml)
Mouse Whole-Genome Dual-gRNA Lentivirus Library	20,493	90,344	Medium (>1.0x 10^8 TU/ml, 1 ml)
			Plus (>1.0x 10^8 TU/ml, 5 ml)

Novel U6 Promoter Sequences



2 variant scaffolds



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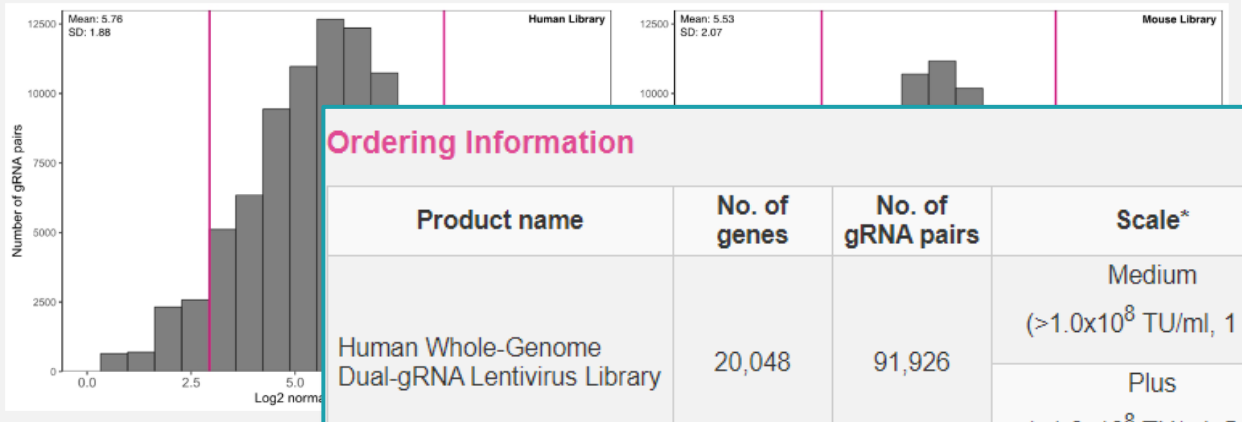
Excellent coverage with low error rate

High uniformity: The representation of gRNA pairs in both libraries are highly uniform (Figure 3).

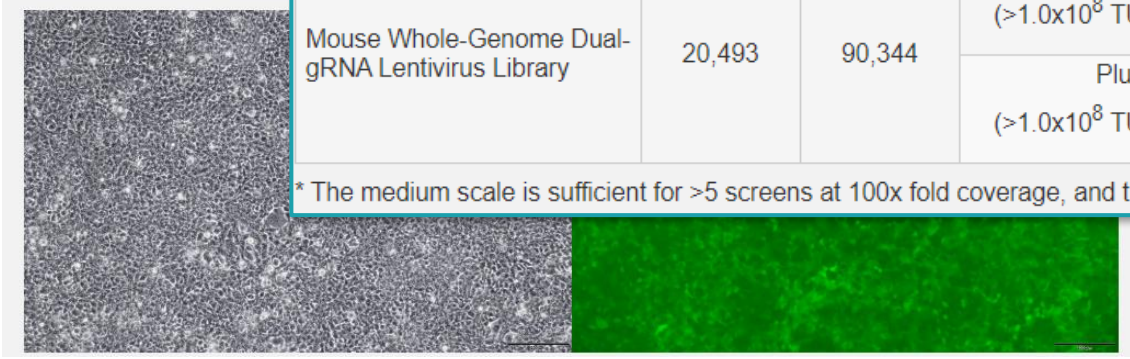


- **>85% and >75%** of total gRNA pairs (human and mouse, respectively) ID's by NGS
- ned gRNA pairs ely) detected by NGS
- **o** allows for efficient

Ordering Information

Product name	No. of genes	No. of gRNA pairs	Scale*	Catalog No.	Price (USD)
Human Whole-Genome Dual-gRNA Lentivirus Library	20,048	91,926	Medium (>1.0x10^8 TU/ml, 1 ml)	LVM(Lib190505-1046fgb)	$3,999 ~~$7,999~~
			Plus (>1.0x10^8 TU/ml, 5 ml)	LV5M(Lib190505-1046fgb)	$8,499 ~~$16,999~~
Mouse Whole-Genome Dual-gRNA Lentivirus Library	20,493	90,344	Medium (>1.0x10^8 TU/ml, 1 ml)	LVM(Lib190505-1050kpm)	$3,999 ~~$7,999~~
			Plus (>1.0x10^8 TU/ml, 5 ml)	LV5M(Lib190505-1050kpm)	$8,499 ~~$16,999~~

* The medium scale is sufficient for >5 screens at 100x fold coverage, and the plus scale is sufficient for >25 screens at 100x fold coverage.



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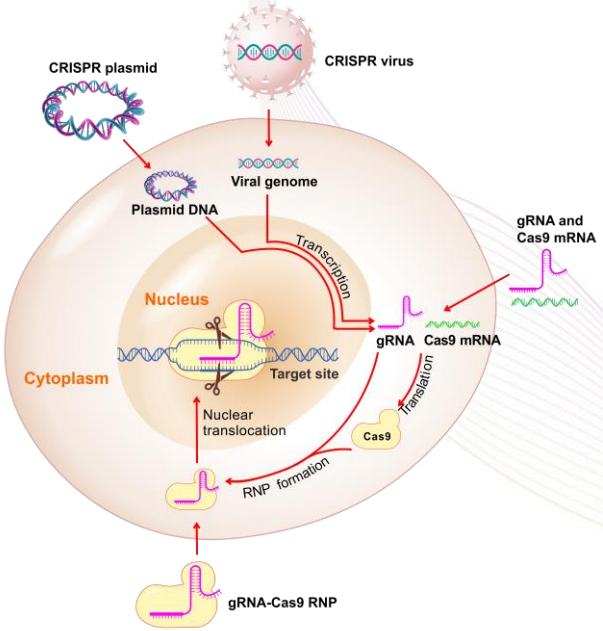
Streamlining research

Engineered cell lines



01 **Tailor-made vectors**

02 **Ectopic exp, KI/KO**

03 **Transfection, electroporation or viral transduction**

04 **Polyclonal or monoclonal cell populations**

05 **Guaranteed with desired genotypes, contaminant-free, ATCC standards**

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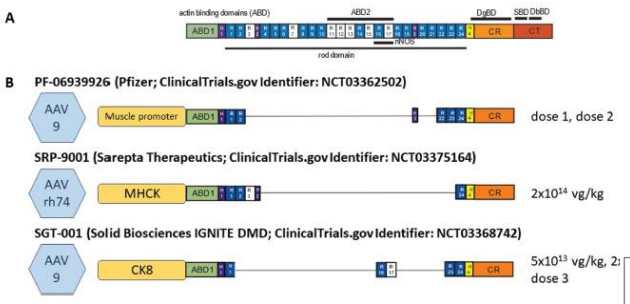
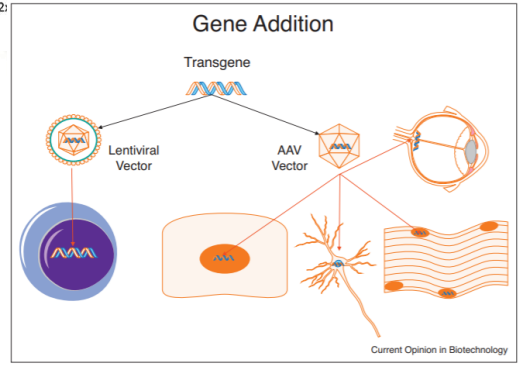
Looking to the future

Viral-mediated gene therapy



Crudele and Chamberlain, 2019



Kohn, 2019

01 **AAV-based approaches for blood & ocular disorders and for MD‘s**

02 **Lentiviral approaches for ex vivo permanent integration (T and lymphocytic disorders)**

03 **FDA approval of AAV9-based SMN1 gene replacement for SMD patients and**

04 **AAV-based gene replacement of RPE65-associated retinal dystrophies**

05 **Ongoing preclinical and clinical trials for gene replacement, modifier gene expression, gene KD, exon splicing/skipping**

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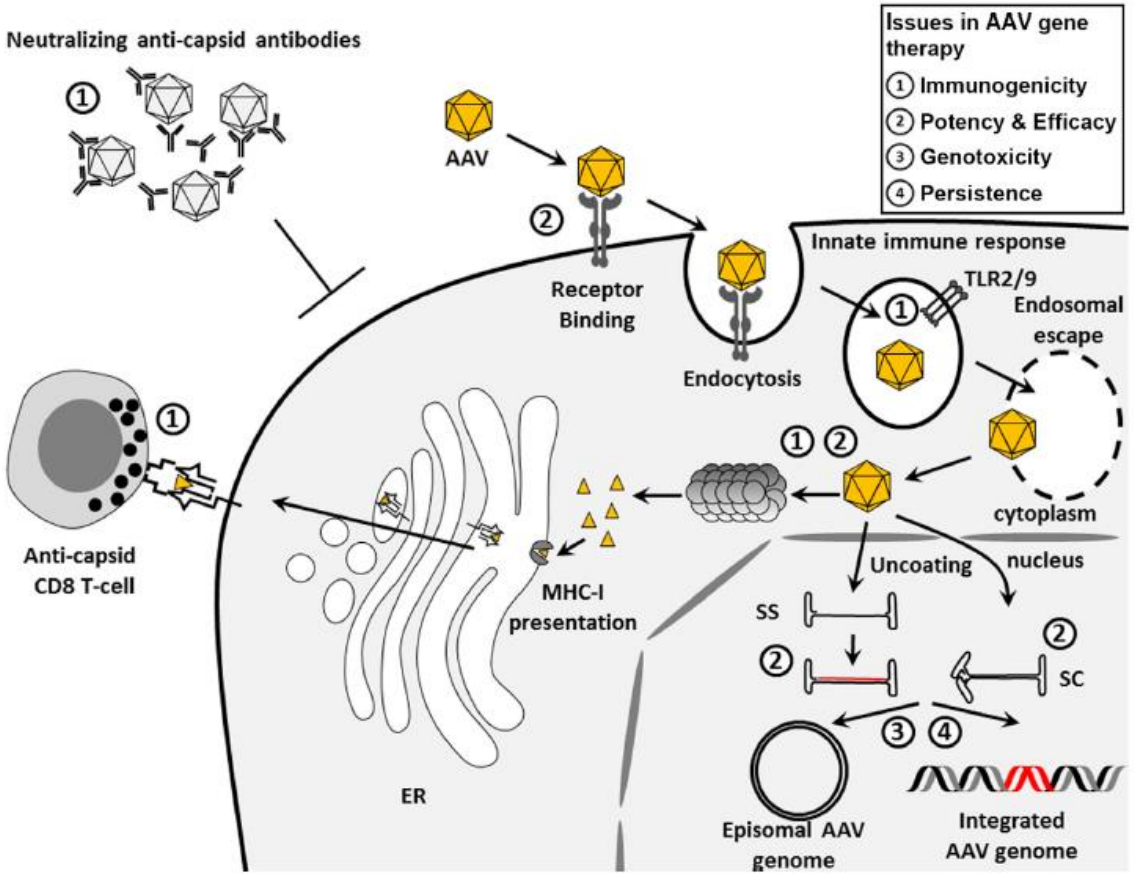
Hurdles to overcome for effective therapies

"The ideal CNS-directed gene therapy will utilize minimally invasive delivery while targeting the appropriate cell type(s) in target tissue(s) to achieve lifelong treatment following a single, low dose. " – Lykken et al., 2018

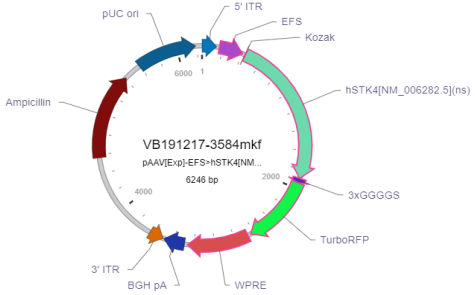


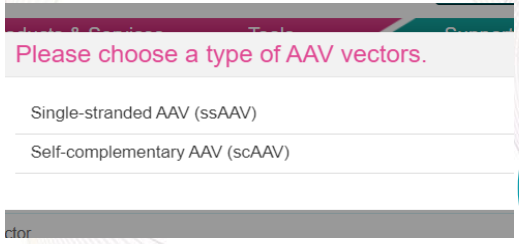
Scalability

Collela, Ronzitti & Mingozzi, 2017

How VB has positioned itself to take on gene therapy projects

- Plethora of AAV options
  - established pipeline for preclinical studies

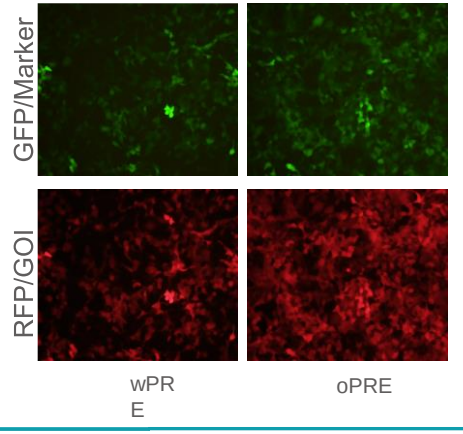




- Product development
  - AAV Panel

- Experience with multiple serotypes
  - 1, 2, 3, 4, 5, 6, 6.2, 7, 8, 9, rh10, DJ, DJ/8, PHP.eB, PHP.S, AAV2-retro and AAV2-QuadYF, AAV2.7m8
  - Multiple scales

- WPRE vs OPRE



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How VB has positioned itself to take on gene therapy projects

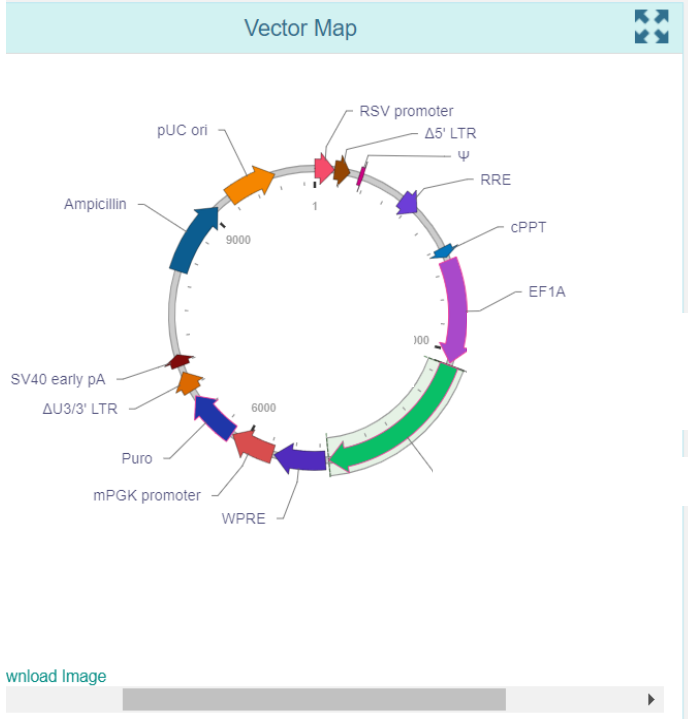
➢ Performance

Vectorbuilder offers a unique platform for easy design of customized DNA vectors and is one of our first-choice providers for molecular biology services. Consistently fast turnover times, high quality of services and excellent customer liaison are the decisive points for us to rely on Vectorbuilder's expertise. I can highly recommend Vectorbuilder as a reliable service provider and thank the whole team for the commitment to meeting our needs.

Eugen Werwein
Evotec

➢ CAR-T

- Extensive Lentivirus expertise
- Experience with custom CAR-T design



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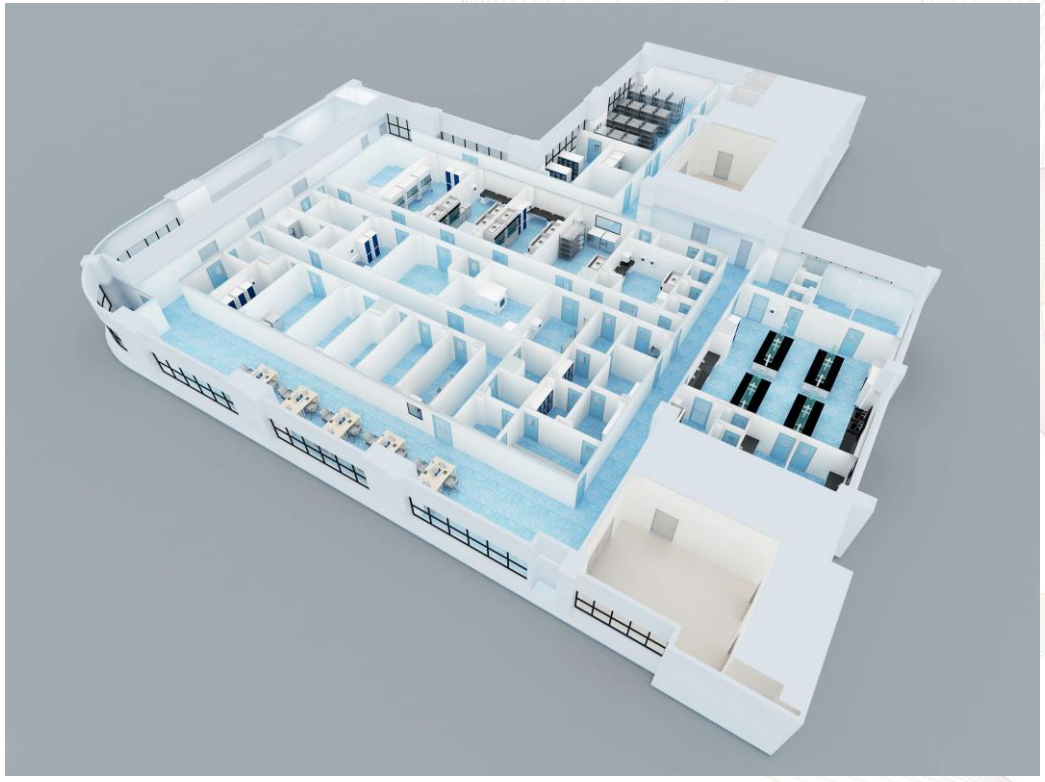
VB Future: CDMO & GMP



- GMP-compliant 52,000 square foot facility
- GMP-grade plasmids, AAVs, lentiviral vectors
- Projects in Sf9 & HEK cells, other common cell types possible
- Improved workflow for higher quality virus yields, lower immunogenicity; lower empty capsid in preparations

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science meets life
Thank you
CLONE less SAVE more
UiO : Life Science
University of Oslo
Weizmann Institute of Science
VE RI TAS
HARVARD
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University of Amsterdam
UCL
Galápagos
UNIVERSITY OF COPENHAGEN
medigene
developing innovative therapies
Yale University
Genentech
KU LEUVEN