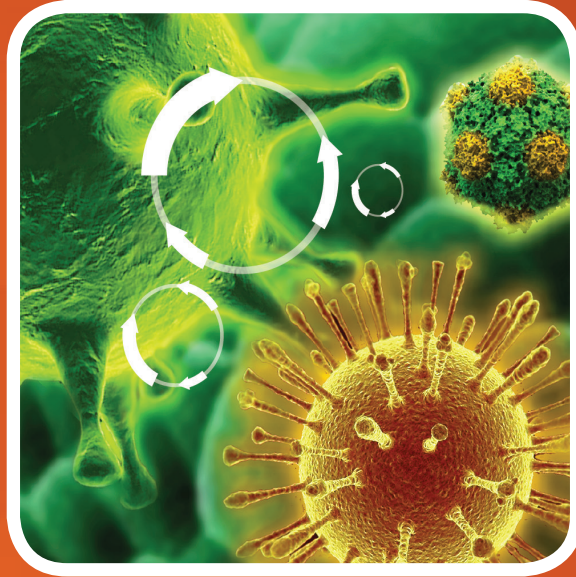


Deliver any gene into any cell type



Viral Expression Systems

Gene delivery is the introduction of genetic material into host cells in order to alter cell function, enabling the study of molecular mechanisms and regulations behind biological functions. Scientists have taken advantage of viruses' natural ability to transport DNA into the cell by engineering recombinant viral delivery systems. These systems can deliver genes safely and with high efficiency, opening a new era for *in vivo* and *in vitro* gene editing and gene therapy. Of the viral expression systems available, the most commonly used include Lenti-, Adeno-, AAV, Retro-, HSV, and Baculoviral systems, with each system offering its own advantages to meet specific project needs. Whether you would like to over-express or knockdown one gene or multiple genes, **abm's** world-leading suite of ready-to-use and custom recombinant viral technologies has something to offer for every project.

Since the 1980s when the concept of gene therapy was first explored, a great number of viral vectors and replication-deficient viruses have since been generated. The table below lists the most popular viral vectors/viruses currently in use - systems that are all available at **abm**. Each system offers unique characteristics:

Characteristic	Lentivirus	Adenovirus	Adeno-Associated virus (AAV)	Retrovirus	Herpes Simplex Virus (HSV)	Baculovirus
Packaging Capacity	8 kb	8 kb	4.7 kb	8 kb	Theoretically up to 150 kb	Theoretically > 100 kb
Transduction Efficiency	●●●	●●●●	●●●	●●●	●●●	●●●
Infection	Most Dividing/ Non-Dividing Cells	Most Dividing/ Non-dividing Cells (with high transduction rate towards Primary Cells)	All Cell Types (depending on Serotype)	Dividing Cells	Most Dividing/Non-Dividing Cells (ideal for Neuronal Cells)	Most Dividing/ Non-Dividing Cells (including Bacterial, Insect, Plant, and Mammalian Cells)
Expression	Stable	Transient	Transient or Stable	Stable	Transient	Transient or Stable
Integrating	Yes	No	Site-specific integration	Yes	No (but may replicate separately from the host)	No
Immune Response	●●●	●●●●●	●●	●●●●	●●●●●	●●●
Genetic Material	RNA	Double Stranded Linear DNA	Single Stranded Linear DNA	RNA	Double Stranded Linear DNA	Double Stranded Linear DNA

● Low ●●●●● High

Lentiviral Vectors and Viruses

The Lentivirus system offers:

- A wide variety of promoters including CMV, EF1α, UbC, and PGK

abm's ready-to-use lentiviral vectors and prepackaged lentiviruses are available for every human, mouse, or rat gene. Select promoters, tags, and reporters to match your project requirements. Our Custom Recombinant Lentivirus Service utilizes our proprietary pLenti-Combo 2nd and 3rd generation packaging mixes as well as streamlined protocols for rapid high titer production.

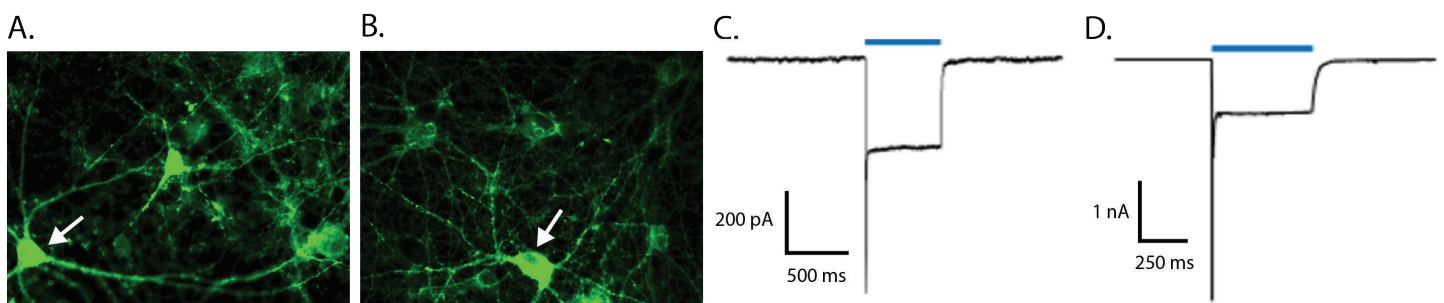


Figure 1: Functional expression of channelrhodopsin-2 Lentivirus *C.reinhardtii* (ABM, LVP010100)
The lentivirus was applied to dissociated embryonic rat neuronal cultures at 1 MOI. A, B, images showing EYFP expression with white arrows highlighting the neurons selected for electrophysiology. C, D, whole-cell patch-clamp recordings showing example photocurrents (from A, B) stimulated by 465nm light. (Data gathered by Dr. Emily Johnson, Andrew Trevelyan's lab, Newcastle University, UK. CANDO project (www.cando.ac.uk/) funded through the Wellcome Trust (102037) and EPSRC (NS/A000026/1)).

Retroviral Vectors and Viruses

The Retrovirus System offers:

- Integration of genetic material specifically into dividing cells
- Broad tropism for infection of a variety of mammalian cells
- Safe to use, the replication-incompetent retrovirus has minimal relation to the wild type Moloney Murine Leukemia Virus (MMLV)

abm's retroviral vectors and retroviruses are available for every human, mouse, and rat gene driven by the CMV promoter and available with either no Tag, HA tag or GFP reporter. Custom Retrovirus Services are also available.

Adenoviruses

abm's adenovirus system offers:

- High infection efficiency
- A broad host range for infection (dividing and non-dividing cells, stem cells, and primary cells)
- Non-integrating gene delivery (no risk of affecting host gene expression due to integration)
- Low immunogenicity (post-infection cell viability is very high)
- Applications in both *in vivo* and *in vitro* projects
- Biosafety due to the use of replication-incompetent (-E1/-E3) human adenovirus type 5 (Ad5)

abm offers a comprehensive library of ready-to-use adenoviruses for every human, mouse and rat gene or miRNA. Our adenoviral systems are driven by a strong CMV promoter, and are available with the choice of no tag, His tag, HA tag, or GFP reporter. For any other unique requirements, our Custom Recombinant Adenovirus Service can be used to produce packaged adenoviruses containing your transgene of interest or RNAi for gene silencing.

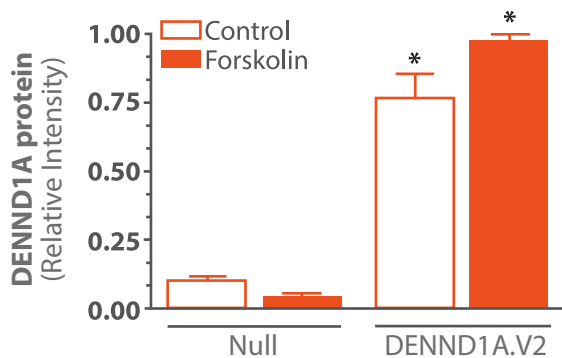


Figure 2: DENND1A.v2 expression in normal theca cells after infection with a cDNA adenovirus. Quantitative Western analysis following infection of normal theca cells with 3 pfu Null or DENND1A.V2 adenovirus to confirm DENND1A.V2 protein expression after adenoviral infection. Cells were treated in the absence or presence of 20 μ M forskolin for 72 h.

Data from Figure 5b of McAllister, J. *et al* (2014). Overexpression of a DENND1A isoform produces a polycystic ovary syndrome theca phenotype. *PNAS*, 111(15), pp.E1519-27.

Adeno-Associated Viral Vectors and Viruses (AAV)

Among all viral vectors, the recombinant Adeno-Associated Virus (AAV) is the most promising candidate for gene therapy and regenerative medicine due to its low pathogenicity and minimally integrating nature. **abm's** Adeno-Associated viral system offers:

- Robust infection of both dividing and non-dividing cells
- Low immunogenicity and pathogenicity in animal models
- Targeting of a broad range of specific tissue types (available serotype capsids include AAV1 to 11 for tissue-specific delivery to CNS/Retina, Heart, Lung, Liver, and Skeletal Muscle tissues). Serotypes DJ and DJ-8 are available as custom services
- Long-term expression in proliferating and non-dividing cells
- No integration into the host genome

abm's complete library of premade AAV vectors and prepackaged AAV particles for human, mouse, and rat genes are available with a wide selection of reporters (GFP and Luciferase) and promoters (CMV, EF1 α , PGK, CAGGS, and MSCV). We also offer a full library of AAV siRNA and miRNA vectors and viruses as well as Custom AAV Services.

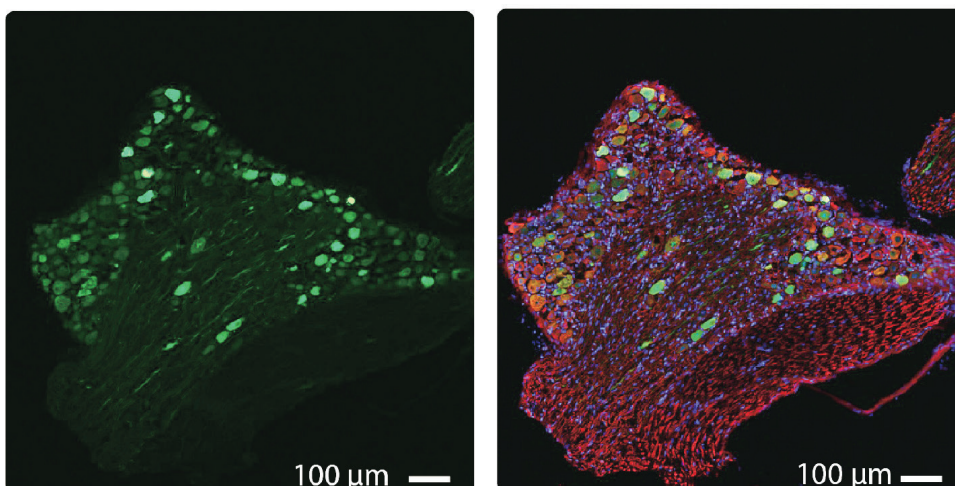


Figure 7: AAV infectivity data

Left: EGFP expression (Green) in lumbar neuronal cells 4 weeks after intrathecal injection of AAV-EGFP Serotype 9 (Cat. # iAAV01509) into mice.

Right: Overlay with β -tubulin (red) and DAPI (blue). Image courtesy of Dr. Douglas Lopes, King's College London

Herpes Simplex Viral Vectors and Viruses (HSV)

The Herpes Simplex Virus is most commonly used for lifelong preventative or therapeutic gene transfer of large transgenes into cells that have low transduction efficiency using other systems (e.g. neurons). The HSV system offers:

- Long-term non-toxic persistent infection of its host (particularly in neurons)
- Transient transgene expression
- Broad tropism for infection of a wide range of host cells
- Ability to deliver genes to post-mitotic cell types
- Delivery of large transgenes to the nucleus of mammalian cells
- Non-integrating gene delivery (but can replicate separately from host cell genome)

abm's Custom HSV Service is available with the CMV promoter (others are available upon request), with or without the addition of a GFP reporter, and includes custom gene synthesis/subcloning and viral production.

Baculoviral Vectors and Viruses

The Baculovirus System is commonly used to deliver genes into insect cells in order to achieve high yields of recombinant protein. This helper-independent system can be used for long-term expression of heterologous genes and offers:

- High yields of recombinant protein compared to bacterial expression systems
- An alternative expression system when bacterial expression is not feasible
- Expression of genes from bacteria, viruses, plants and mammals at levels from 1-500 mg/l
- Post-translational modifications such as phosphorylation, glycosylation and acylation (similar to mammalian processes) suitable for functional/structural studies
- Biosafety Level 1, as the baculovirus does not infect human cells

abm's Custom Baculovirus System includes both gene synthesis/subcloning and viral production. In addition, large-scale protein production service (involving culturing and infection of insect cells) is also available.

Summary of **abm's** Viral Vectors and Viruses

	Lentivirus	Adenovirus	Adeno-associated virus (AAV)	Retrovirus	Herpes Simplex Virus (HSV)	Baculovirus
Genes available	Ready-to-use vectors and prepackaged viruses containing any human, mouse, or rat gene. Custom Services also available			Custom Service	Custom Service	Custom Service
Gene Over-expression	ORF, miRNA inhibitors	ORF, miRNA inhibitors	ORF, miRNA inhibitors	ORF	ORF	ORF
Gene Silencing	siRNA, miRNA, shRNA	siRNA, miRNA	siRNA, miRNA	-	-	-
Promoters available	CMV, EF1 α , UbC, PGK, H1 (for RNA; miRNA)	CMV	CMV, EF1 α , PGK, CAGGS, MSCV	CMV	CMV	polh and p10
Other types of expression	CRISPR/Cas9, lncRNA, antisense RNA	CRISPR/Cas9, lncRNA, antisense RNA	CRISPR/Cas9	-	-	-
Tags/Reporters available	No tag or with HA tag, GFP and RFP available	No tag, His tag, HA tag, GFP Reporter	No tag, GFP, Luciferase	No tag, HA tag, GFP Reporter	GFP, no RFP	No reporter, GFP, RFP
Titers available	10 ⁷ , 10 ⁸ , 10 ⁹ , 10 ¹⁰ IU/ml	10 ⁶ , 10 ¹⁰ , 10 ¹² pfu/ml	10 ¹¹ , 10 ¹² , 10 ¹³ GC/ml	10 ⁶ , 10 ⁷ , 10 ⁸ IU/ml	10 ⁶ , 10 ⁷ IU/ml	10 ⁶ , 10 ⁷ IU/ml

Gene Regulation (siRNA, miRNA, lncRNA) Vectors and Viruses

For gene regulation and functional studies, **abm** offers a variety of expression systems for:

- **siRNA:** efficiently express any target siRNA to knockdown any gene (lentiviral vector comes with either GFP or no reporter) without the need to design hair-pin loop structures using our unique iLenti™ RNAi Expression System
- **miRNA:** inhibit or over-express any miRNA for studies of post-translational gene regulation in mammalian systems using our ready-to-use viral vectors and packaged viruses
- **lncRNA/antisense RNA:** select from premade vectors and viruses for overexpression or knockdown of lncRNAs or antisense RNAs for studies of embryonic regulation, histone modification, post-transcriptional gene regulation, mRNA processing, and more
- **UTR and Promoter Reporter platform:** quantitatively study a specific miRNA's regulation of its target gene using our 3'UTR platform or study promoter and 5'UTR elements using our custom reporters, all available as a library of premade lentiviral vectors and lentiviruses for any human or mouse gene

	Lenti-		AAV-		Adenovirus
	Vector	Virus	Vector	Virus	
siRNA/shRNA	✓	✓	✓	✓	✓
miRNA overexpression/inhibition	✓	✓	✓	✓	✓
lncRNA/antisense RNA	✓	✓	✓	✓	✓
5' UTR, 3'UTR or Promoter Reporter Platform	✓	✓			

CRISPR Gene Editing Vectors and Viruses

The CRISPR gene editing system is the biotech breakthrough of the century, enabling more cost-effective, rapid, and specific gene editing of almost any region of the genome. **abm** offers one of the most comprehensive selection of gene editing tools, available in lenti & AAV vector and virus, as well as adenovirus formats

	Lenti-		AAV-		Adenovirus	Non-Viral Vector
	Vector	Virus	Vector	Virus		
sgRNA for spCas9	✓	✓	✓	✓	✓	✓
sgRNA for saCas9			✓	✓		
All-in-One (sgRNA & spCas9 Nuclease)	✓	✓				✓
All-in-One (sgRNA & saCas9 Nuclease)			✓	✓		
Multiplex sgRNA with spCas9	✓	✓				

Non-viral ORFs and Protein Vectors

In addition to our extensive viral vector library, **abm** also offers a complete library of sequence verified human, mouse, and rat ORFs. These ORFs are ready for insertion into a protein expression system from our non-viral protein vector library, which offers:

- Option of expression in either a prokaryotic (*E. coli*, T7 promoter-based) or eukaryotic (mammalian cell, CMV promoter-based) system
- A choice of 5 tags (His, HA, MBP, GST, and D-tag)
- A complete list of human, mouse, or rat genes

Can't find what you need? Take advantage of our complete **custom services menu!**

At **abm**, we pride ourselves in our flexibility and expertise in generating whatever gene expression system you need! If you can't find a suitable promoter, reporter, or any other specification in our catalogue, please inquire with us as we also offer custom gene synthesis, cloning, and viral packaging.

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