abm
PRODUCT
OVERVIEW

Alfamed

Agenda

01 Company introduction

02 Molecular biology

03 Vector & Virus- CRISPR

04 Cell biology

05 Q&A

About us

Let discover more

FROM THEN TO NOW

About us

Company started
2005

HQ in Vancouver, Canada

US operations in Bellingham (2018)

France warehouse in Toulouse (2023)

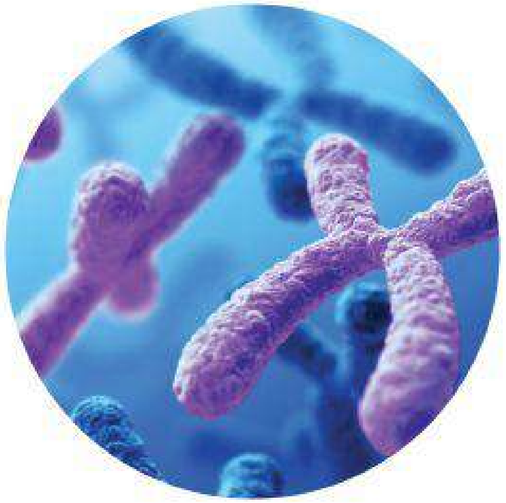
Chinese branch in Zhenjiang, China

Today

About us

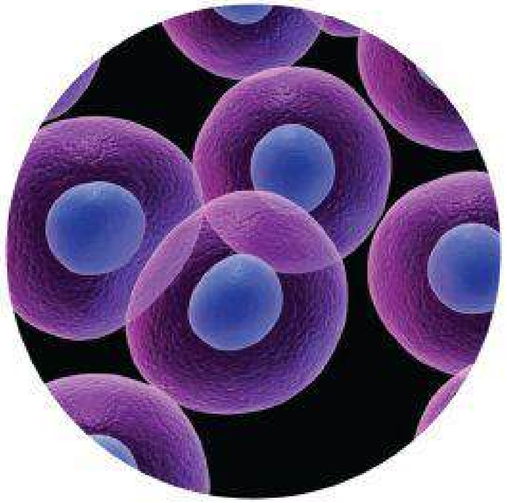
General biological materials

+10K Products



Genetic materials

+700K Products



Cell lines

+300K Products

1 Million products and counting...

PCR & Enzymes

Molecular biology products

PCR & Enzymes

As an industry-leading and global biotech company, abm offers a wide range of PCR, RT-PCR and qPCR enzyme products:

- DNA & RNA Purification Kits
- Reverse Transcriptase
- Taq Polymerase
- PCR & qPCR MasterMixes
- Safe DNA Stains
- DNA Markers
- Cloning & Special Enzymes

ThermoFisher SCIENTIFIC

"Supermarket" for life science

QIAGEN

Sample prep kits

BIO-RAD

Instrument focus with bundled reagents

Featured product

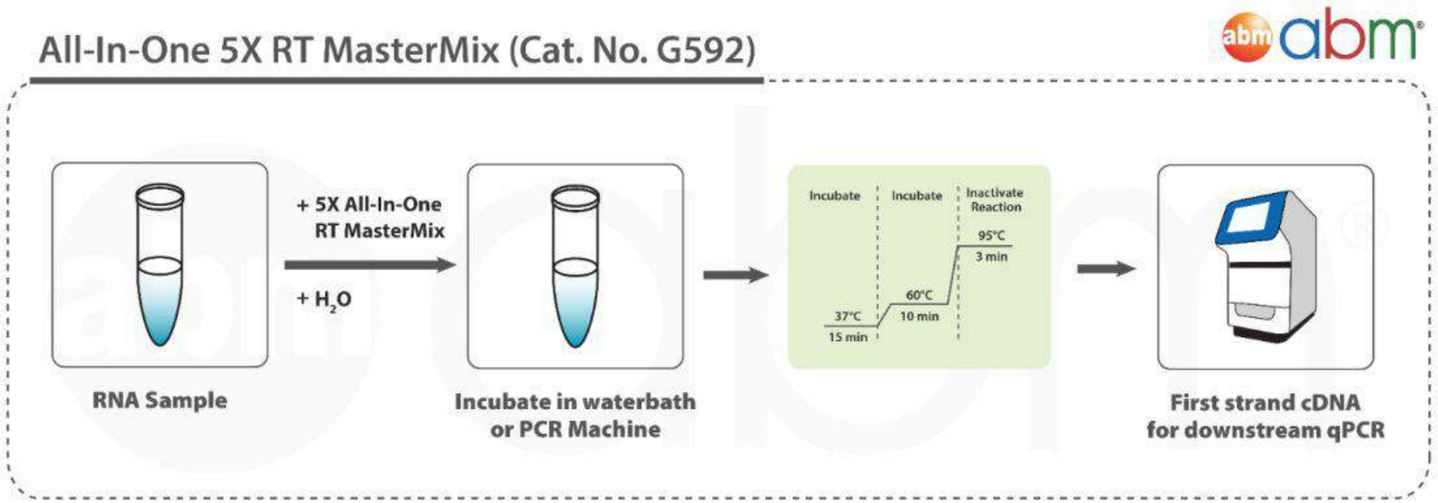
Unique in the growing market

All-In-One 5X RT MasterMix

(CAT. NO. G592)

All-In-One 5X RT MasterMix is a convenient and ready-to-use formulation for first-strand cDNA synthesis, including genomic DNA (gDNA) removal.

abm´s kit



Traditional cDNA Synthesis

01 DNase and Stop Solution

02 Reverse transcriptase

03 RNase inhibitor

04 Reaction buffer

05 dNTPs

06 Oligo dT

07 Random primers

Combines genomic DNA removal with efficient and sensitive reverse transcription

RNase R

(CAT. NO. E049)

RNase R is a potent 3’-5’ exoribonuclease that degrades linear RNA (mRNA and tRNA).

It is required in almost **all** circRNA isolation / detection protocol.

Species	Percentage of total RNA
rRNA	~>80%
tRNA	~15%
mRNA	~1-5%
circRNA	**~<1%**
snRNA	~<1%
lncRNA	~<1%
miRNA	~0.01%

What is circular RNA?

- New family member of RNA gaining recognition in recent years due to rapid progress in high-throughput RNA-Seq
- Functional roles in cellular physiology and disease
- Previously thought to be a by -product of unusual splicing with no function

What is RNase R?

- Rnase R is a potent 3’-5’ exoribonuclease
- Degrades linear RNA (mRNA and tRNA)
- Does NOT digest circular or lariat or dsRNA
- Enriches circRNA (0.1 – 0.01% of total RNA)

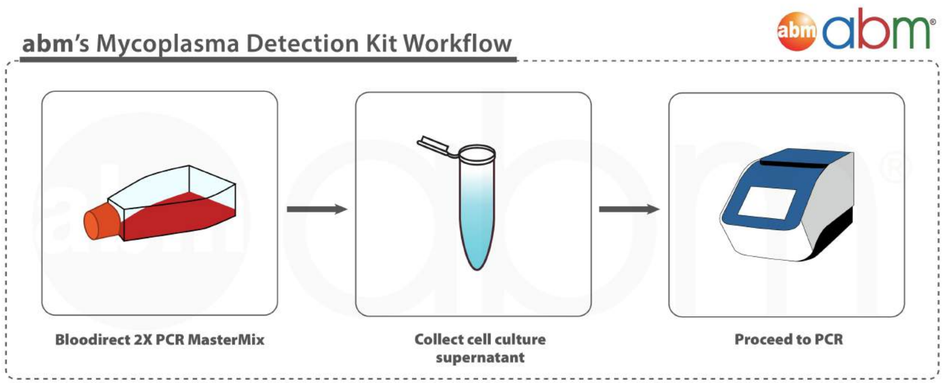
Mycoplasma Pro PCR Detection Kit

(CAT. NO. G239)

abm's Mycoplasma PCR Detection Kit offers **highly specific and sensitive detection of** 300+ strains of Mycoplasmas and Acholeplasma in less than 2 hours.

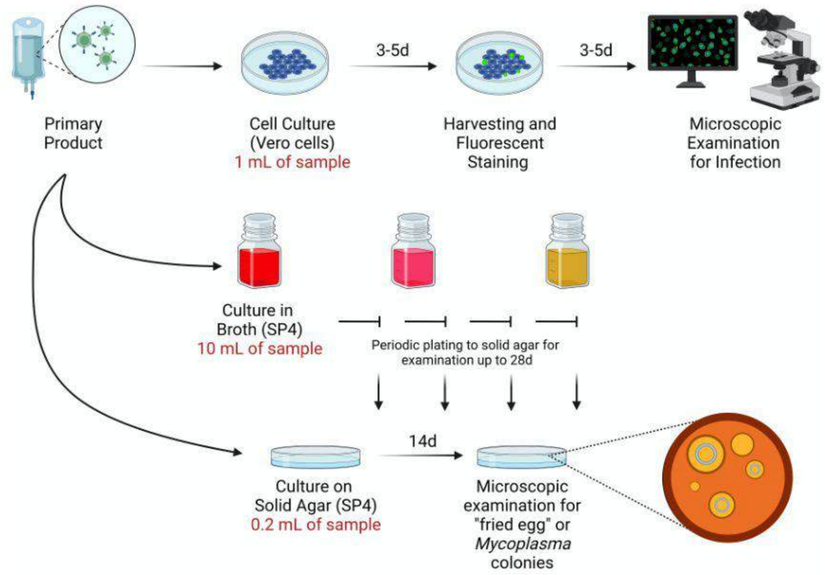
PCR Detection Kit

Result in < 2 hours



Traditional methods

Result in 3d, 14d, 28d



Example of

PRODUCT DIFFERENTIATION

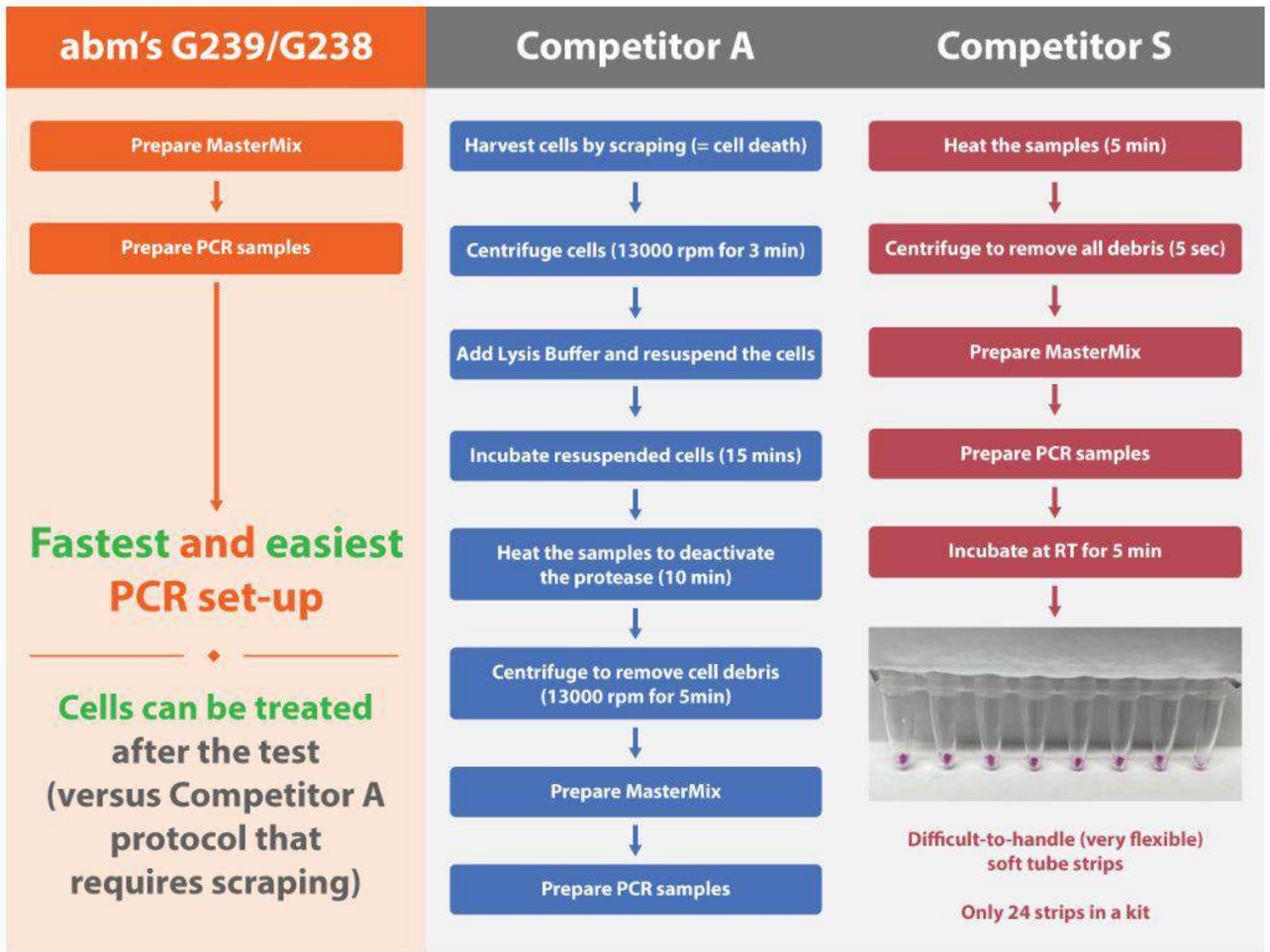
Buzz word: Cell Biology
Cross Selling: Mycoplasma Elimination Kit

Research (+Industrial R&D)

01 **Manuscript & "Entry" Must -**
Many journals require cell lines to be tested for mycoplasma (For industrial R&D, only mycoplasma negative cells can enter the facility)

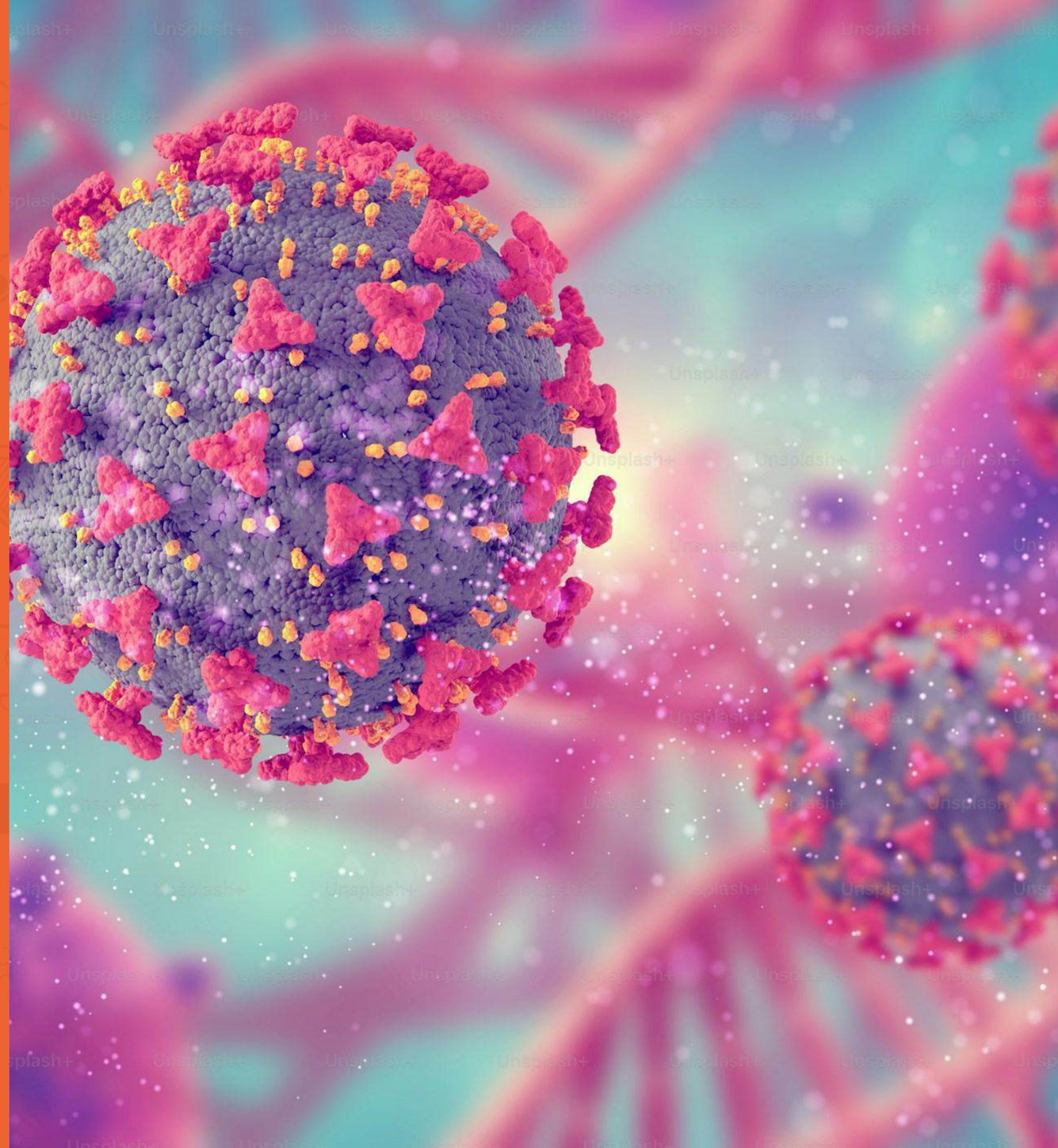
02 **Market Leading Breadth of Species -**
Including 300+ mycoplasma and acholeplasma

03 **Rapid and Simple Workflow -**
In both detection workflow & cell culture workflow



Vector & Virus

CRISPR: The future of Biotech

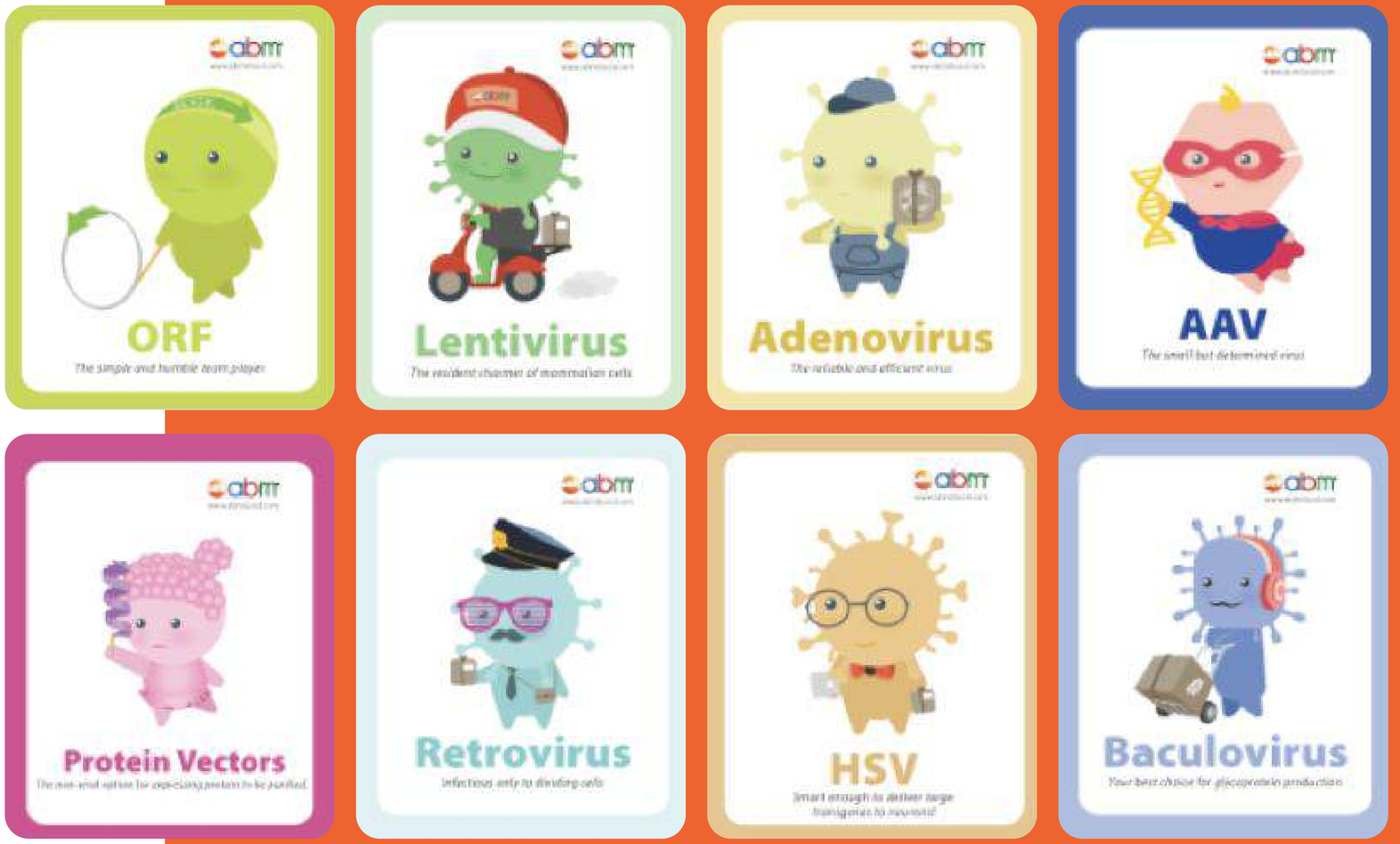


Vector & Virus

We provide the most comprehensive collection of Lentiviral, AAV, Adenoviral vectors & viruses for various applications, including:

- Gene Editing : CRISPR
- Gene Expression: ORF cDNA
- Gene Regulation: miRNA, siRNA

Custom gene synthesis, Cloning and Viral packaging available



TWIST BIOSCIENCE
Oligo Synthesis
Into std vector

IDT INTEGRATED DNA TECHNOLOGIES
Oligo Synthesis
Into std vector

GenScript Make Research Easy
Oligo Synthesis, Cloning,
Packaging of Lenti & AAV

Flexible & Customizable

OPTIONS

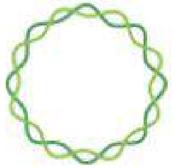
Service differentiation

01 Personalized solution for your unique needs

02 Expert guidance every step of the way

03 Comprehensive one-stop solution provider

Vector transfection



- All-in-One
- Cas9 Only
- dCas9 Modifications
- sgRNA Only

Viral infection

Lentivirus

- Integrates into genome: long term expression
- Commonly used

Adenovirus

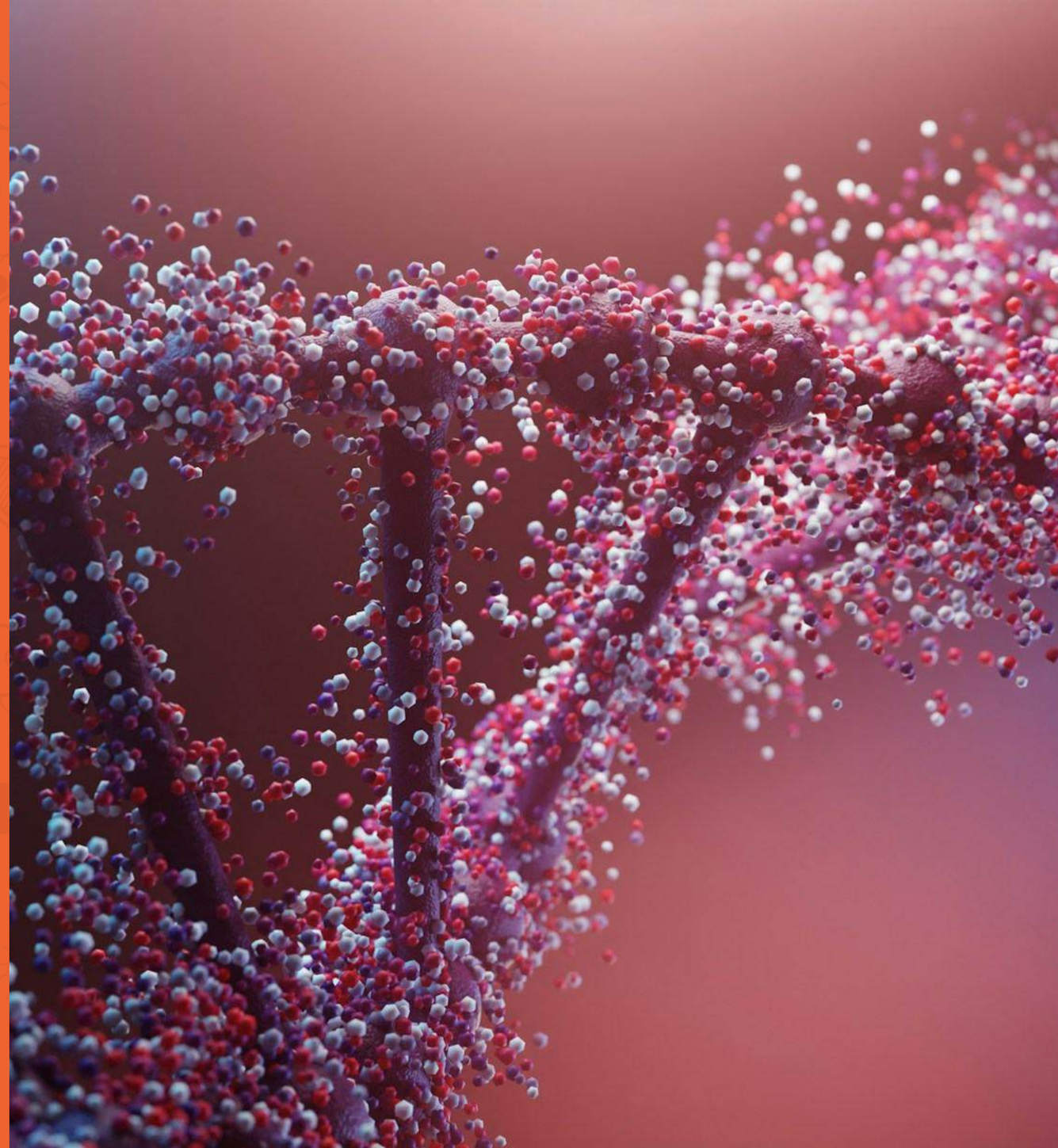
- Short term expression
- High infection efficiency
- Immune response *in vivo*

AAV

- Short term expression
- Small virus – fits saCas9 (not spCas9)
- Low immune response

Featured product

All in one CRISPR Lentivector

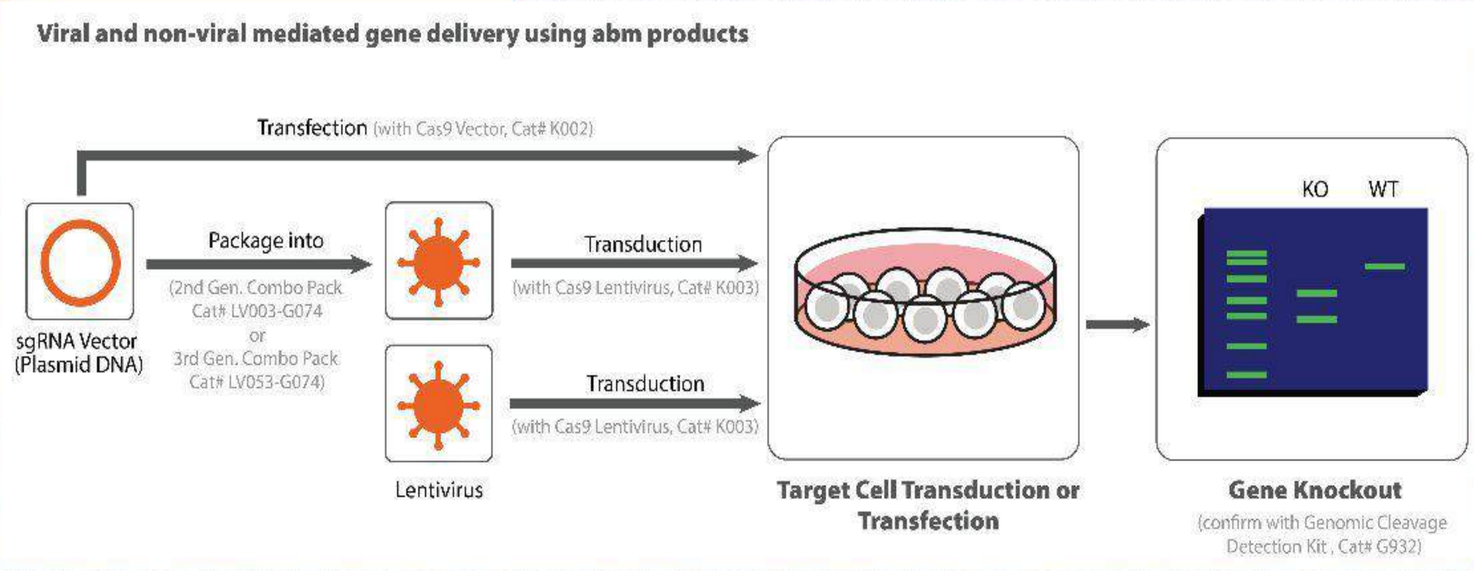


CRISPR

ALL-IN-ONE LENTIVIRAL VECTOR AND VIRUS

All-in-One combines spCas9 Nuclease and sgRNA expression into one single vector for simple gene knockout workflow.

sgRNA Vectors workflow



Advantages

01 Targets wider range of cell types

02 Single reagent – easy to operate

03 Versatile (Promoter, selection options)

Cell biology products

The largest collection on the world

abm Cell biology and cell engineering

COMPREHENSIVE SOLUTIONS

Cell and Cell lines

01 Primary Cells

02 **Immortalized cell lines**
Cell immortalization reagents

03 Tumor cell lines

04 Stable cell lines

05 **CRISPR cell lines**
Cas9 Expressing Cell Lines
Knockout (KO) Cell Lines

Reagents

01 Mediums and coating reagents

02 Transfection reagents

03 Growth factors

04 **Contamination solutions**
Mycoplasma Pro Detection Kit (G239)
Mycyplasma Elimination Cocktail (G398)

Our position

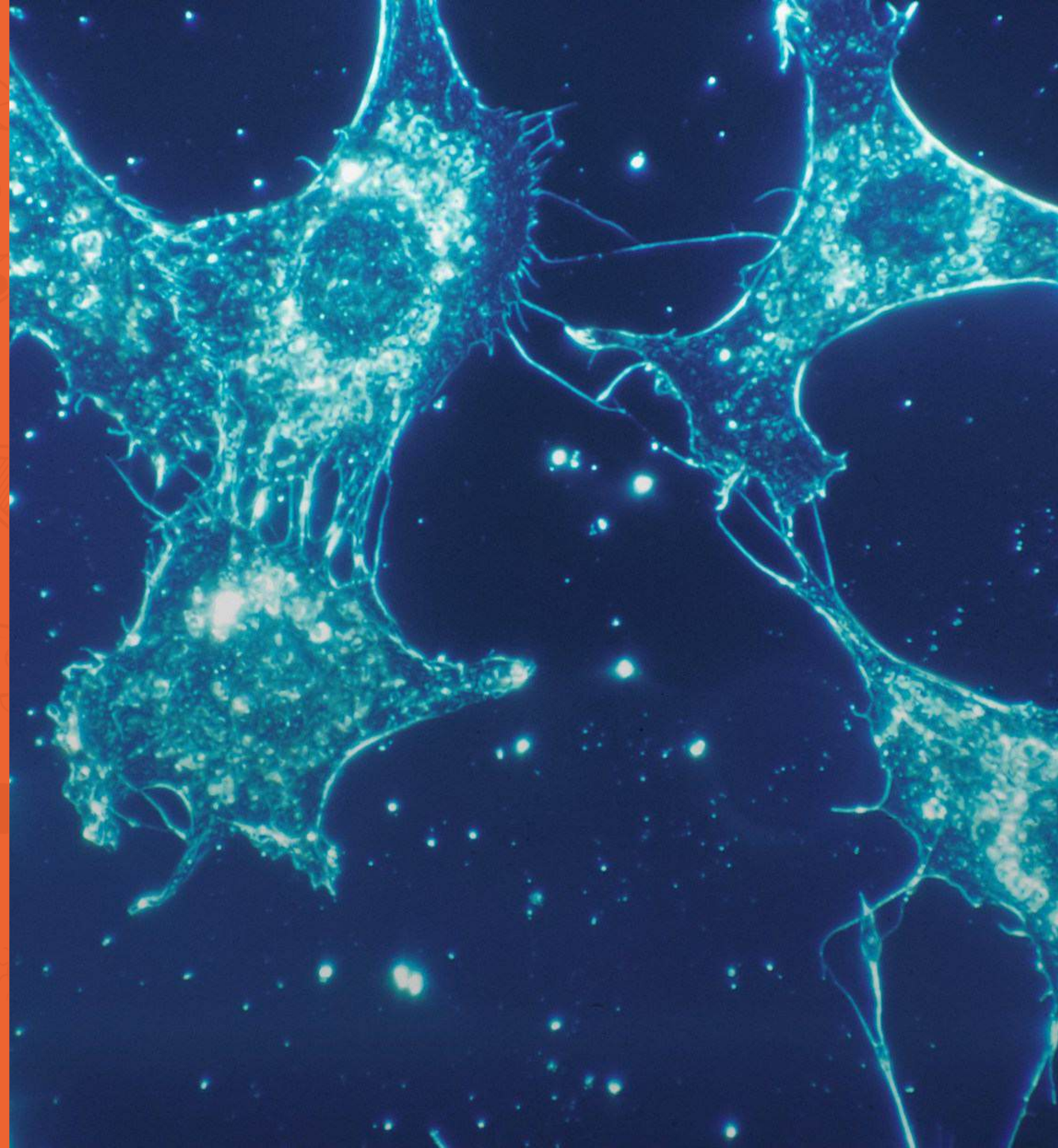
IN THE CELL MARKET

Companies	Primary	Immortalized	Stable cell lines	Tumor
Applied Biological Materials	**>322**	**>586**	**>979**	**>346**
ATTC	~57	~64	~146	~636
Merck Sigma	~283*	N/A	N/A	~2606**
AddexBio	~31	~27	~6	~182
Cell Applications	<75	N/A	N/A	N/A
CLS (Cytion)	N/A	N/A	~43	~790
CellnTec	5	3	N/A	N/A

*Merck offers primary cells from Cell Applications and PromoCell (Selected Geographics). Overlap exists between suppliers and deliverable format. e.g. types offered is less than 283
**Partner with ECACC

Engineered cell lines

Feature: Immortalized & CRISPR knockout solutions



We are the pioneers for Cell Immortalization

Produced by the Science/AAAS Custom Publishing Office

LIFE SCIENCE TECHNOLOGIES

CELL CULTURE TECHNOLOGIES

The Art of Culture: Developing Cell Lines

Immortalized cell lines are critical for biomedical research, but establishing new lines can be tricky and frustrating. Researchers who've succeeded at it recommend a combination of old and new tools and techniques. **By Alan Dove**

On February 8, 1951, George Gey of Johns Hopkins University isolated some cells from a cervical cancer biopsy and placed them into a petri dish with some medium. Unlike all of the other cells Gey and his colleagues had tested, these—from a patient named Henrietta Lacks—adapted to their new environment beautifully. Lacks died of her cancer eight months later, but her cells, dubbed HeLa, became the first immortalized cell line, capable of renewing itself in artificial culture indefinitely. In the decades since their isolation, scientists have grown an estimated twenty tons of them.

Meanwhile, researchers have identified numerous ways to transform primary tissues from humans and animals into immortalized cell lines, and now laboratory supply vendors and nonprofit repositories carry hundreds of lines specifically adapted for everything from protein production to virus propagation. Embryonic and induced pluripotent stem cells may get more media attention, but ordinary somatic cell lines still form the backbone of biomedical research.

The selection extends across a zoo of species. "Within [our] general collection, we actually have more than sixty different species, and some are exotic," says Fang Tian, lead scientist and head of the cell biology group at the **American Type Culture Collection** (ATCC) in Manassas, Virginia. ATCC currently holds more than three thousand lines. The **Coriell Institute for Medical Research** in Camden, New Jersey maintains several thousand more, with an emphasis on human lines representing specific diseases.

A problem of scales

Even with thousands of cell lines just a click or phone call away, though, scientists may still find the selection inadequate. That's what happened to Mark Stenglein, a postdoctoral scholar at the **University of California, San Francisco School of Medicine** (UCSF), when he tried to study inclusion body disease (IBD) in snakes.

The project began when a snake enthusiast contacted Joseph DeRisi, Howard Hughes Medical Institute (HHMI) investigator and professor of biochemistry and biophysics at UCSF and Stenglein's boss. The serpent fan explained that IBD triggers behavioral changes, followed by wasting, secondary infections, and death, and is a major problem in the pet snake trade. Veterinarians had no idea what caused it. Intrigued, DeRisi and Stenglein decided to see if a virus might be responsible.

Working with snake owners and veterinarians, the team performed metagenomic sequencing and uncovered evidence of arenavirus infections in snakes with IBD. The trouble started when Stenglein tried to grow the new viruses. Common arenavirus-friendly mammalian cells didn't work. "There were four reptile cell lines total available at the ATCC, so we ordered all of them," says Stenglein, but the viruses didn't replicate in any of those lines, either.

Through their new veterinary contacts, Stenglein and DeRisi collected tissues from a boa constrictor named Juliet, which had died of lymphoma. Stenglein then tried numerous isolation techniques to immortalize Juliet's cells. "It's sort of one of those multiplication problems, you can start changing conditions and it can get out of control fast," says Stenglein, adding that he had between fifty and a hundred different plates of cells in the incubator at one time. To keep the problem manageable, he minimized as many variables as he could.

For example, he used only one recipe for the cell culture media: minimal essential medium (MEM)—a liquid solution, originally invented by Harry Eagle, which meets the basic requirements for many cells. These days a wide array of other media have been developed to help cater to specific needs, including sensitive and difficult-to-culture cells and lines from different species.

Stenglein also incubated all of the plates at the same temperature, varying only the cell isolation method. The protocol that finally worked involved simply slicing tissues into pieces with scalpels, then immersing them in trypsin overnight. That eventually yielded two new cell lines, one from Juliet's kidneys and one from her spleen.

The new lines have now propagated through continued>

Upcoming Features

Lipidomics—February 13, 2015 ■ *Cell Culture: Stem Cells—March 13, 2015* ■ *Proteomics—April 17, 2015*

multiple passages. Using these, the researchers developed a test for the new arenaviruses and used it on snakes with and without IBD. The work showed a strong correlation between arenavirus infection and IBD, suggesting that the viruses may cause the disease.

Stenglein now sees developing new cell lines as simply another laboratory technique that he could use in the future—though he has no immediate plans to do so. "I would only do it if ... I needed it for a project," he says. However, he urges others to consider creating new lines, especially if they work on a species that isn't well represented in the big repositories. "It's not as hard as you might think, it's worth a try if you are in a situation where your research question would benefit from having a [new] cell line."

For those studying reptiles, DeRisi's lab now distributes Juliet's cells to anyone interested in studying or using them. They did offer them to a repository, but were declined.

Repositories of culture

Indeed, researchers who create other lines from "exotic" species may receive similar responses from repositories, chiefly because of funding. "Previously ATCC did have government funding support, so we took whatever researchers requested ... did cell banking, did cell authentication to make sure the line is what it's supposed to be, and expanded them and distributed them worldwide," says Tian. Government funding for the repository ended 20 years ago, though, forcing ATCC to become more selective. Now, the nonprofit organization only adds new lines for which they anticipate high demand and widespread scientific interest.

The Coriell Institute still receives substantial federal funding, but focuses on human and nonhuman primate cells. The institute maintains a trove of several thousand samples that range from umbilical cord blood to clinical isolates from patients with rare genetic diseases.

Besides banking established cell lines, repositories are also at the forefront of creating new ones.

The simplest way to create a new cell line is to modify an existing one, a common strategy when an established line already comes close to meeting the requirements. Cells optimized to grow particular viruses or maximize recombinant protein production often come from such modifications. Establishing an entirely novel cell line can require more exotic techniques. While traditional cut-and-try methods such as those used by Stenglein may work, professional cell line developers constantly look for ways to accelerate the process. Partly as a result of studying researchers now know that one of the quickest routes to immortalization is through viral infection. For example, the T antigen from SV40 virus, the E6 and E7 oncogenes from human papilloma virus, quickly turn a primary cell into an immortalized tumor, they tend to change the cells' characteristics. Because viral oncogenes essentially turn cells into available oncogenes also have somewhat restricted ranges, and don't work on all cell types. That's why many cell culture experts are now using a gene called human telomerase reverse instead of or in addition to viral oncogenes. Developed in 1999, the hTERT technique like primary cultures but propagate indefinitely. For lymphoblasts, the Epstein-Barr virus the cells but allows them to maintain physiology.

Tian advises researchers who line to start by reviewing the literature bases. A similar line can often be scientist's needs, which is faster and line from scratch. If no existing line next step is to decide on a genera primary cells. "If you want to establish spontaneously established cell lines if you want to get a non-cancer line lished, then hTERT ... and viral in use," says Tian.

Companies such as **Applied Biological Materials** in Richmond, British Columbia offer cells with hTERT, with or without Cat GmbH in Heidelberg, Germany lines and reagents for cell immortalization. researchers can send their primary let the company develop the cell line.

Regardless of the specific technique new line, the usual problems of arise, such as microbial contamination particularly hard to handle, as they culture and quietly ruin experiments.

Featured Participants

- American Type Culture Collection — www.atcc.org
- Applied Biological Materials — www.abmgood.com
- Coriell Institute for Medical Research — www.coriell.org
- Food and Drug Administration — www.fda.gov
- Institute of Virology, University of Bonn Medical Center — www.virology-bonn.de
- InvivoGen — www.invivogen.com
- Lonza — www.lonza.com
- Save Lucy Campaign — savelucythebat.org
- University of Maryland School of Medicine — medschool.umaryland.edu
- University of California, San Francisco — www.ucsf.edu

We are the experts in cell immortalization since 2015

American Type Culture Collection
www.atcc.org

Applied Biological Materials
www.abmgood.com

Coriell Institute for Medical Research
www.coriell.org

Food and Drug Administration
www.fda.gov

Institute of Virology, University of Bonn Medical Center
www.virology-bonn.de

InvivoGen
www.invivogen.com

Lonza
www.lonza.com

Save Lucy Campaign
savelucythebat.org

University of Maryland School of Medicine
medschool.umaryland.edu

University of California, San Francisco
www.ucsf.edu

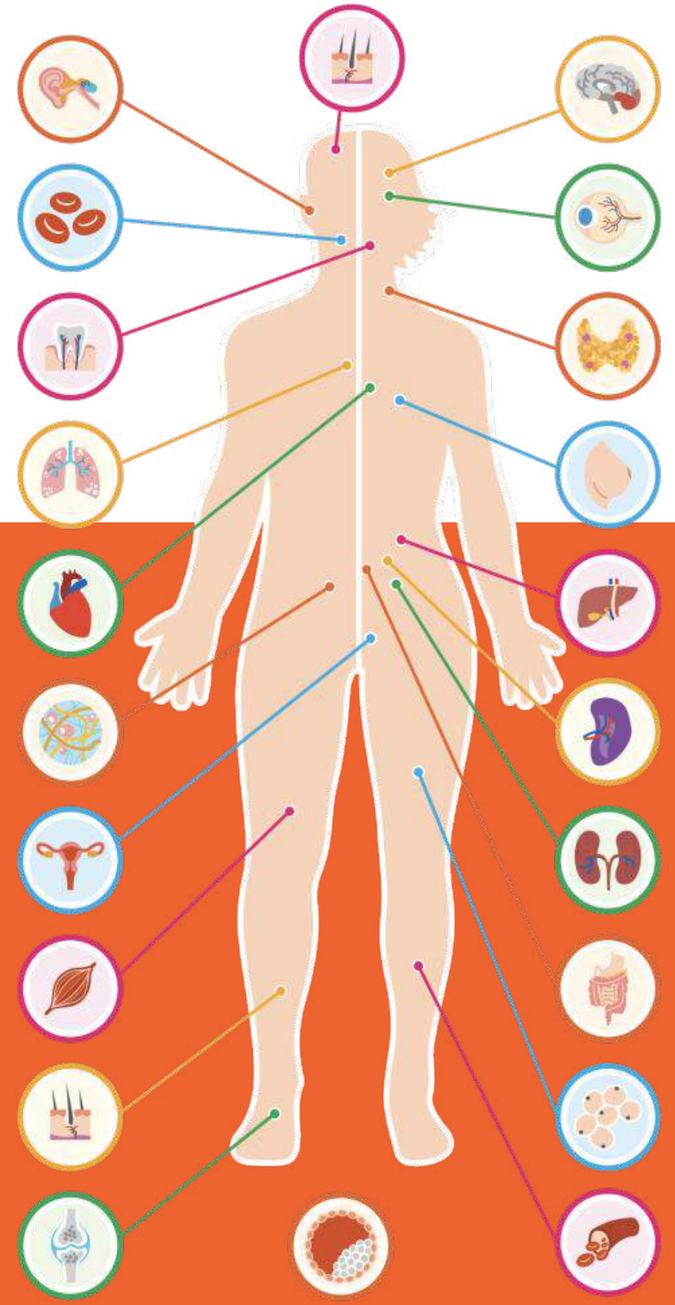
Experience & Resulting collection

Solid scientific foundation with:

01 Diverse viral vector options

02 Skillful expression cloning

900+
Cell lines



- Continuous source of cells
- Retain characteristics of the primary parental cells
- Less batch-to-batch variation
- Saves money

Popular immortalized cell lines/ Tumor cells

Human

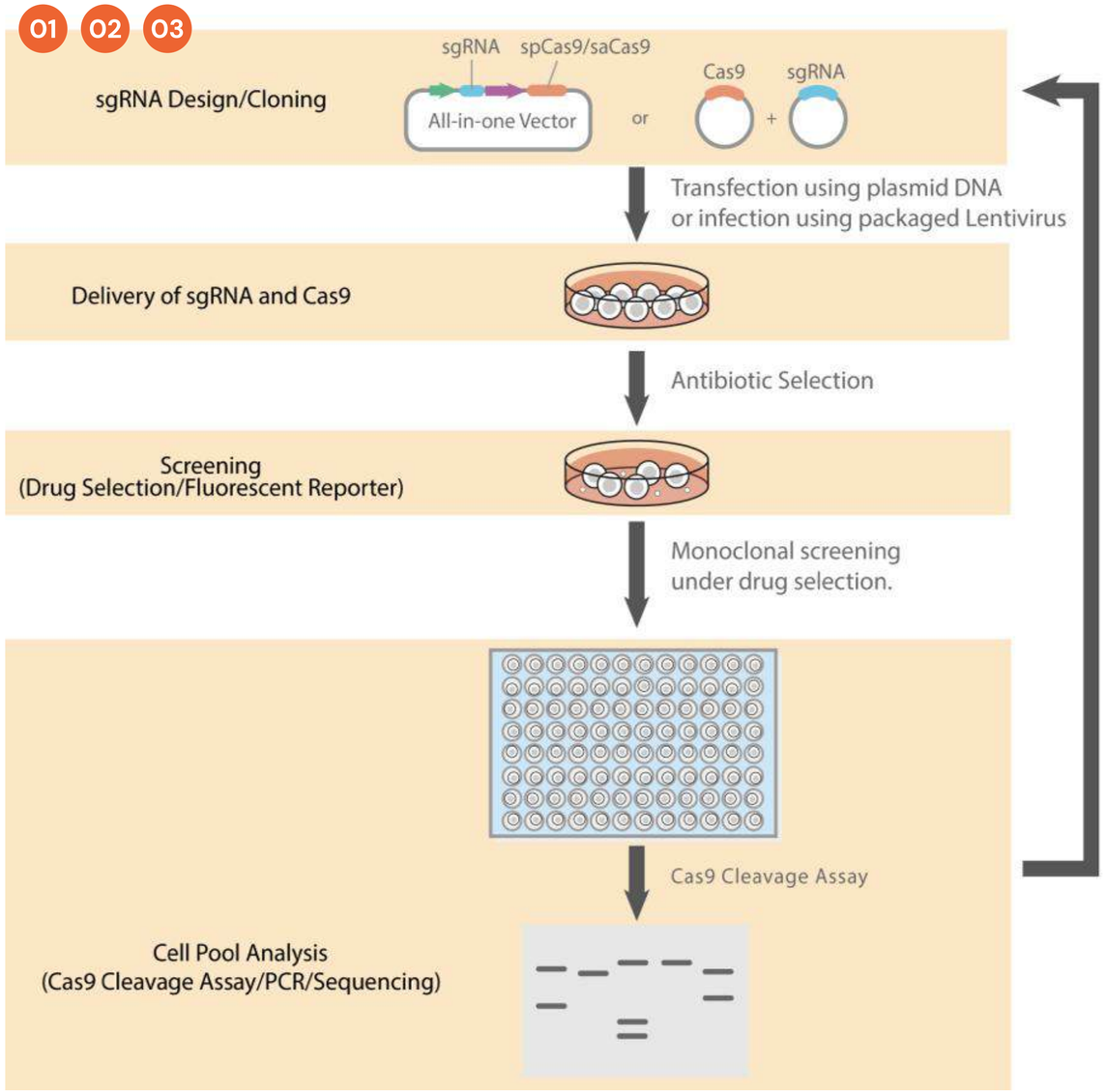
01	Microglia (T0251)
02	Dopaminergic neuronal precursor cell (LUHMES, T0284)
03	Mast cell lines LADR, LAD2 (T8156, T8157)
04	Skeletal muscle myoblasts (T0033)
05	Bone marrow mesenchymal stem cells (T0529)
06	Hepatocytes (T0063)

Animal

01	Mouse dendritic cells (MutuDC, T0528)
02	Rat retinal müller cells (rMC-1, T0576)
03	Canine skin microvascular endothelial cells (CSkMEC, T0839)
04	Equine lung cells (extEqFL, T0095)
05	Porcine alveolar macrophage cells (PAM-KNU, T0741)
06	Feline laryngeal, gingival & lingual squamous cell carcinoma (SCCF1~3, T8294-6)

Custom service

OUR FLEXIBILITY & SCALABILITY



Our methods for Cas9/sgRNA delivery

01 Cas9 Proteins

02 Cas9/sgRNA All-in-One vector:

- Lentiviral
- AAV
- Adenovirus
- Non-viral

03 Cas9 expressing cell lines

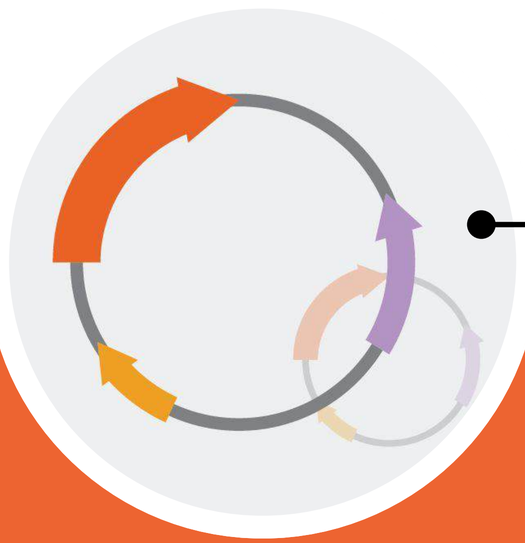
500+
Projects/Year

From start to finish

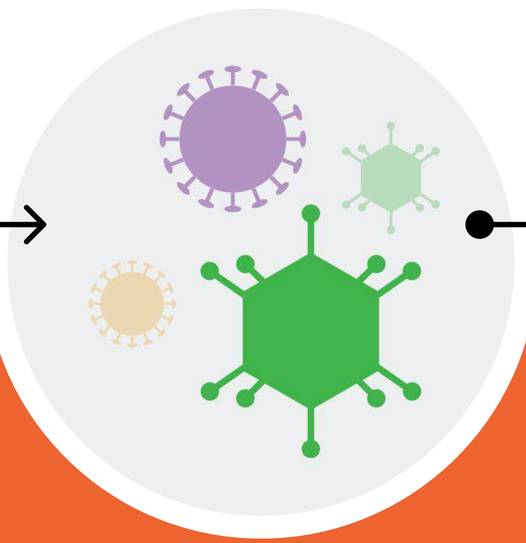
WE DO IT ALL

From project inception...

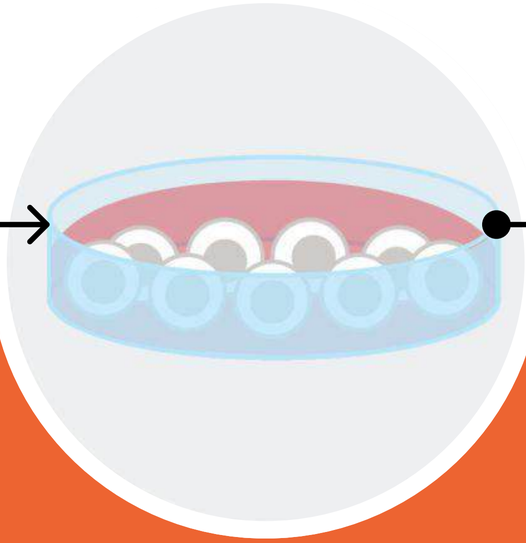
... To asset delivery and beyond!



Gene editing & cloning



Gene delivery



Cell engineering

Validation

Q&A

Ask us anything!

Alfamed

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