

PR_11 Экспрессия в клетках млекопитающих

- Сигналы транскрипции, процессинга мРНК трансляции – консервативны у высших эукариот

= возможность экспрессировать как кДНК так и ДНК

- Посттрансляционные модификации и экспрессия белков в стабильной функциональной форме)
- Секреция

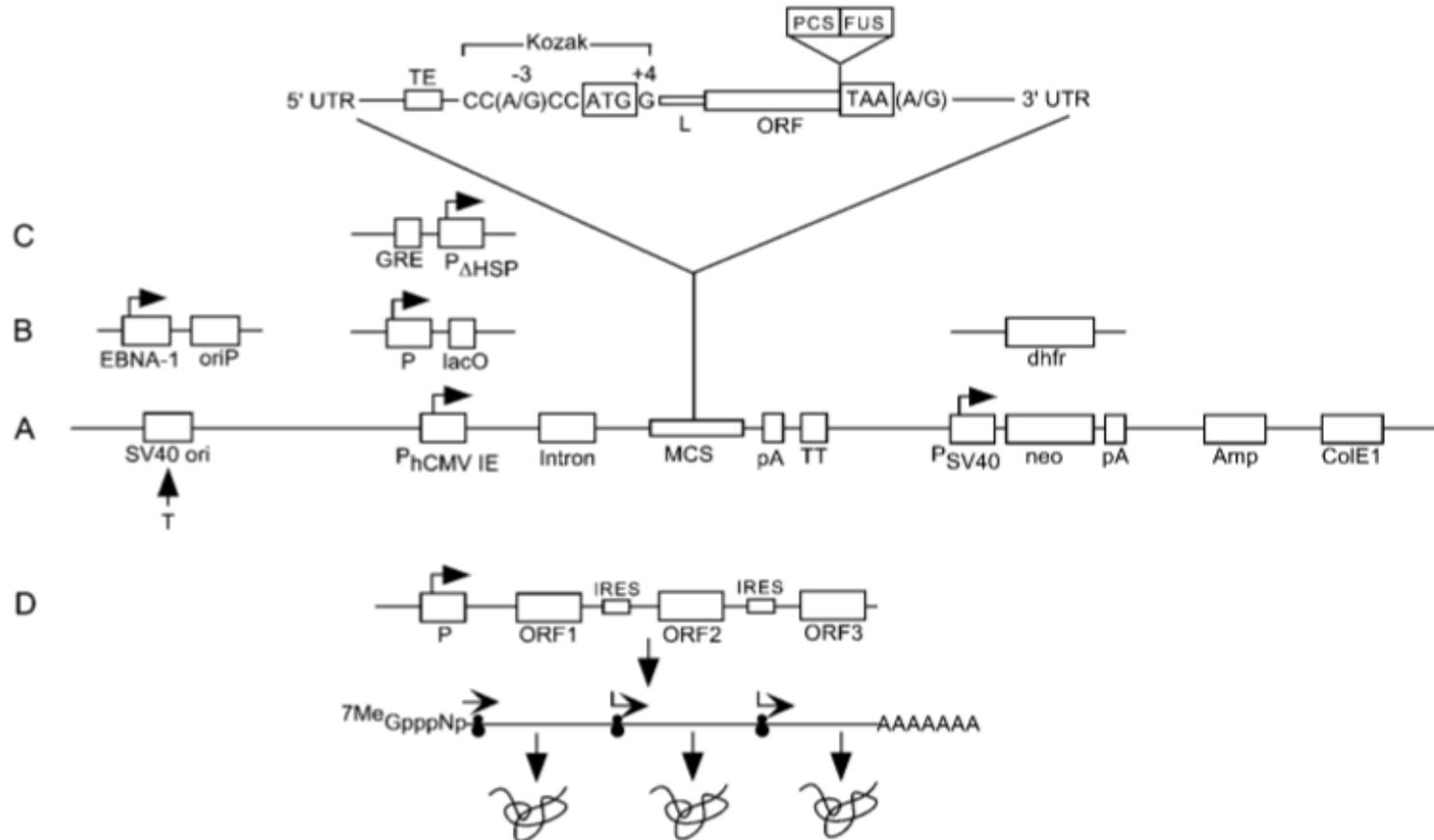


FIG. 1. Configuration of genetic elements in monocistronic (A) and polycistronic (D) expression vectors. Specific elements are shown for illustrative purposes and are not drawn to scale. The source, position, and combination of different components in the vector may vary in order to meet specific experimental requirements. SV40 ori is required for transient gene expression in COS cells. EBNA-1 and oriP facilitate high-copy episomal replication in primate and canine cell lines. The various promoter (P) elements allow constitutive (A) or inducible (B, C) expression. The optimal translational initiation sequence (Kozak) and termination codon followed by purines are shown. The ColE1 origin of replication and the ampicillin-resistance gene allow vector propagation in bacteria. The neomycin-resistance gene facilitates selection in mammalian cells, and the *dhfr* gene allows both selection and gene amplification. In a polycistronic vector (D) IRES elements allow multiple ORFs to be efficiently translated from a single transcript. See text for details. Amp, ampicillin resistance gene; ColE1, prokaryotic origin of replication; *dhfr*, dihydrofolate reductase; EBNA, Epstein-Barr virus nuclear antigen; FUS, fusion moiety; GRE, glucocorticoid response element; hCMV IE, human cytomegalovirus immediate early enhancer/promoter; HSP, heat shock protein; IRES, internal ribosome entry site; lacO, *lac* operator; L, leader (targeting sequence); MCS, multiple cloning site; neo, neomycin resistance gene; ORF, open reading frame; ori, origin of replication; oriP, Epstein-Barr virus origin of replication; P, promoter; pA, polyadenylation signal; PCS, protease cleavage site; T, SV40 large tumor (T) antigen; TE, translational enhancer; TT, transcription terminator; UTR, untranslated region.

Промоторы

Вирусные:

SV40 early promoter

- Rous sarcoma virus promoter
- adenovirus major late promoter
- human cytomegalovirus immediate early promoter

Эукариотические:

- human elongation factor 1 α promoter (pEF-1 α)

Регулируемые (гибридные)

- T-Rex
- pEF-LAC

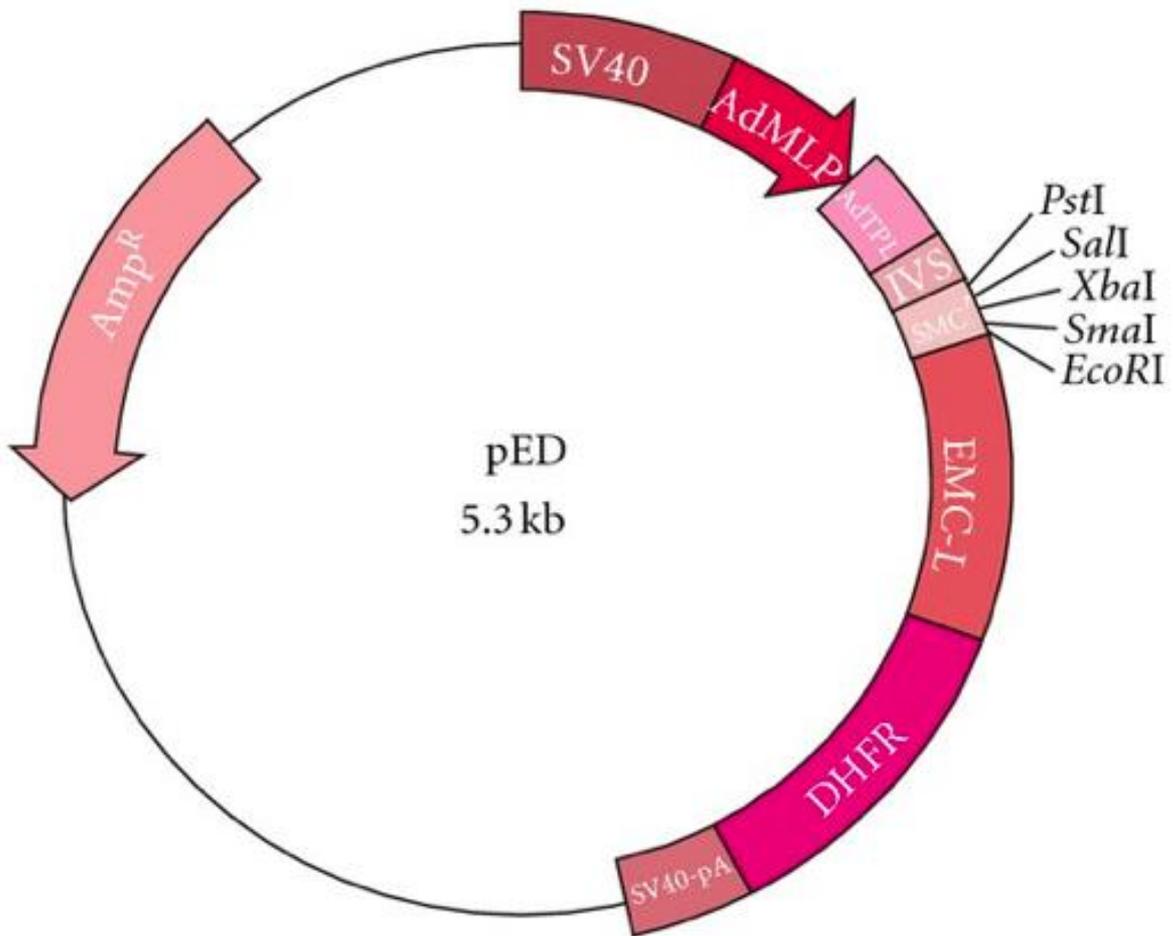


TABLE 4

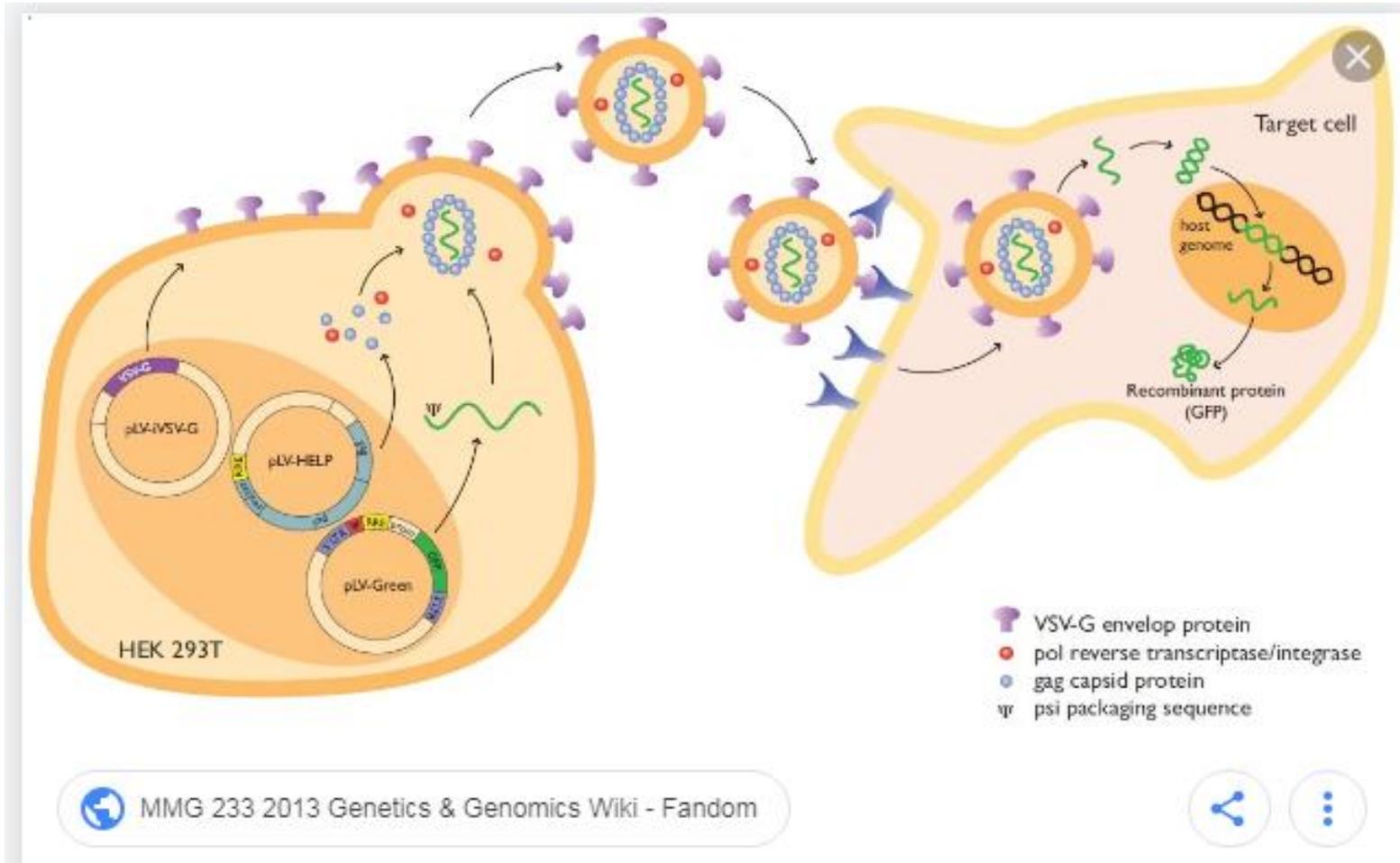
Selection Markers for Gene Expression in Mammalian Cells

Gene	Phenotype	Action of selective agent	Reference
<i>dhfr</i> (dihydrofolate reductase)	Positive selection Resistance to MTX ^c	MTX is a competitive inhibitor of DHFR	194, 195, 196
<i>xgppt</i> (<i>gpt</i>) (xanthine-guanine phosphoribosyl transferase)	Xanthine as the source for guanine synthesis	Aminopterin and mycophenolic acid in dialyzed medium block <i>de novo</i> synthesis of GMP	197
<i>aph</i> (<i>neo</i>) (aminoglycoside phosphotransferase)	Resistance to G418 ^b	G418 blocks mammalian protein synthesis	198, 199
<i>hph</i> (<i>hyg</i>) (hygromycin-B-phosphotransferase)	Resistance to hygromycin B	Hygromycin B blocks protein synthesis	200, 201
<i>pac</i> (<i>puro</i>) (puromycin-N-acetyl transferase)	Resistance to puromycin	Puromycin blocks protein synthesis	202, 203
<i>ble</i> (bleomycin)	Resistance to bleomycin, phleomycin, or zeocin	Bleomycin belongs to a group of related glycopeptide antibiotics which are believed to cause DNA strand scission	204, 205
<i>hisD</i> (histidinol dehydrogenase)	Resistance to histidinol	Histidinol is cytotoxic; HD oxidizes histidinol to histidine	206
<i>trpB</i> (tryptophan synthase (β subunit))	Indole as the source for tryptophan synthesis		206
<i>atpA</i> (Na^+ , K^+ -ATPase α subunit)	Resistance to ouabain	Ouabain belongs to a group of related cardiac glycosides which block transport of Na^+ and K^+ by intact cell membranes	207, 208
<i>ada</i> (adenosine deaminase)	Resistance to Xyl-A ^c	Xyl-A is converted to Xyl-ATP, which damages nucleic acids	209, 210
<i>codA</i> (cytosine deaminase)	Resistance to PALA ^d	PALA blocks <i>de novo</i> synthesis of pyrimidines; CD converts cytosine in the medium to uracil	211, 212
<i>codA</i> (cytosine deaminase)	Negative selection Cell death	CD converts 5-fluorocytosine to 5-fluorouracil	213
HSV-TK (Herpes simplex virus thymidine kinase)	Cell death	TK phosphorylates the selection drug ganciclovir which incorporates into DNA; ganciclovir is a poor substrate for mammalian TK	214
Fusion: <i>hyg</i> - <i>tk</i>	Positive or negative selection Positive: resistance to hygromycin Negative: cell death		215
Fusion: <i>tk</i> - <i>neo</i>	Positive: resistance to G418 Negative: cell death		216
Fusion: <i>tk</i> - <i>bsd</i>	Positive: resistance to blasticidin S Negative: cell death		217
Fusion: <i>pac</i> - <i>tk</i>	Positive: resistance to puromycin Negative: cell death		218
Fusion: <i>hyg</i> - <i>codA</i>	Positive: resistance to hygromycin Negative: cell death		218
Fusion: <i>codA</i> - <i>neo</i>	Positive: resistance to G418 Negative: cell death		218
Fusion: <i>codA</i> - <i>bsd</i>	Positive: resistance to blasticidin S Negative: cell death		218
Fusion: <i>pac</i> - <i>codA</i>	Positive: resistance to puromycin Negative: cell death		218

TABLE 5
Fusion Moieties for Gene Expression in Mammalian Cells^a

Fusion partner (source)	Ligand/substrate	Detection	Application	Reference ^b	Commercial source of expression vector ^c
FLAG peptide	Anti-FLAG monoclonal antibodies M1 and M2	Antibody	Purification, detection	219, 220	Stratagene (www.stratagene.com)
(Histidine) ₆	Ni ²⁺ -nitrilotriacetic acid	Antibody	Purification, detection	221, 222	Invitrogen (www.invitrogen.com) Qiagen (www.qiagen.com)
Glutathione <i>S</i> -transferase (<i>Schistosoma japonicum</i>) c-myc epitope Calmodulin-binding peptide Fc-Hinge	Glutathione Antibody Calmodulin Protein A	Biochemical, antibody Antibody	Purification, detection Purification, protein dimerization, higher protein yield, longer protein half-life	223, 224, 225 226, 227	Amersham Pharmacia (www.apbiotech.com) Invitrogen Stratagene
IgG1 and IgM heavy chain constant regions Streptococcal protein G	Serum albumin		Longer protein half-life Purification, longer protein half-life	228 229	
Serum albumin Viral glycoprotein transmembrane domain Platelet-derived growth factor receptor (PDGFR) transmembrane domain Herpes simplex virus glycoprotein D (gD) domain Epstein-Barr virus nuclear antigen 1 GGAGAGAG			Longer protein half-life Surface expression for vaccination Surface expression for ligand-binding interactions Purification	229, 230 ^d 231	Invitrogen
Growth hormone (human, rat)	Antibody	Antibody	Longer protein half-life	125	
Alkaline phosphatase (mammalian/bacterial)	<i>p</i> -Nitrophenyl phosphate	Immunoassay	Detection, monitor promoter activity	233, 234	
Alkaline phosphatase (mammalian/bacterial)	<i>p</i> -Nitrophenyl phosphate	Electrochemical, chemiluminescence, fluorescence	Detection, monitor promoter activity, biosensors	143, 234, 235, 236	Clontech (www.clontech.com)
β -Galactosidase (<i>Escherichia coli</i>)	β -Galactosides	Electrochemical, chemiluminescence, fluorescence	Detection, monitor promoter activity, biosensors	143, 234, 237	Clontech
Chloramphenicol acetyltransferase (<i>E. coli</i>)	Chloramphenicol or its derivatives	Radioisotope, fluorescence	Detection, monitor promoter activity, biosensors	143, 234, 238, 239	Promega (www.promega.com)
Luciferase (<i>Photinus pyralis</i>) (<i>Luciola mingrelica</i>)	Firefly luciferin	Bioluminescence	Detection, monitor promoter activity, biosensors	143, 234, 240–242	Promega
Luciferase (<i>Vibrio harveyi</i>)	<i>n</i> -Decyl aldehyde	Bioluminescence	Detection, monitor promoter activity, biosensors	143, 240 ^e	
Luciferase (<i>Renilla reniformis</i>)	Coelenterazine	Bioluminescence	Detection, monitor promoter activity, biosensors	243	
Green fluorescent protein and its variants (<i>Aequorea victoria</i>)		Fluorescence	Detection, monitor promoter activity, higher protein yield, biosensors	143, 144, 244, 245, 246	Clontech, Invitrogen
Aequorin (<i>A. victoria</i>)	Coelenterazine	Bioluminescence	Immunoassay, hybridization assay, Ca ²⁺ reporter	143	

Лентивирусные векторы



Экспрессия

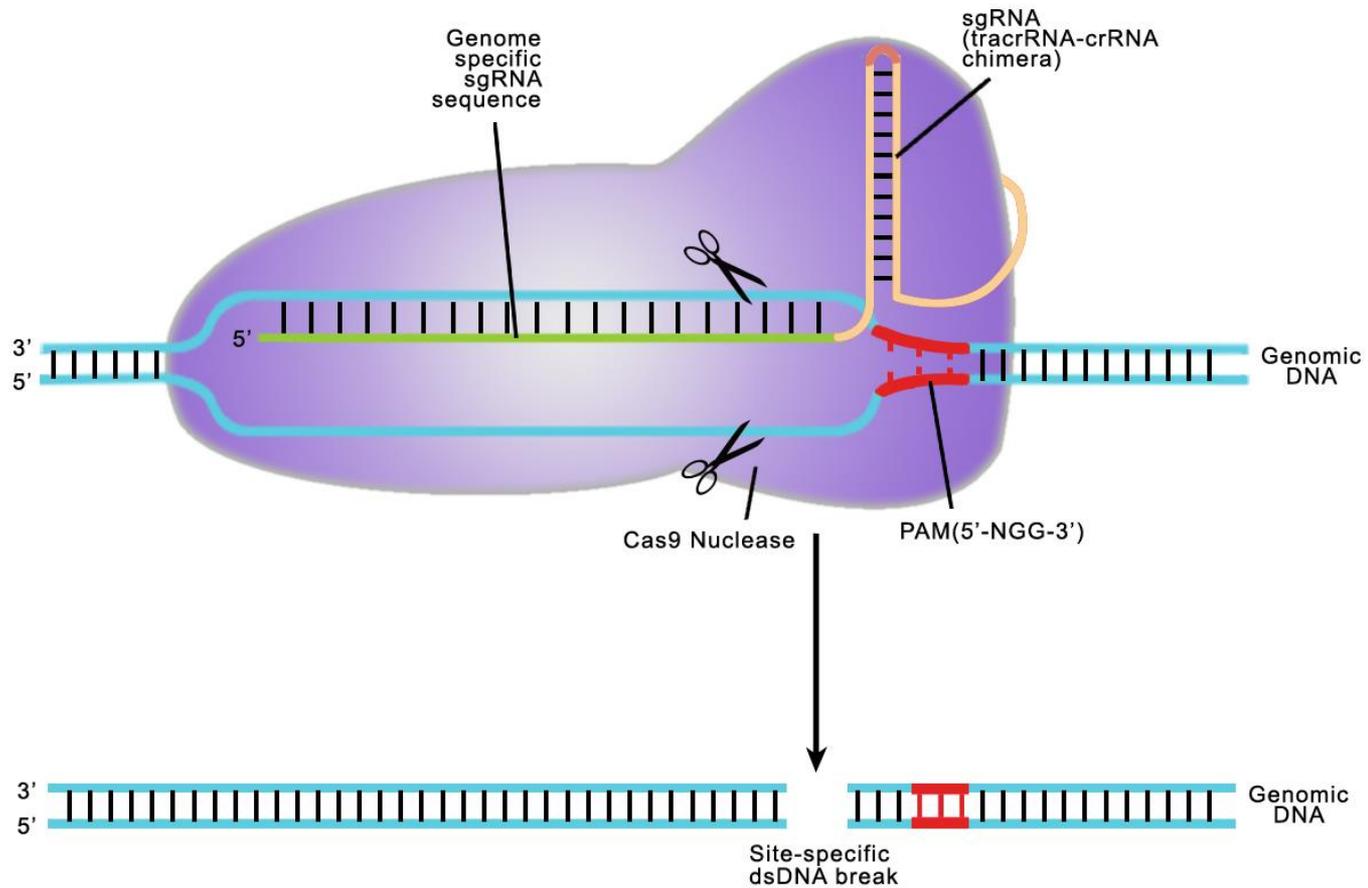
- Транзиентная
(плазмида)

Cell line: COS, HEK-293

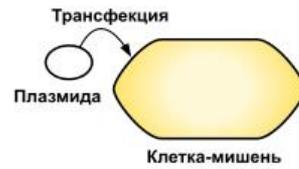
- Стабильная
(интеграция в геном)

- Cell line: CHO

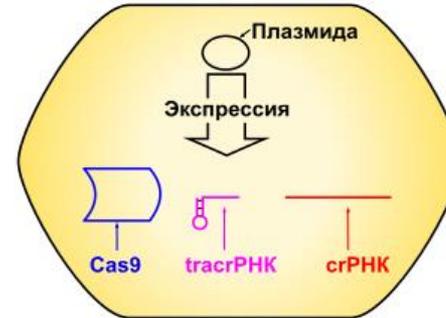
CRISPER/Cas9



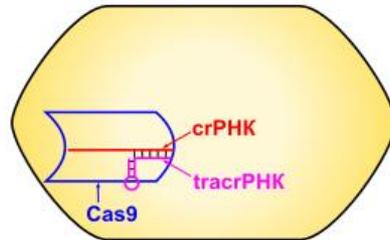
1: Трансфекция



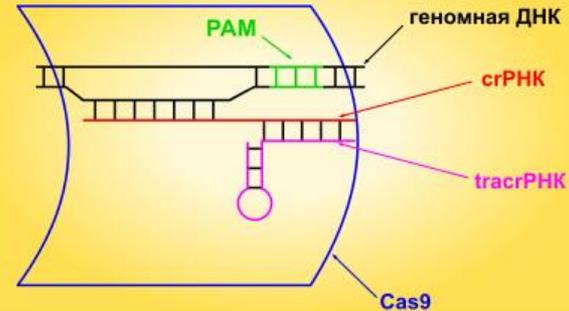
2: Экспрессия плазмиды



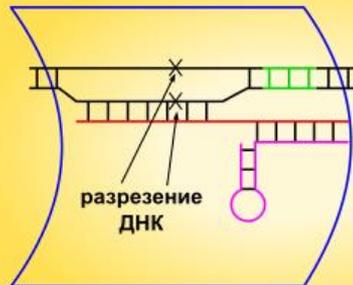
3: Активация Cas9



4: Связывание с последовательностью-мишенью в геноме



5: Разрезание геномной ДНК



6: ДНК готова к репарации



Биоинформатический анализ



Создание TALEN или CRISPR/Cas



Доставка TALEN и CRISPR/Cas

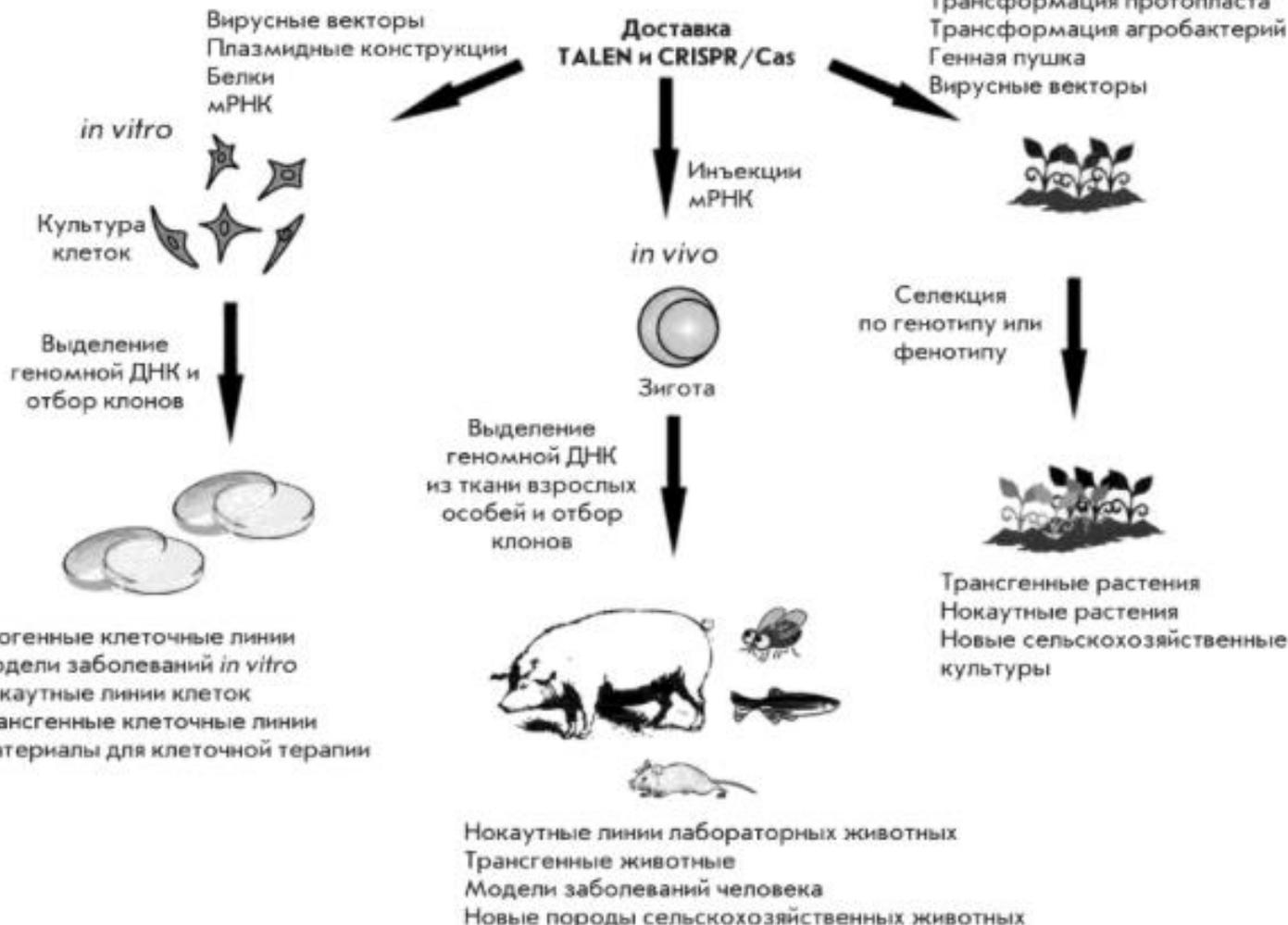


Рис. 3. Общая схема стратегии применения систем TALEN и CRISPR/Cas в геномной инженерии

TALEN

L TALEN

