BUILD YOUR PLASMID
Educational Game

"**Build your plasmid**" is a free educational resource created by *Dr. Marco Straccia* to teach, in an attractive way, gamma-retrovirus or lentivirus-based *ex-vivo* gene therapy' strategies to undergraduate university students.

However it can be used and modified for non commercial reuse, to teach also molecular biology, genetic engineering or any other matter that you can figure out at any educational level.

It would be great if anyone, who uses this resource to generate new ones, will freely share their own projects with us, in order to exchange ideas and improve the life science and biomedical education of our future generations.

All suggestions, tips and critiques to improve the educational nature of this material are welcome and if you want to receive the modifiable *.ppt* file, please feel free to contact us at:

FRESCI

fre-sci@fre-sci.info
www.FRE-SCI.info

ACKNOWLEDGEMENTS

This game has been created using free available information from:

- Plasmid 101: A Desktop Resource by Addgene. March 2017 (3rd Edition)
- The portal to gene-specific content based on NCBI's RefSeq project (www.ncbi.nlm.nih.gov/gene/).
- Nikon's **MicroscopyU** website features technical support and timely information about all aspects of optical microscopy, photomicrography, and digital imaging. (www.microscopyu.com/techniques/fluorescence/introduction-to-fluorescent-proteins)

EDUCATIONAL PERSPECTIVE

Aims Of The Game:

The aim of this game is to simulate a research strategy that uses students' knowledge acquired during the theoretical class. Since they are not allowed to manipulate virus and human cells, at least this game force them to think on how to plan a gene therapy strategy based on a clinical case or disease feature.

Generic Skills This Game Helps To Develop:

- Team building.
- Collaborative critical thinking.
- Logic processes.
- Problem solving.
- Lateral-thinking.
- Communication.
- Public speaking.
- Innovation.

Specific Skills This Game Helps To Develop:

- Therapeutic strategies.
- Molecular Biology concepts application.
- Genetic Engineering theory application.
- Gene therapy strategy logic flow.
- Gene therapy strategy knowledge application.
- Transient versus Stable gene expression.
- Constitutive versus Inducible gene expression.
- Cell Selection strategies.
- Protein Biochemistry concepts.

GAME RULES

"Build your plasmid – The Game" can be played, used and customized depending on your public, your class, your background etc. etc.
We play this game after 3 hours of frontal class we usually taught one month in advance.

Here we explain how we play:

FEATURES:

Time:	90 min
Nº of participants:	2 or more (each team should have max. 4 students)
Teams:	2 (4 vs. 4) per session or disease case
Educational level:	university undergraduate students

1. We prepare in advance one disease case per session, which could be theoretically approached by gene therapy strategies.
2. We explain the 3 viral plasmids general features (Envelope, Packaging and Transfer plasmids), then we focus on the Transfer plasmid and we explain its components.
3. Then, we split the group in 2.
4. Each team has 45 min max. to develop a strategy that could solve the clinical case.
5. The first step for them is to select what source of cells they will use.
6. Then they will have to decide how to build the plasmid that matches their strategy, using Promoter, Insert 1, Insert 2, Tag, Origin of Replication, Antibiotic Resistance and Selection Marker cards.
7. At the end they have to present their strategy (15 min per team) and justify the reason for each selected feature card.
8. The teacher has to lead the session and encourage to develop a critical logic thinking and innovation.

NOTES:
- The teacher can guide the team and solve doubts answering questions.
- There is not a unique solution, but there are many alternatives which should be accepted based on their strategy and defense.
- Not all cards must be used.
- You can assume everything will fit into the plasmid without size restriction or you can be more realistic. You set the difficulty level.
- Promoters should be considered as the minimal responsive element that would fit into the construct, however for educational reason this information is skipped and we suppose that minimal promoters are available for any gene.

CASE STUDIES

We have used the following "straight forward" case studies prepared by *Dr. Albert Giralt*, which already suggest the first step type of strategy to follow (overexpression, silencing or genome editing). However the level can be changed and new cases can be proposed to students depending on the target public and educational aim.
The Promoter and Insert cards were prepared to solve the following cases. New cards can be created to solve these same cases or new ones.

1. Alzheimer's disease is a neurodegenerative disease that affects the entire brain. The cause is in the 90% sporadic and the 10% with genetic links. Among the main hallmarks of the disease are neuron atrophy, alterations in synaptic plasticity, astrogliosis and reduction of trophic factors such as GDNF, BDNF and NGF. One of the current attractive strategies is the ex vivo genetic therapy. Concretely, we would like to design **astroglial** cells to express **BDNF** and to **graft** them in the most affected brain regions in patients. Astrocytes are the most common cell type in the brain and are very important for the normal functioning of the neurons.

2. Liver Fibrosis: Prolonged challenge to the liver such as Hepatitis B or Hepatitis C infection, or chronic alcoholism leads to deposition of type-I collagen to the extracellular space as a healing mechanism, which finally impairs the normal function of the liver. Clinically, this stage is called liver fibrosis. In the experimental models of liver fibrosis, **CCR2$^+$ monocytes** migrate rapidly into the liver and accelerate the fibrosis by producing pro-inflammatory and pro-fibrogenic cytokines, such as interleukin-6 (IL-6). Thus, we would like to modify monocytes *in vitro* **to prevent** the excess of **IL-6** production in such pathological condition.
Monocytes are the largest leukocyte cell type and are produced by the bone marrow. Monocytes circulate in the bloodstream for about one to three days and then typically move into tissues throughout the body where they differentiate into macrophages and dendritic cells.

3. X-linked retinoschisis (XLRS) is juvenile-onset macular degeneration caused by haploinsufficiency of the extracellular cell adhesion protein retinoschisin (RS1). *RS1* mutations can lead to either a non-functional protein or the absence of protein secretion, and it has been established that extracellular deficiency of RS1 is the underlying cause of the phenotype. Therefore, we hypothesized that an ex vivo gene therapy strategy could be used to deliver sufficient extracellular RS1 to reverse the phenotype seen in XLRS. Here, we want to use adipose-derived, syngeneic mesenchymal stem cells (**MSCs**). We want **to modify them to secrete human RS1 in a constitutive fashion** and then delivered these cells by intravitreal injection to the retina.
MSCs cells are localized in the adipose tissue, they have a great capacity for self-renewal while maintaining their multipotency and they display the potential to differentiate to (at least) osteoblasts, adipocytes and chondrocytes.

4. Acute lung injury (ALI): Genetic defects in the purine salvage enzyme adenosine deaminase (ADA) lead to severe combined immunodeficiency (SCID) with profound depletion of T, B, and natural killer cell lineages. Human leukocyte antigen–matched allogeneic hematopoietic stem cell transplantation (HSCT) offers a successful treatment option. However, individuals who lack a matched donor must receive mismatched transplants, which are associated with considerable morbidity and mortality. Enzyme replacement therapy (ERT) for ADA-SCID is available, but the associated suboptimal correction of immunological defects leaves patients susceptible to infection. In the present case we want to treat in a children population with **autologous hematopoietic bone marrow stem cells transduced with a conventional gamma retroviral vector encoding the human ADA gene**.
Hematopoietic bone marrow stem cells are the stem cells that give rise to mature blood cells. This process is called haematopoiesis. This process occurs in the red bone marrow, in the core of most bones.

PROMOTER CARD
GFAP

Official Full Name: *Glial fibrillary acidic protein*

LOCATION: 17q21.31 **EXON COUNT:** 10

Summary: This gene encodes one of the major intermediate filament proteins of mature astrocytes. It is used as a marker to distinguish astrocytes from other glial cells during development. Mutations in this gene cause Alexander disease, a rare disorder of astrocytes in the central nervous system. Alternative splicing results in multiple transcript variants encoding distinct isoforms. [provided by RefSeq, Oct 2008]

Expression: Restricted expression toward brain (RPKM 1208.4)

Organism: *H. sapiens*

PROMOTER CARD
BDNF

Official Full Name: *Brain derived neuro-trophic factor*

LOCATION: 11p14.1 **EXON COUNT:** 12

Summary: This gene encodes a member of the nerve growth factor family of proteins. Alternative splicing results in multiple transcript variants, at least one of which encodes a preproprotein that is proteolytically processed to generate the mature protein. Binding of this protein to its cognate receptor promotes neuronal survival in the adult brain. Expression of this gene is reduced in Alzheimer's, Parkinson's, and Huntington's disease patients. This gene may play a role in the regulation of the stress response and in the biology of mood disorders. [provided by RefSeq, Nov 2015]

Expression: Biased expression in brain (RPKM 3.0), lung (RPKM 1.2) and 11 other tissues

Organism: *H. sapiens*

PROMOTER CARD
GDNF

Official Full Name: *Glial cell derived neurotrophic factor*

LOCATION: 5p12.2 **EXON COUNT:** 6

Summary: This gene encodes a secreted ligand of the TGF-beta superfamily of proteins, leading to recruitment and activation of SMAD family transcription factors. The encoded preproprotein is proteolytically processed to generate each subunit of the disulfide-linked homodimer. The recombinant form of this protein, a highly conserved neurotrophic factor, was shown to promote the survival and differentiation of dopaminergic neurons in culture, and was able to prevent apoptosis of motor neurons induced by axotomy. This protein is a ligand for the product of the RET protooncogene. [provided by RefSeq, Aug 2016]

Expression: Broad expression in placenta (RPKM 1.7), ovary (RPKM 1.5) and 18 other tissues

Organism: *H. sapiens*

PROMOTER CARD
NGF

Official Full Name: *Nerve growth factor*

LOCATION: 1p13.2 **EXON COUNT:** 4

Summary: his gene is a member of the NGF-beta family and encodes a secreted protein which homodimerizes and is incorporated into a larger complex. This protein has nerve growth stimulating activity and the complex is involved in the regulation of growth and the differentiation of sympathetic and certain sensory neurons. Mutations in this gene have been associated with hereditary sensory and autonomic neuropathy, type 5 (HSAN5), and dysregulation of this gene's expression is associated with allergic rhinitis. [provided by RefSeq, Jul 2008]

Expression: Broad expression in ovary (RPKM 1.3), heart (RPKM 0.5) and 18 other tissues

Organism: *H. sapiens*

PROMOTER CARD

CCR2

Official Full Name: *C-C motif chemokine receptor 2*

LOCATION: 3q21.31 **EXON COUNT:** 3

Summary: The protein encoded is a receptor for monocyte chemoattractant protein-1, a chemokine which specifically mediates monocyte chemotaxis. Monocyte chemoattractant protein-1 is involved in monocyte infiltration in inflammatory diseases such as rheumatoid arthritis as well as in the inflammatory response against tumors. The encoded protein mediates agonist-dependent calcium mobilization and inhibition of adenylyl cyclase. This protein can also be a coreceptor with CD4 for HIV-1 infection. [provided by RefSeq, Aug 2017]

Expression: Biased expression in appendix (RPKM 15.2), lymph node (RPKM 5.1) and 12 other tissues

Organism: *H. sapiens*

PROMOTER CARD

IL6

Official Full Name: *Interleukin 6*

LOCATION: 7p15.3 **EXON COUNT:** 6

Summary: This gene encodes a cytokine that functions in inflammation and the maturation of B cells. In addition, it has been shown to be an endogenous pyrogen capable of inducing fever in people with autoimmune diseases or infections. The protein is primarily produced at sites of acute and chronic inflammation, where it is secreted into the serum and induces a transcriptional inflammatory response through interleukin 6 receptor, alpha. It is implicated in a wide variety of inflammation-associated disease states. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Dec 2015]

Expression: Broad expression in urinary bladder (RPKM 9.7), gall bladder (RPKM 7.1) and 14 other tissues

Organism: *H. sapiens*

PROMOTER CARD

COL1A2

Official Full Name: *Collagen type I alpha 2 chain*

LOCATION: 7q21.3 **EXON COUNT:** 52

Summary: This gene encodes the pro-alpha2 chain of type I collagen whose triple helix comprises two alpha1 chains and one alpha2 chain. Type I is a fibril-forming collagen found in most connective tissues and is abundant in bone, cornea, dermis and tendon. Symptoms associated with mutations in this gene, however, tend to be less severe than mutations in the gene for the alpha1 chain of type I collagen (COL1A1) reflecting the different role of alpha2 chains in matrix integrity. Three transcripts, resulting from the use of alternate polyadenylation signals, have been identified for this gene. [provided by R. Dalgleish, Feb 2008]

Expression: Broad expression in gall bladder (RPKM 891.0), urinary bladder (RPKM 521.6) and 14 other tissues

Organism: *H. sapiens*

PROMOTER CARD

EEF1A1

Official Full Name: *Eukaryotic translation elongation factor 1 alpha 1*

LOCATION: 6q13.2 **EXON COUNT:** 8

Summary: This gene encodes an isoform of the alpha subunit of the elongation factor-1 complex, which is responsible for the enzymatic delivery of aminoacyl tRNAs to the ribosome. This isoform (alpha 1) is expressed in brain, placenta, lung, liver, kidney, and pancreas, and the other isoform (alpha 2) is expressed in brain, heart and skeletal muscle. This isoform is identified as an autoantigen in 66% of patients with Felty syndrome. This gene has been found to have multiple copies on many chromosomes, some of which, if not all, represent different pseudogenes. [provided by RefSeq, Jul 2008]

Expression: Ubiquitous expression in ovary (RPKM 2994.9), thyroid (RPKM 2551.7) and 25 other tissues

Organism: *H. sapiens*

PROMOTER CARD

CMV

Promoter from the human cytomegalovirus

Primarily used for:	General Expression
RNA transcript:	mRNA
Expression:	Constitutive
Description:	Strong mammalian expression
Additional considerations:	May contain an enhancer region. Can be silenced in some cell types

PROMOTER CARD

SV40

Promoter from the simian vacuolating virus 40

Primarily used for:	General Expression
RNA transcript:	mRNA
Expression:	Constitutive
Description:	Mammalian expression
Additional considerations:	May include an enhancer.

PROMOTER CARD

PGK1

Official Full Name: *Phosphoglycerate kinase 1*

LOCATION: Xq21.1 **EXON COUNT:** 11

Summary: It codifies for a glycolytic enzyme that catalyzes the conversion of 1,3-diphospho-glycerate to 3-phosphoglycerate. It may also act as a cofactor for polymerase alpha. Additionally, this protein is secreted by tumor cells where it participates in angiogenesis by functioning to reduce disulfide bonds in the serine protease, plasmin, which consequently leads to the release of the tumor blood vessel inhibitor angiostatin. It is a moonlighting protein based on its ability to perform mechanistically distinct functions. Deficiency of the enzyme is associated with a wide range of clinical phenotypes hemolytic anemia and neurological impairment. [provided by RefSeq, Jan 2014]

Expression: Ubiquitous expression in kidney (RPKM 128.3), heart (RPKM 120.1) and 25 other tissues

Organism: *H. sapiens*

PROMOTER CARD

UBC

Official Full Name: *Ubiquitin C*

LOCATION: 12q24.31 **EXON COUNT:** 2

Summary: This gene represents a ubiquitin gene, ubiquitin C. The encoded protein is a polyubiquitin precursor. Conjugation of ubiquitin monomers or polymers can lead to various effects within a cell, depending on the residues to which ubiquitin is conjugated. Ubiquitination has been associated with protein degradation, DNA repair, cell cycle regulation, kinase modification, endocytosis, and regulation of other cell signaling pathways. [provided by RefSeq, Aug 2010]

Expression: Ubiquitous expression in bone marrow (RPKM 1559.8), gall bladder (RPKM 1270.7) and 25 other tissues

Organism: *H. sapiens*

PROMOTER CARD

ACTB

Mammalian Promoter

Primarily used for:	General Expression
RNA transcript:	mRNA
Expression:	Constitutive
Description:	Mammalian promoter from β-Actin gene.
Additional considerations:	Ubiquitous. Chicken version is commonly used in promoter hybrids.

PROMOTER CARD

CAG

Hybrid Promoter

Primarily used for:	General Expression
RNA transcript:	mRNA
Expression:	Constitutive
Description:	Strong hybrid mammalian promoter
Additional considerations:	Contains CMV enhancer, chicken beta actin promoter, and rabbit beta-globin splice acceptor.

PROMOTER CARD

TRE

Inducible Promoter

Primarily used for:	General Expression
RNA transcript:	mRNA
Expression:	Inducible with Tetracyline or its derivatives.
Description:	Tetracycline response element promoter
Additional considerations:	Typically contains a minimal promoter with low basal activity and several tetracycline operators. Transcription can be turned on or off depending on what tet transactivator is used.

PROMOTER CARD

RS1

Official Full Name: *Retinoschisin 1*

LOCATION: Xp22.13 **EXON COUNT:** 6

Summary: This gene encodes an extracellular protein that plays a crucial role in the cellular organization of the retina. The encoded protein is assembled and secreted from photoreceptors and bipolar cells as a homo-oligomeric protein complex. Mutations in this gene are responsible for X-linked retinoschisis, a common, early-onset macular degeneration in males that results in a splitting of the inner layers of the retina and severe loss in vision. [provided by RefSeq, Oct 2008]

Expression: Low expression observed in reference dataset

Organism: *H. sapiens*

PROMOTER CARD

ADA

Official Full Name: *Adenosine deaminase*

LOCATION: 20q13.12 **EXON COUNT:** 12

Summary: This gene encodes an enzyme that catalyzes the hydrolysis of adenosine to inosine. Various mutations have been described for this gene and have been linked to human diseases. Deficiency in this enzyme causes a form of severe combined immunodeficiency disease (SCID), in which there is dysfunction of both B and T lymphocytes with impaired cellular immunity and decreased production of immunoglobulins, whereas elevated levels of this enzyme have been associated with congenital hemolytic anemia. [provided by RefSeq, Jul 2008]

Expression: Biased expression in duodenum (RPKM 234.2) and lymph node (RPKM 16.6)

Organism: *H. sapiens*

PROMOTER CARD

SYN1

Official Full Name: *Synapsin I*

LOCATION: Xp11.3-p11.23 **EXON COUNT:** 13

Summary: This gene is a member of the synapsin gene family. Synapsins encode neuronal phosphoproteins which associate with the cytoplasmic surface of synaptic vesicles; implicated in synaptogenesis and the modulation of neurotransmitter release, suggesting a potential role in several neuropsychiatric diseases. This member of the synapsin family plays a role in regulation of axonogenesis and synaptogenesis. The protein encoded serves as a substrate for several different protein kinases and phosphorylation may function in the regulation of this protein in the nerve terminal. [provided by RefSeq, Jul 2008]

Expression: Biased expression in brain (RPKM 99.5) and adrenal (RPKM 5.6)

Organism: *H. sapiens*

PROMOTER CARD

CamKIIa

Human RNA Promoter

Primarily used for:	Gene expression for optogenetics
RNA transcript:	mRNA
Expression:	Specific
Description:	Ca2+/calmodulin-dependent protein kinase II promoter
Additional considerations:	Used for neuronal/CNS expression. Modulated by calcium and calmodulin.

Organism: *H. sapiens*

PROMOTER CARD

H1

Human Pol III RNA Promoter

Primarily used for:	Small RNA expression
RNA transcript:	shRNA
Expression:	Constitutive
Description:	From the human polymerase III RNA promoter
Additional considerations:	May have slightly lower expression than U6. May have better expression in neuronal cells.

Organism: *H. sapiens*

PROMOTER CARD

U6

Human U6 small nuclear Promoter

Primarily used for:	Small RNA expression
RNA transcript:	shRNA
Expression:	Constitutive
Description:	From the human U6 small nuclear promoter
Additional considerations:	Murine U6 is also used, but may be less efficient.

Organism: *H. sapiens*

PROMOTER CARD

MAP2

Official Full Name: *Microtubule associated protein-2*

LOCATION: 2q34 **EXON COUNT:** 21

Summary: This gene encodes a protein that belongs to the microtubule-associated protein family. The proteins of this family are thought to be involved in microtubule assembly, which is an essential step in neurogenesis. The products of similar genes in rat and mouse are neuron-specific cytoskeletal proteins that are enriched in dentrites, implicating a role in determining and stabilizing dentritic shape during neuron development. A number of alternatively spliced variants encoding distinct isoforms have been described. [provided by RefSeq, Jan 2010]

Expression: Biased expression in brain (RPKM 82.4), thyroid (RPKM 4.7) and 1 other tissue

Organism: *H. sapiens*

PROMOTER CARD

CEBPB

Official Full Name: *CCAAT enhancer binding protein beta*

LOCATION: 20q13.13 **EXON COUNT:** 1

Summary: This intronless gene encodes a transcription factor that contains a basic leucine zipper (bZIP) domain. The encoded protein functions as a homodimer but can also form heterodimers with CCAAT/enhancer-binding proteins alpha, delta, and gamma. Activity of this protein is important in the regulation of genes involved in immune and inflammatory responses, among other processes. The use of alternative in-frame AUG start codons results in multiple protein isoforms, each with distinct biological functions. [provided by RefSeq, Oct 2013]

Expression: N/A

Organism: *H. sapiens*

PROMOTER CARD

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Organism: *H. sapiens*

INSERT CARD

GFAP

Official Full Name: *Glial fibrillary acidic protein*

LOCATION: 17q21.31 **EXON COUNT:** 10

Summary: This gene encodes one of the major intermediate filament proteins of mature astrocytes. It is used as a marker to distinguish astrocytes from other glial cells during development. Mutations in this gene cause Alexander disease, a rare disorder of astrocytes in the central nervous system. Alternative splicing results in multiple transcript variants encoding distinct isoforms. [provided by RefSeq, Oct 2008]

mRNA lenght: V1=3097bp; V2=1839bp; V3=2193

Expression: Restricted expression toward brain (RPKM 1208.4)

Organism: *H. sapiens*

INSERT CARD

BDNF

Official Full Name: *Brain derived neuro-trophic factor*

LOCATION: 11p14.1 **EXON COUNT:** 12

Summary: This gene encodes a member of the nerve growth factor family of proteins. Alternative splicing results in multiple transcript variants, at least one of which encodes a preproprotein that is proteolytically processed to generate the mature protein. Binding of this protein to its cognate receptor promotes neuronal survival in the adult brain. Expression of this gene is reduced in Alzheimer's, Parkinson's, and Huntington's disease patients. This gene may play a role in the regulation of the stress response and in the biology of mood disorders. [provided by RefSeq, Nov 2015]

mRNA lenght: 1335bp

Expression: Biased expression in brain (RPKM 3.0), lung (RPKM 1.2) and 11 other tissues

Organism: *H. sapiens*

INSERT CARD

GDNF

Official Full Name: *Glial cell derived neurotrophic factor*

LOCATION: 5p12.2 **EXON COUNT:** 6

Summary: This gene encodes a secreted ligand of the TGF-beta superfamily of proteins, leading to recruitment and activation of SMAD family transcription factors. The encoded preproprotein is proteolytically processed to generate each subunit of the disulfide-linked homodimer. The recombinant form of this protein, a highly conserved neurotrophic factor, was shown to promote the survival and differentiation of dopaminergic neurons in culture, and was able to prevent apoptosis of motor neurons induced by axotomy. This protein is a ligand for the product of the RET protooncogene. [provided by RefSeq, Aug 2016]

mRNA lenght: ~3700bp

Expression: Broad expression in placenta (RPKM 1.7), ovary (RPKM 1.5) and 18 other tissues

Organism: *H. sapiens*

INSERT CARD

NGF

Official Full Name: *Nerve growth factor*

LOCATION: 1p13.2 **EXON COUNT:** 4

Summary: his gene is a member of the NGF-beta family and encodes a secreted protein which homodimerizes and is incorporated into a larger complex. This protein has nerve growth stimulating activity and the complex is involved in the regulation of growth and the differentiation of sympathetic and certain sensory neurons. Mutations in this gene have been associated with hereditary sensory and autonomic neuropathy, type 5 (HSAN5), and dysregulation of this gene's expression is associated with allergic rhinitis. [provided by RefSeq, Jul 2008]

mRNA lenght: 1052bp

Expression: Broad expression in ovary (RPKM 1.3), heart (RPKM 0.5) and 18 other tissues

Organism: *H. sapiens*

INSERT CARD

CCR2

Official Full Name: *C-C motif chemokine receptor 2*

LOCATION: 3q21.31 **EXON COUNT:** 3

Summary: The protein encoded is a receptor for monocyte chemoattractant protein-1, a chemokine which specifically mediates monocyte chemotaxis. Monocyte chemoattractant protein-1 is involved in monocyte infiltration in inflammatory diseases such as rheumatoid arthritis as well as in the inflammatory response against tumors. The encoded protein mediates agonist-dependent calcium mobilization and inhibition of adenylyl cyclase. This protein can also be a coreceptor with CD4 for HIV-1 infection. [provided by RefSeq, Aug 2017]

mRNA lenght: ~2300bp

Expression: Biased expression in appendix (RPKM 15.2), lymph node (RPKM 5.1) and 12 other tissues

Organism: *H. sapiens*

INSERT CARD

IL6

Official Full Name: *Interleukin 6*

LOCATION: 7p15.3 **EXON COUNT:** 6

Summary: This gene encodes a cytokine that functions in inflammation and the maturation of B cells. In addition, it has been shown to be an endogenous pyrogen capable of inducing fever in people with autoimmune diseases or infections. The protein is primarily produced at sites of acute and chronic inflammation, where it is secreted into the serum and induces a transcriptional inflammatory response through interleukin 6 receptor, alpha. It is implicated in a wide variety of inflammation-associated disease states. Alternative splicing results in multiple transcript variants. [provided by RefSeq, Dec 2015]

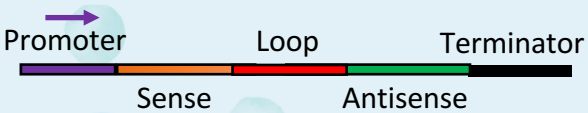
mRNA lenght: V1= ~1197bp

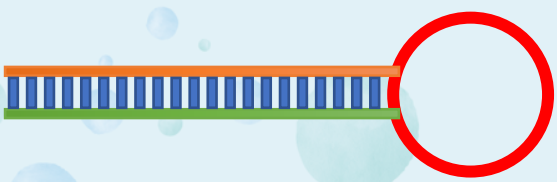
Expression: Broad expression in urinary bladder (RPKM 9.7), gall bladder (RPKM 7.1) and 14 other tissues

Organism: *H. sapiens*

INSERT CARD

Sh-C/EBPβ

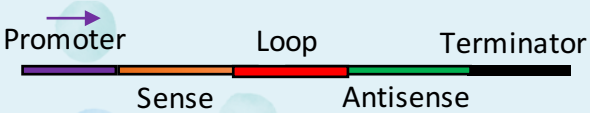




Organism: *H. sapiens*

INSERT CARD

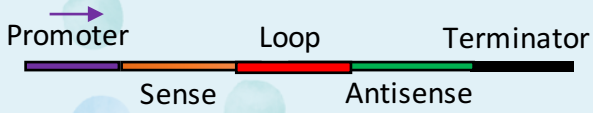
Sh-IL6

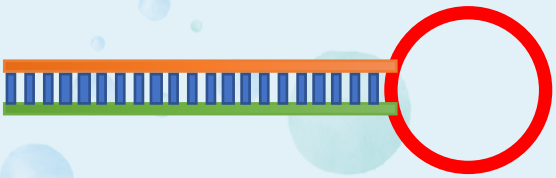


Organism: *H. sapiens*

INSERT CARD

Sh-BDNF

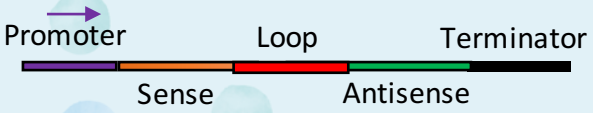




Organism: *H. sapiens*

INSERT CARD

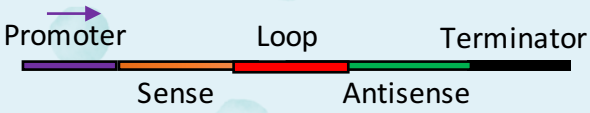
Sh-GFAP



Organism: *H. sapiens*

INSERT CARD

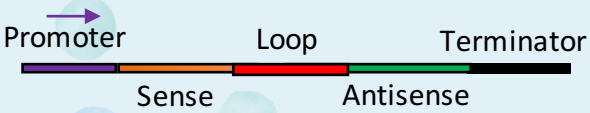
Sh-ADA



Organism: *H. sapiens*

INSERT CARD

Sh-RS1



Organism: *H. sapiens*

INSERT CARD

EGFP

Gene for Fluorescent Protein

Excitation max (nm):	484
Emission max (nm):	507
Molecular extinction coefficient:	56.000
Quantum yield:	0.60
***In vivo* structure:**	Monomer (weak dimer)
Relative Brightness (% of EGFP)	100

INSERT CARD

mCherry

Gene for Fluorescent Protein

Excitation max (nm):	587
Emission max (nm):	610
Molecular extinction coefficient:	72.000
Quantum yield:	0.22
***In vivo* structure:**	Monomer
Relative Brightness (% of EGFP)	47

INSERT CARD

EYFP

Gene for Fluorescent Protein

Excitation max (nm):	514
Emission max (nm):	527
Molecular extinction coefficient:	83.400
Quantum yield:	0.61
***In vivo* structure:**	Monomer
Relative Brightness (% of EGFP)	151

INSERT CARD

EBFP

Gene for Fluorescent Protein

Excitation max (nm):	383
Emission max (nm):	445
Molecular extinction coefficient:	29.000
Quantum yield:	0.31
***In vivo* structure:**	Monomer (weak dimer)
Relative Brightness (% of EGFP)	27

INSERT CARD

ECFP

Gene for Fluorescent Protein

Excitation max (nm):	439
Emission max (nm):	476
Molecular extinction coefficient:	32.500
Quantum yield:	0.40
***In vivo* structure:**	Monomer (weak dimer)
Relative Brightness (% of EGFP)	39

INSERT CARD

mCitrine

Gene for Fluorescent Protein

Excitation max (nm):	516
Emission max (nm):	529
Molecular extinction coefficient:	77.000
Quantum yield:	0.76
***In vivo* structure:**	Monomer
Relative Brightness (% of EGFP)	174

INSERT CARD

Cerulean

Gene for Fluorescent Protein

Excitation max (nm):	433
Emission max (nm):	475
Molecular extinction coefficient:	43.000
Quantum yield:	0.62
***In vivo* structure:**	Monomer (weak dimer)
Relative Brightness (% of EGFP)	79

INSERT CARD

dsRED

Gene for Fluorescent Protein

Excitation max (nm):	558
Emission max (nm):	583
Molecular extinction coefficient:	75.000
Quantum yield:	0.79
***In vivo* structure:**	Tetramer
Relative Brightness (% of EGFP)	176

INSERT CARD

RS1

Official Full Name: *Retinoschisin 1*

LOCATION: Xp22.13 **EXON COUNT:** 6

Summary: This gene encodes an extracellular protein that plays a crucial role in the cellular organization of the retina. The encoded protein is assembled and secreted from photoreceptors and bipolar cells as a homo-oligomeric protein complex. Mutations in this gene are responsible for X-linked retinoschisis, a common, early-onset macular degeneration in males that results in a splitting of the inner layers of the retina and severe loss in vision. [provided by RefSeq, Oct 2008]

mRNA lenght: 3040bp

Expression: Low expression observed in reference dataset

Organism: *H. sapiens*

INSERT CARD

ALB

Official Full Name: *Albumin*

LOCATION: 4q13.3 **EXON COUNT:** 15

Summary: This gene encodes the most abundant protein in human blood. This protein functions in the regulation of blood plasma colloid osmotic pressure and acts as a carrier protein for a wide range of endogenous molecules including hormones, fatty acids, and metabolites, as well as exogenous drugs. Additionally, this protein exhibits an esterase-like activity with broad substrate specificity. The encoded preproprotein is proteolytically processed to generate the mature protein. A peptide derived from this protein, EPI-X4, is an endogenous inhibitor of the CXCR4 chemokine receptor. [provided by RefSeq, Jul 2016]

mRNA lenght: 2335bp

Expression: Restricted expression toward liver (RPKM 41385.4)

Organism: *H. sapiens*

INSERT CARD

_____

Organism: *H. sapiens*

INSERT CARD

ACTB

Official Full Name: β-*Actin*

LOCATION: 7p22.1 **EXON COUNT:** 6

Summary: This gene encodes one of six different actin proteins. Actins are highly conserved proteins that are involved in cell motility, structure, integrity, and intercellular signaling. The encoded protein is a major constituent of the contractile apparatus and one of the two nonmuscle cytoskeletal actins that are ubiquitously expressed. Mutations in this gene cause Baraitser-Winter syndrome 1, which is characterized by intellectual disability with a distinctive facial appearance in human patients. Numerous pseudogenes of this gene have been identified throughout the human genome. [provided by RefSeq, Aug 2017]

mRNA lenght: 1940bp

Expression: Ubiquitous expression in appendix (RPKM 2395.4), lymph node (RPKM 2072.0) and 24 other tissues.

Organism: *H. sapiens*

INSERT CARD

ADA

Official Full Name: *Adenosine deaminase*

LOCATION: 20q13.12 **EXON COUNT:** 12

Summary: This gene encodes an enzyme that catalyzes the hydrolysis of adenosine to inosine. Various mutations have been described for this gene and have been linked to human diseases. Deficiency in this enzyme causes a form of severe combined immunodeficiency disease (SCID), in which there is dysfunction of both B and T lymphocytes with impaired cellular immunity and decreased production of immunoglobulins, whereas elevated levels of this enzyme have been associated with congenital hemolytic anemia. [provided by RefSeq, Jul 2008]

mRNA lenght: ~1500bp

Expression: Biased expression in duodenum (RPKM 234.2) and lymph node (RPKM 16.6)

INSERT CARD

SYN1

Official Full Name: *Synapsin I*

LOCATION: Xp11.3-p11.23 **EXON COUNT:** 13

Summary: This gene is a member of the synapsin gene family. Synapsins encode neuronal phosphoproteins which associate with the cytoplasmic surface of synaptic vesicles; implicated in synaptogenesis and the modulation of neurotransmitter release, suggesting a potential role in several neuropsychiatric diseases. This member of the synapsin family plays a role in regulation of axonogenesis and synaptogenesis. The protein encoded serves as a substrate for several different protein kinases and phosphorylation may function in the regulation of this protein in the nerve terminal. [provided by RefSeq, Jul 2008]

mRNA lenght: ~3200bp

Expression: Biased expression in brain (RPKM 99.5) and adrenal (RPKM 5.6)

INSERT CARD

CamKIIa

Official Full Name: *Calcium/calmodulin dependent protein kinase II gamma*

LOCATION: 10q22.2 **EXON COUNT:** 24

Summary: The product of this gene is one of the four subunits of an enzyme which belongs to the serine/threonine protein kinase family, and to the Ca(2+)/calmodulin-dependent protein kinase subfamily. Calcium signaling is crucial for several aspects of plasticity at glutamatergic synapses. In mammalian cells the enzyme is composed of four different chains: alpha, beta, gamma, and delta. The product of this gene is a gamma chain. Many alternatively spliced transcripts encoding different isoforms have been described but the full-length nature of all the variants has not been determined.[provided by RefSeq, Mar 2011]

mRNA lenght: ~4900bp

Expression: Ubiquitous expression in brain (RPKM 28.7), prostate (RPKM 10.9) and 24 other tissues

INSERT CARD

TAG CARD

CBP

Calmodulin binding peptide

Epitope:	KRRWKKNFIAVSAANRFK KISSSGAL
Mass (kDa):	4
Function:	Affinity and Purification
Notes:	Binding and elution steps use very moderate buffer conditions

TAG CARD

FLAG

Epitope:	DYKDDDD or DYKDDDDK or DYKDDDK
Mass (kDa):	1
Function:	Affinity and Purification
Notes:	Good for antibody-based purification; has inherent enterokinase cleavage site

TAG CARD

GST

Glutathione S-transferase

Epitope:	Large Protein
Mass (kDa):	26
Function:	Purification and Stability
Notes:	Good for purification with glutathione; protects against proteolysis, but may reduce solubililty

TAG CARD

HA

Hemaglutinin

Epitope:	YPYDVPDYA or YAYDVPDYA or YDVPDYASL
Mass (kDa):	1.1
Function:	Affinity
Notes:	Frequently used for western blots, IP, co-IP, IF, flow cytometry; can occasionally interfere with protein folding

TAG CARD

HBH

Epitope: HHHHHHAGKAGEGEIPA PLAGTVSKILVKEGDTVKA GQTVLVLEAMKMETEIN APTDGKVEKVLVKERDAV QGGQGLIKI GVHHHHHH

Mass (kDa): 9

Function: Combo

Notes: Consists of a bacterially-derived in-vivo biotinylation signaling peptide (Bio), flanked by hexahistidine motifs (6xHis)

TAG CARD

MBP

Maltose Binding Protein

Epitope: Large Protein

Mass (kDa): 40

Function: Solubility and Purification

Notes: Can improve solulibility and folding of eukaryotic proteins in prokaryotes; single step purification with amylose, but wicked huge

TAG CARD

Myc

Epitope: EQKLISEEDL

Mass (kDa): 1.2

Function: Affinity

Notes: Frequently used for western blots, IP, co-IP, IF, flow cytometry, but rarely used for purification as elution requires low pH

TAG CARD

Poly-His

Hemaglutinin

Epitope: HHHHHH

Mass (kDa): 0,8

Function: Affinity and Purification

Notes: Very small size, rarely affects function

TAG CARD

S-tag

Epitope: KETAAAKFERQHMDS

Mass (kDa): 1.8

Function: Solubility and Affinity

Notes: Abundance of charged and polar residues improves solubility; good for antibody-based detection

TAG CARD

SUMO

Small ubiquitin-related modifier

Epitope: About 100 aminoacid protein

Mass (kDa): 12

Function: Stability

Notes: At N-terminus, promotes folding and structural integrity; cleavable. Not great for purification; too cleavable in eukaryotes

TAG CARD

TAP

Tandem Affinity Purification

Epitope: Large Peptide

Mass (kDa): 21

Function: Combo

Notes: Comprised of a calmodulin binding peptide (CBP), a TEV cleavage site (more on that in a moment), and 2 ProtA IgG-binding domains

TAG CARD

TRX

Thioredoxin

Epitope: Large Peptide

Mass (kDa): 12

Function: Solubility

Notes: Assists in proper folding

TAG CARD

V5

Epitope: GKPIPNPLLGLDST

Mass (kDa): 1.4

Function: Affinity and Purification

Notes: Good for antibody-based purification

TAG CARD

TAG CARD

TAG CARD

RESISTANCE CARD

Kanamycin

Class:	Amino-glycoside
Mode of Action in prokaryotes:	Binds 30S ribosomal subunit; causes mistranslation
Effect:	Bactericidal
Working Concentration:	50-100 µg/mL in dH2O

RESISTANCE CARD

Ampicillin

Class:	Beta-Lactam
Mode of Action in prokaryotes:	Inhibits cell wall synthesis
Effect:	Bactericidal
Working Concentration:	100-200 µg/mL in dH2O

RESISTANCE CARD

Bleomycin

Class:	Glycopeptide
Mode of Action in prokaryotes:	Induces DNA breaks
Effect:	Bactericidal
Working Concentration:	5-100 µg/mL in dH2O

RESISTANCE CARD

Carbenicillin

Class:	Beta-Lactam
Mode of Action in prokaryotes:	Inhibits cell wall synthesis
Effect:	Bactericidal
Working Concentration:	100 µg/mL in dH2O

RESISTANCE CARD

Chloram-phenicol

Class: N/A

Mode of Action in prokaryotes: Binds 50S ribosomal subunit; inhibits peptidyl translocation

Effect: Bacteriostatic

Working Concentration: 5-25 µg/mL in EtOH

RESISTANCE CARD

Erythro-mycin

Class: Macrolide

Mode of Action in prokaryotes: Blocks 50S ribosomal subunit; inhibits aminoacyl translocation

Effect: Bacteriostatic

Working Concentration: 50-100 µg/mL in EtOH

RESISTANCE CARD

Spectino-mycin

Class: Aminoglycoside

Mode of Action in prokaryotes: Binds 30S ribosomal subunit; interrupts protein synthesis

Effect: Bactericidal

Working Concentration: 7.5-50 µg/mL In dH2O

RESISTANCE CARD

Polymyxin B

Class: PolyPeptide

Mode of Action in prokaryotes: Alters outer membrane permeability

Effect: Bactericidal

Working Concentration: 10-100 µg/mL in dH2O

RESISTANCE CARD

Strepto-mycin

Class: Amino-glycoside

Mode of Action in prokaryotes: Inhibits initiation of protin synthesis

Effect: Bactericidal

Working Concentration: 25-100 µg/mL In dH2O

RESISTANCE CARD

Tetra-cycline

Class: Tetracyclin

Mode of Action in prokaryotes: Binds 30S ribosomal subunit; inhibits protein synthesis (elongation step)

Effect: Bacteriostatic

Working Concentration: 10 µg/mL In dH2O

RESISTANCE CARD

_____

RESISTANCE CARD

_____

SELECTION MK CARD

Blasticidin

Gene conferring resistance:	bsd
Some Cell types:	HeLa, NIH3T3, CHO, COS-1, 293HEK
Mode of Action:	Inhibits termination step of translation
Working Concentration:	2-10 µg/mL

SELECTION MK CARD

G418 / Geneticin

Gene conferring resistance:	neo
Some Cell types:	HeLa, NIH3T3, CHO, 293HEK Jurkat T Cells
Mode of Action:	Blocks polypeptide synthesis at 80S; inhibits chain elongation
Working Concentration:	100-800 µg/mL

SELECTION MK CARD

Hygro-mycin B

Gene conferring resistance:	bsd
Some Cell types:	HeLa, NIH3T3, CHO, COS-1, Jurkat T Cells
Mode of Action:	Blocks polypeptide synthesis at 80S; inhibits chain elongation
Working Concentration:	50-500 µg/mL

SELECTION MK CARD

Puromycin

Gene conferring resistance:	pac
Some Cell types:	HeLa, 293HEK Jurkat T Cells
Mode of Action:	Inhibits protein synthesis; premature chain termination
Working Concentration:	1-10 µg/mL

SELECTION MK CARD

Zeocin

Gene conferring resistance: Sh bla

Some Cell types: HeLa, NIH3T3, CHO, COS-1, 293HEK, Jurkat T cells

Mode of Action: Complexes with DNA; Causes strand scissions

Working Concentration: 100-400 µg/mL

SELECTION MK CARD

SELECTION MK CARD

SELECTION MK CARD

ORI CARD

pMB1

pMB1 derivative

Copy number: around 500-700

Common vector: pUC

Incompatibility group: A

Control: Relaxed

ORI CARD

pMB1

Copy number: around 15-20

Common vector: pBR322

Incompatibility group: A

Control: Relaxed

ORI CARD

pBR322

pMB1 derivative

Copy number: around 15-20

Common vector: pET

Incompatibility group: A

Control: Relaxed

ORI CARD

ColE1

Copy number: around 15-20

Common vector: pColE1

Incompatibility group: A

Control: Relaxed

ORI CARD

R6K

pMB1 derivative

Copy number:	around 15-20
Common vector:	pR6K
Incompatibility group:	B
Control:	Stringent
Notes:	Requires *pir* gene for replication

ORI CARD

P15A

Copy number:	around 10
Common vector:	pACYC
Incompatibility group:	B
Control:	Relaxed

ORI CARD

pSC101

Copy number:	around 5
Common vector:	pSC101
Incompatibility group:	C
Control:	Stringent

ORI CARD

ColE1 & F1

ColE1 derivative

Copy number:	around 300-500
Common vector:	pBluescript
Incompatibility group:	A
Control:	Relaxed
Notes:	F1 is a phage-derived ORI that allows for the replication and packaging of ssDNA into phage particles. Plasmids with phage-derived ORIs are referred to as phagemids.

ORI CARD

pUC & F1

Copy number: around 300-500

Common vector: pGEM

Incompatibility group: A

Control: Relaxed

Notes: F1 is a phage-derived ORI that allows for the replication and packaging of ssDNA into phage particles. Plasmids with phage-derived ORIs are referred to as phagemids

ORI CARD

ORI CARD

ORI CARD

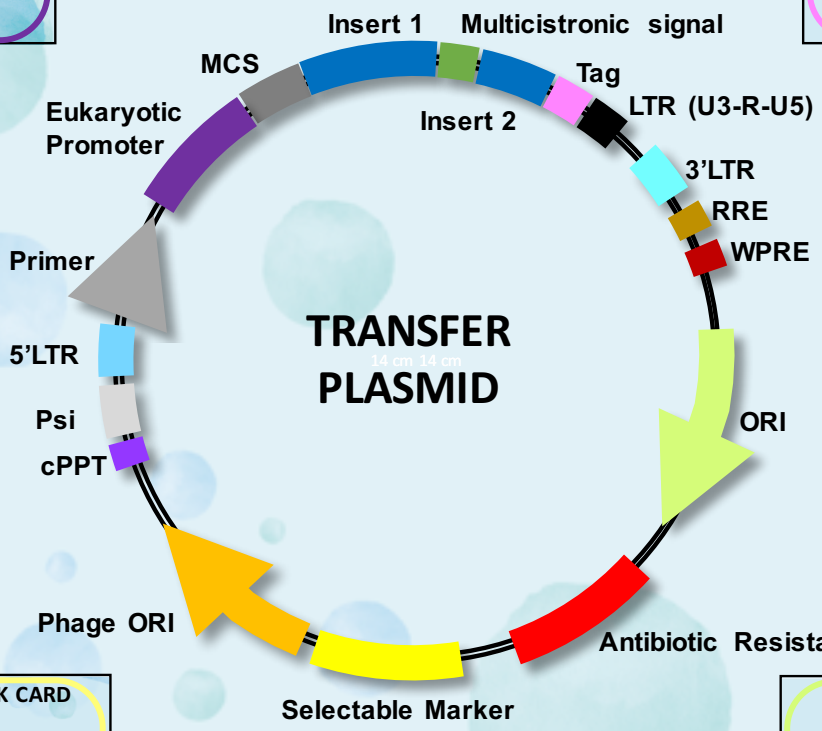
Build your Plasmid

PROMOTER CARD

INSERT CARD

INSERT CARD

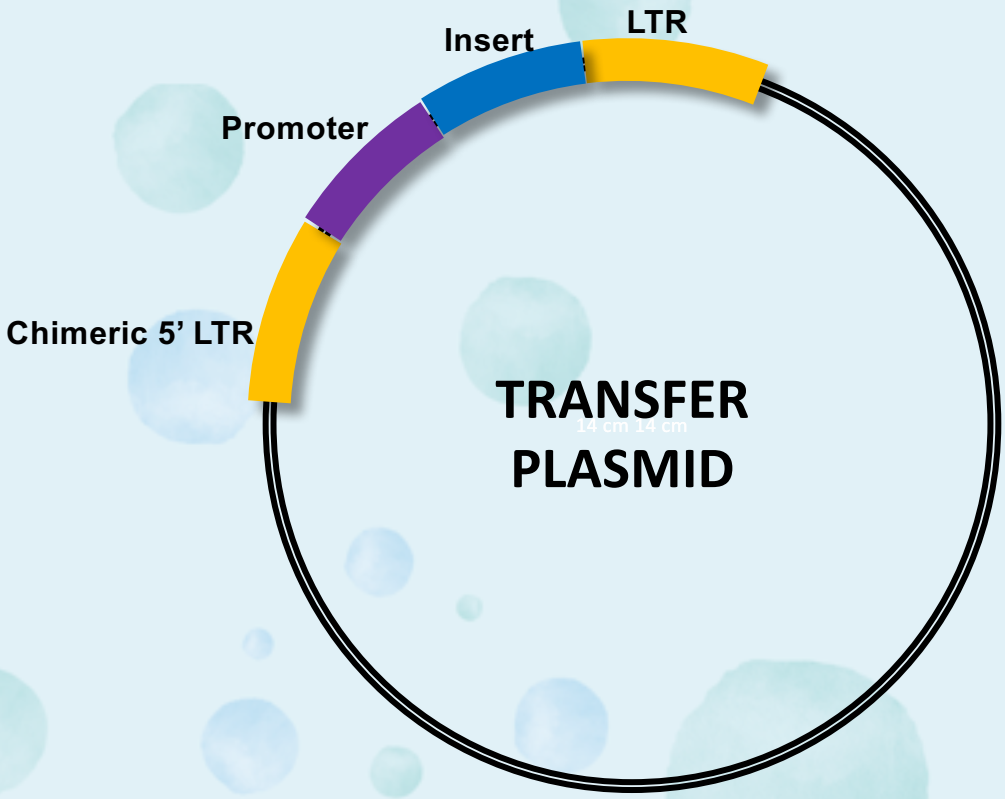
TAG CARD



SELECTION MK CARD

RESISTANCE CARD

ORI CARD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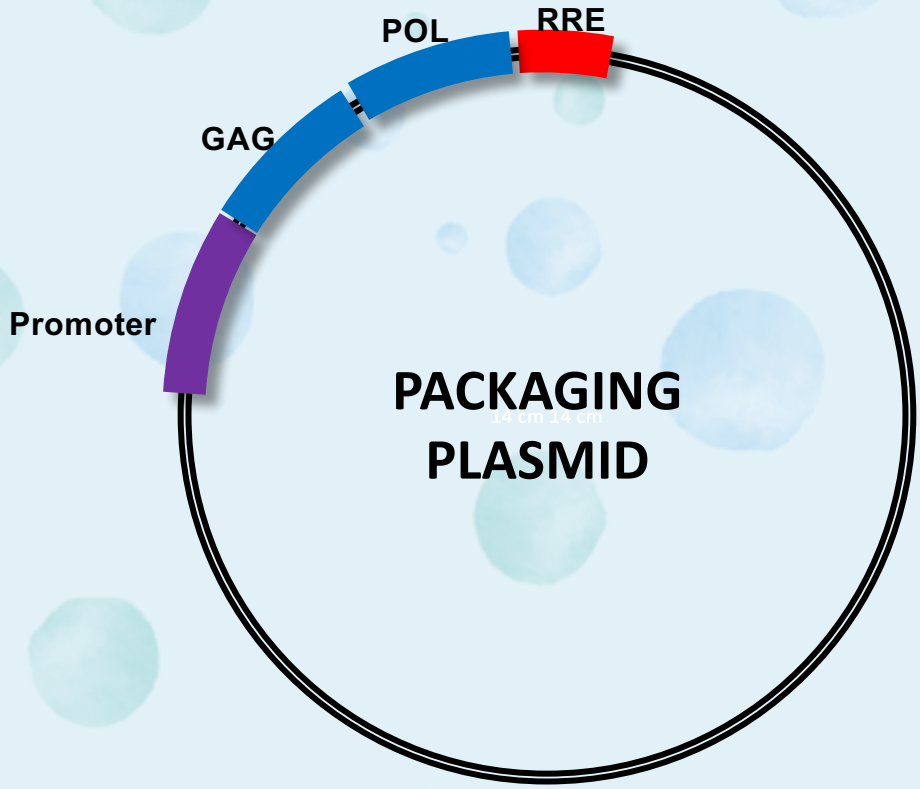
Promoter
Chimeric 5' LTR
TRANSFER
PLASMID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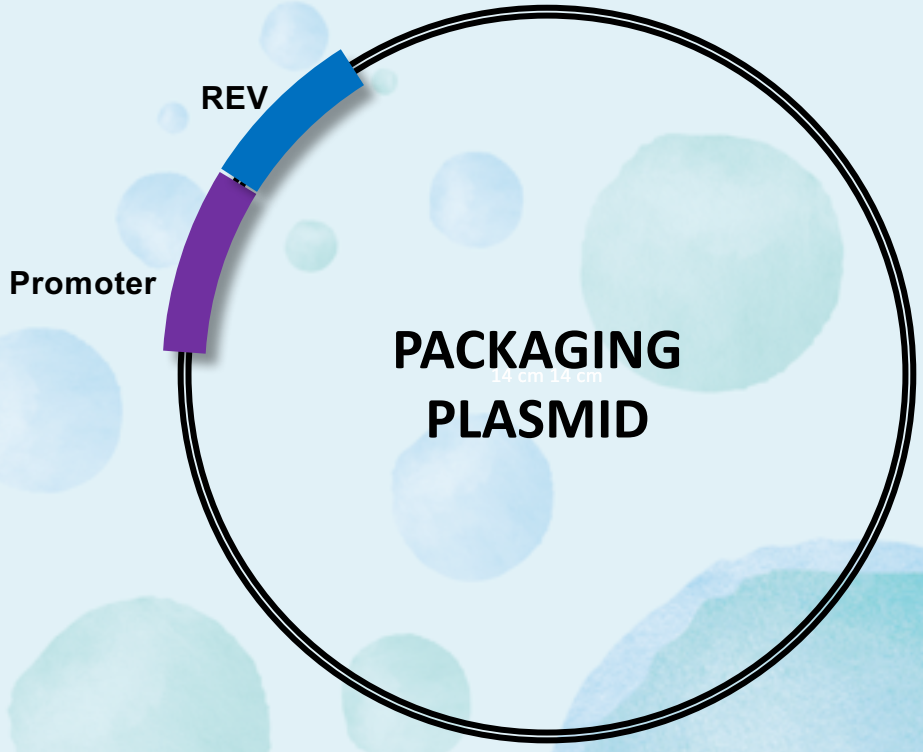
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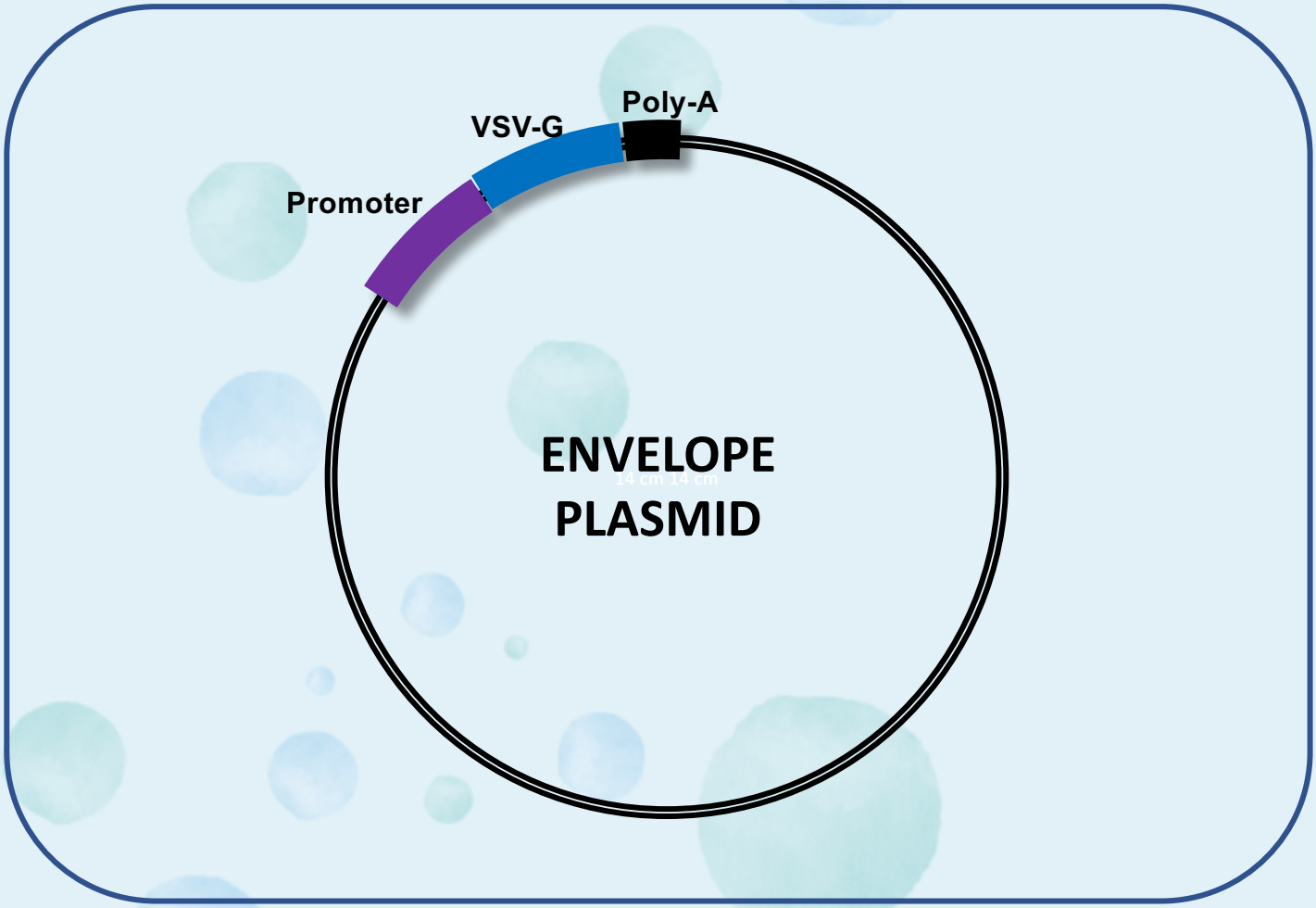
Poly-A
VSV-G
Promoter
ENVELOPE
PLASMID