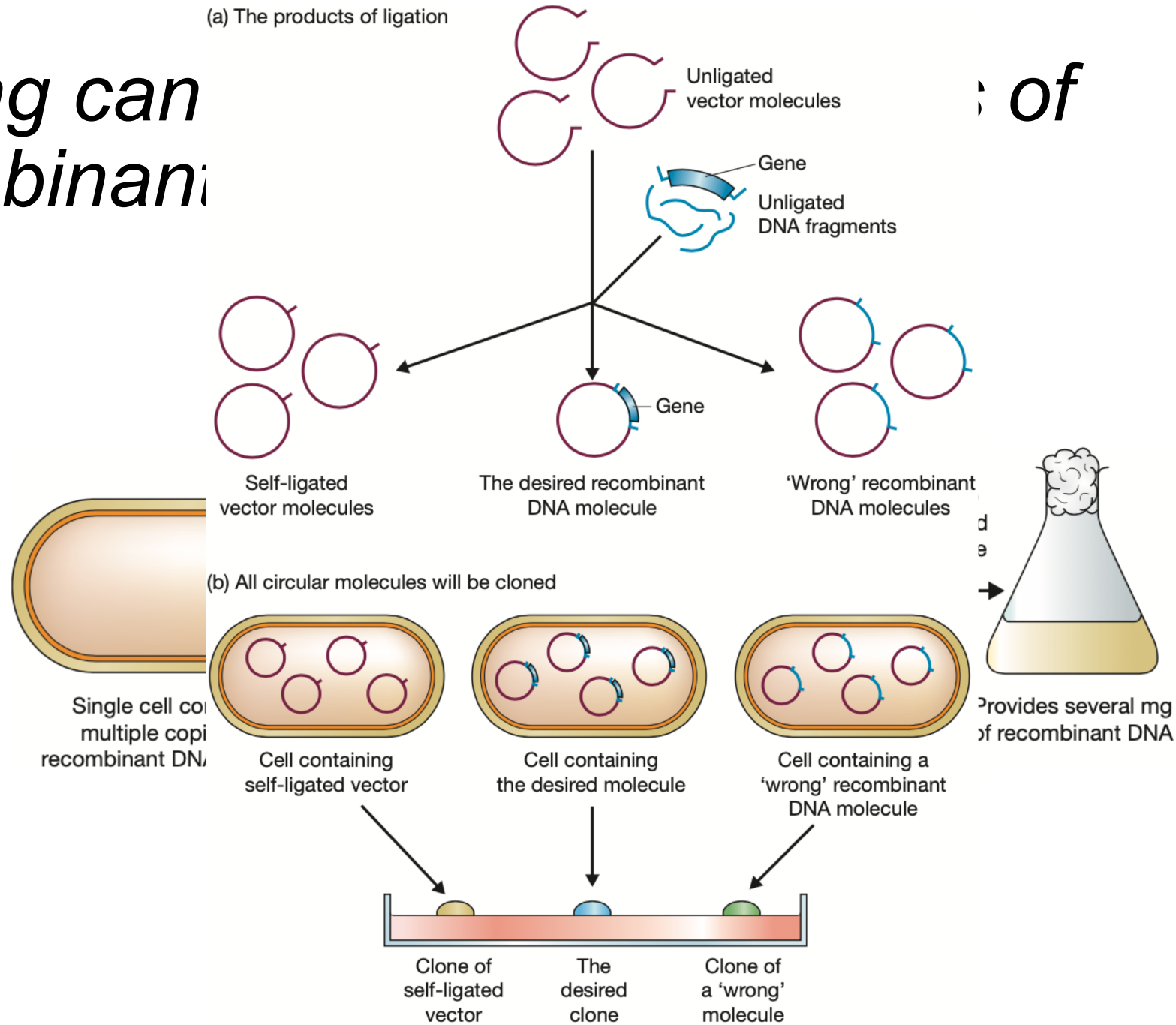


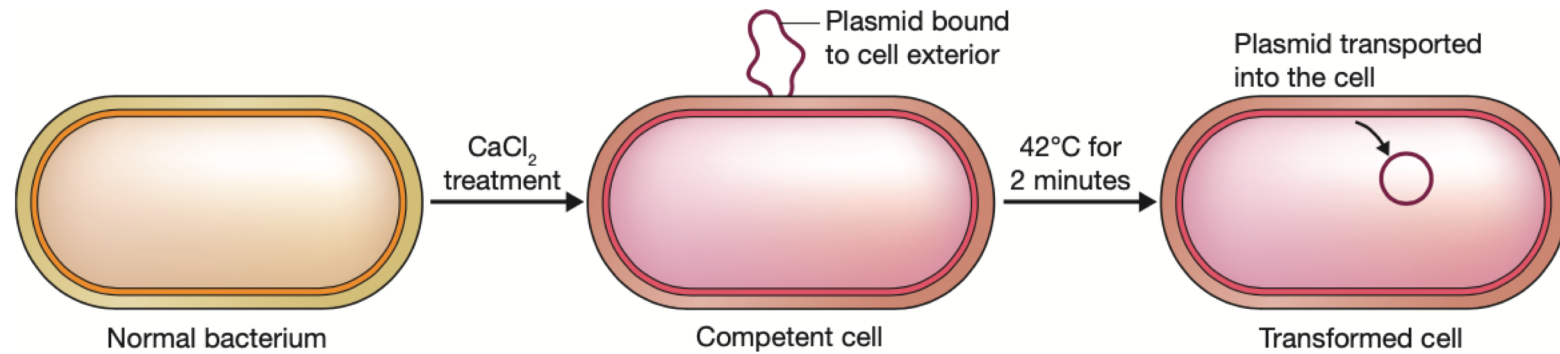
INTRODUCTION OF DNA INTO LIVING CELLS

Cloning can recombinant

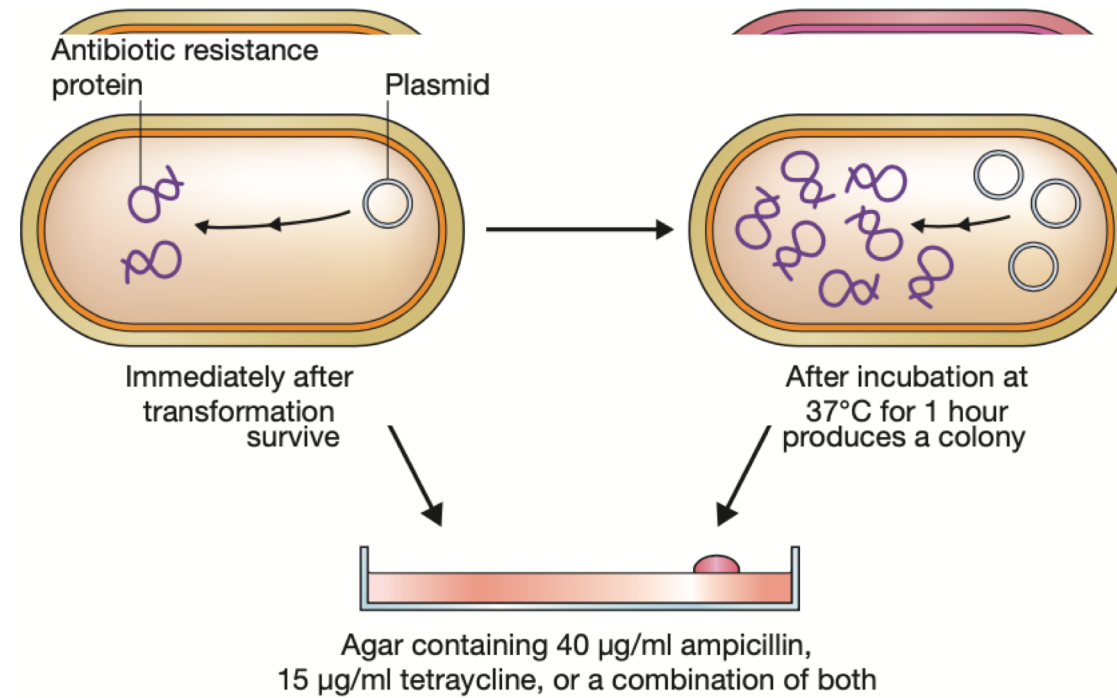
of



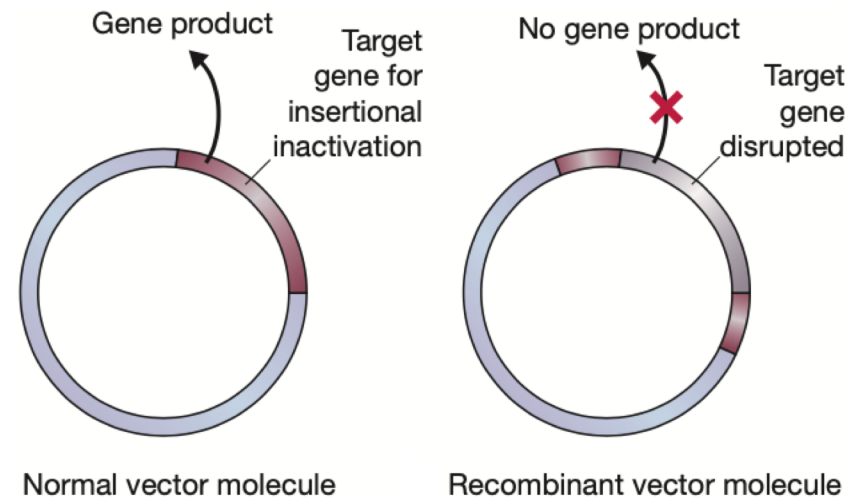
Transformation—the uptake of DNA by bacterial cells



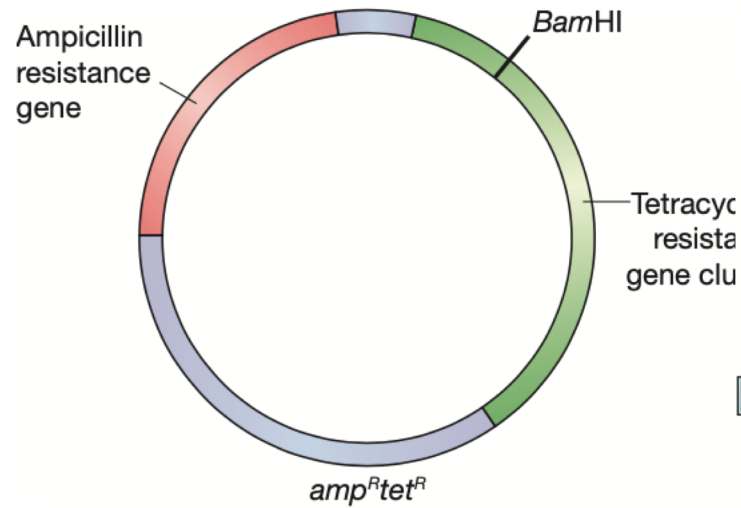
Selection for transformed cells



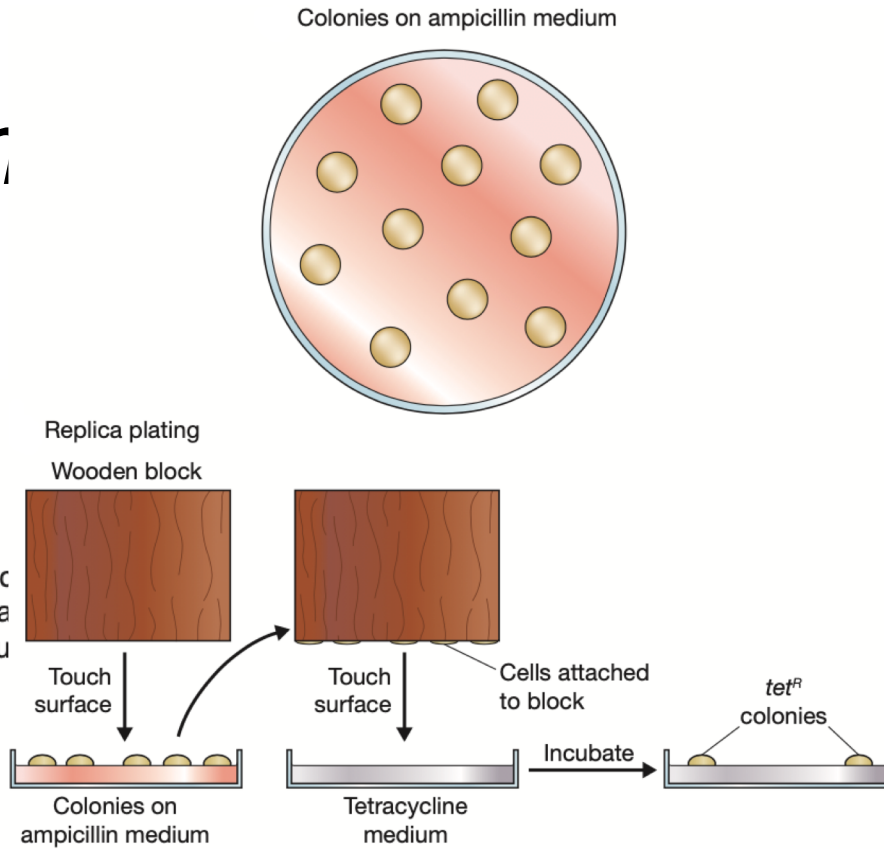
Identification of recombinants



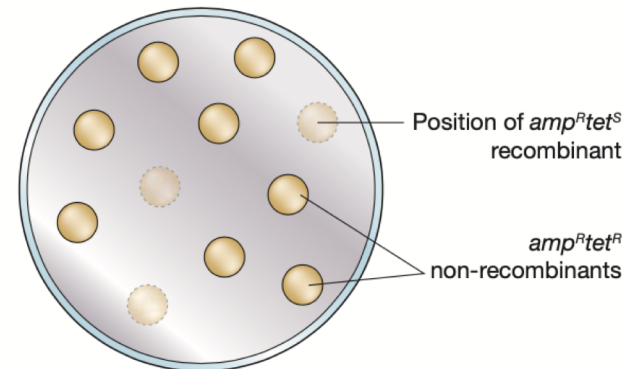
Identification of recombinants



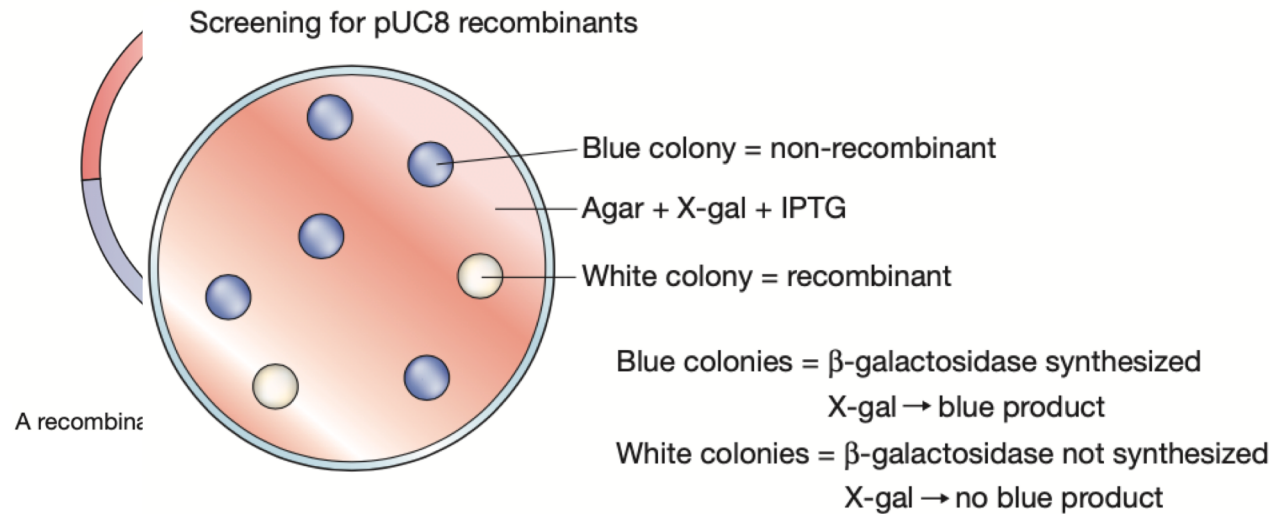
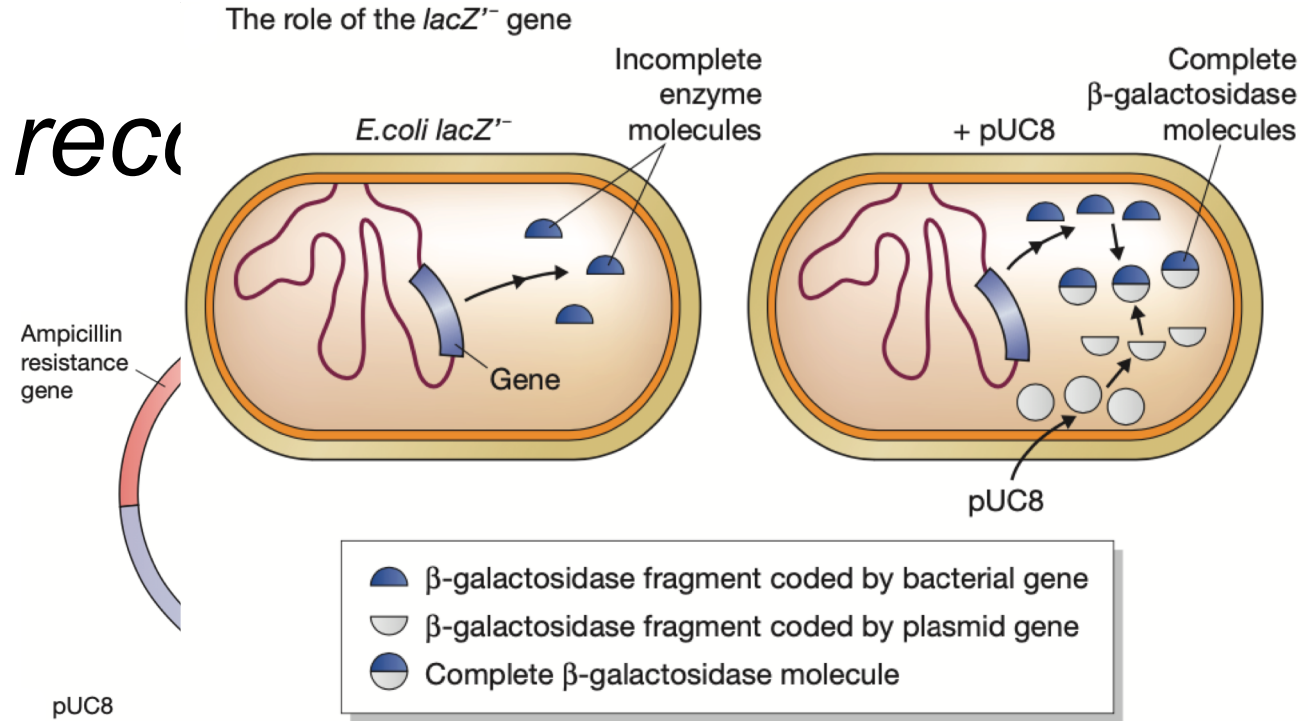
The normal vector molecule



amp^R*tet*^R colonies grow on tetracycline medium



Identification of recombinants



Introduction of phage DNA into bacterial cells

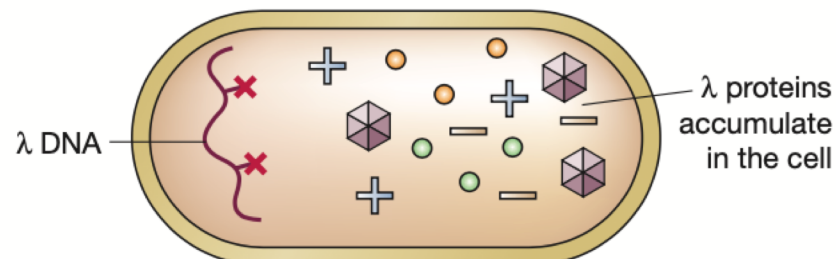
- Transfection

Equivalent to transformation. The purified phage DNA, or recombinant phage molecule, is mixed with competent *E. coli* cells and DNA uptake induced by heat shock.

- In vitro packaging of cloning vectors

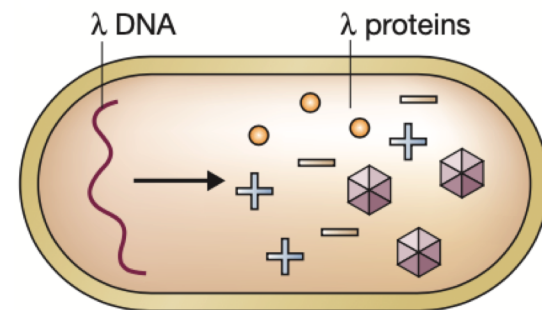
- Cos mutation -> cannot replicate but protein synthesis is on

A single-strain packaging system

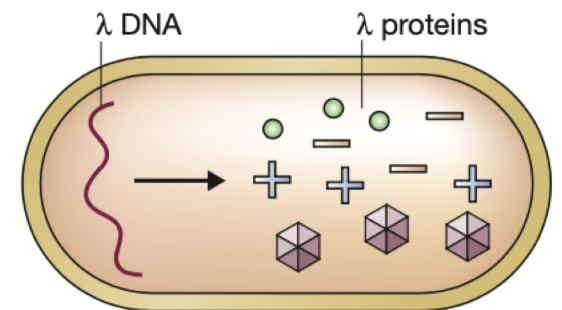


E. coli SMR10 - λ DNA has defective *cos* sites

A two-strain packaging system

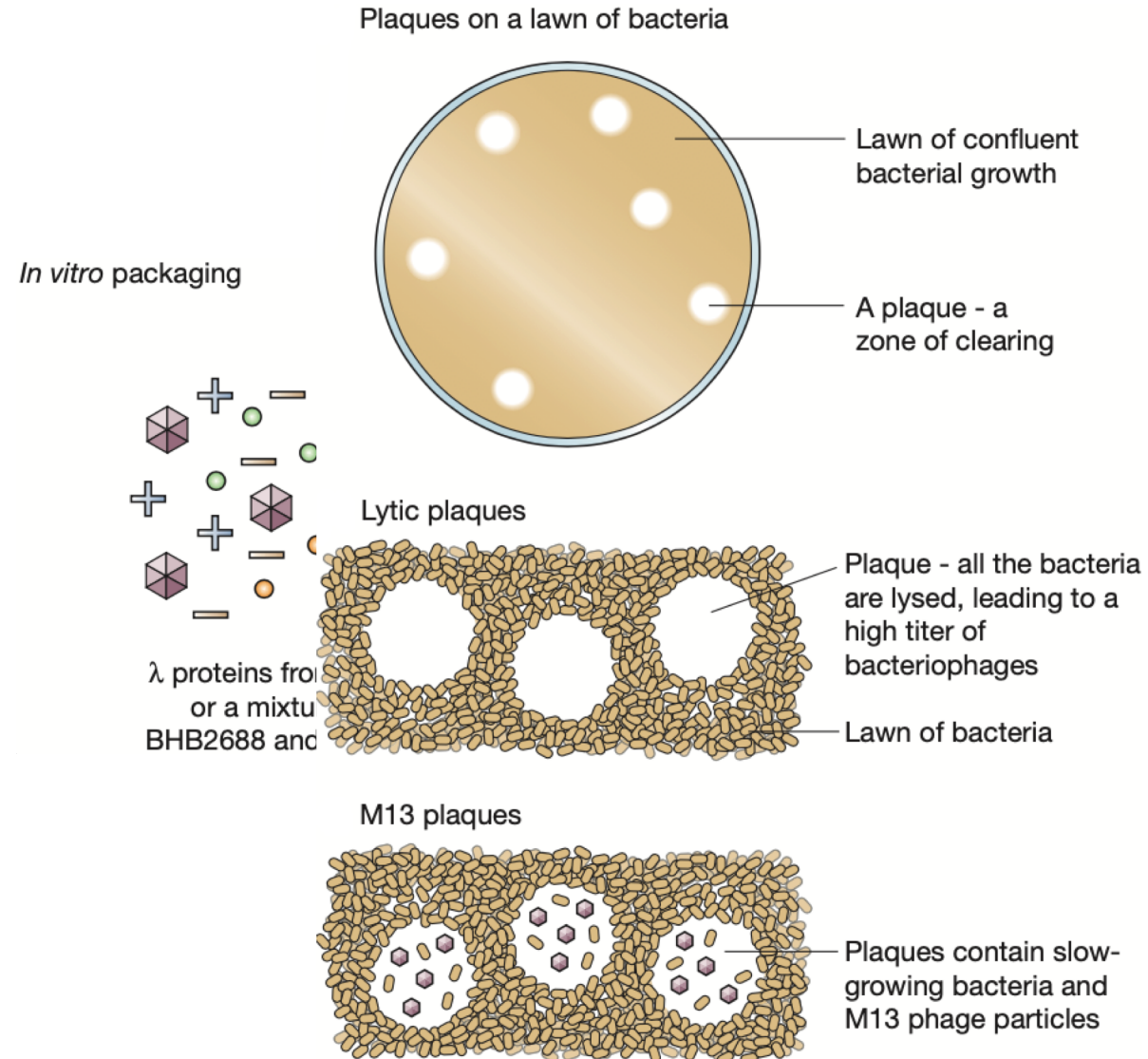


E. coli BHB2688 - λ defective for synthesis of protein E (●)



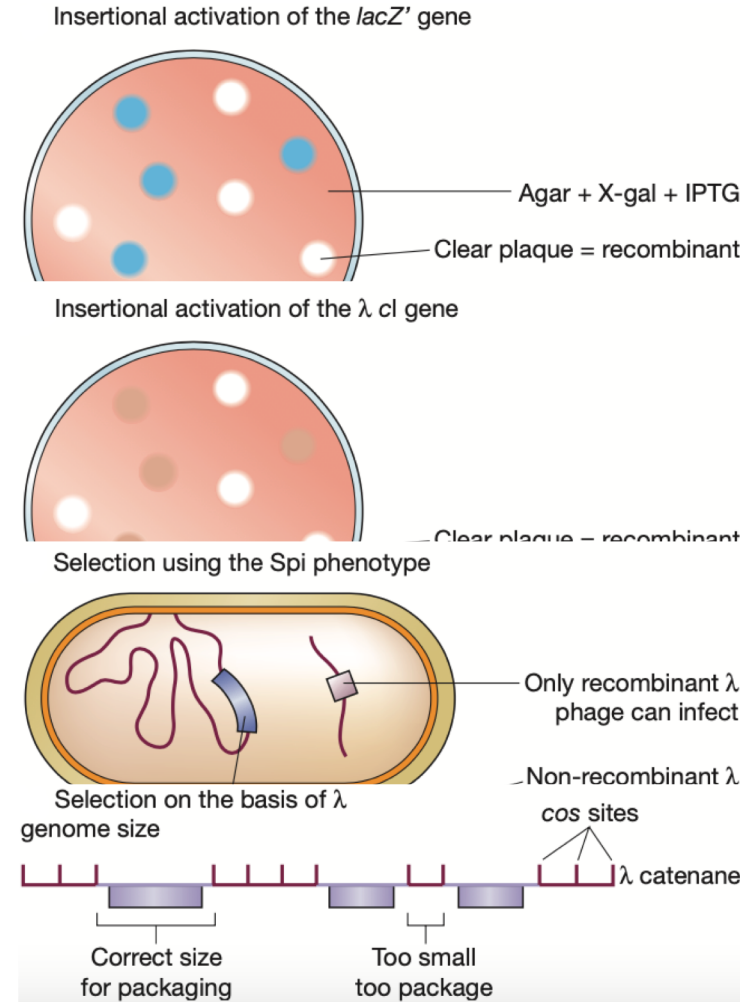
E. coli BHB2690 - λ defective for synthesis of protein D (●)

Introduction of phage DNA into bacterial cells

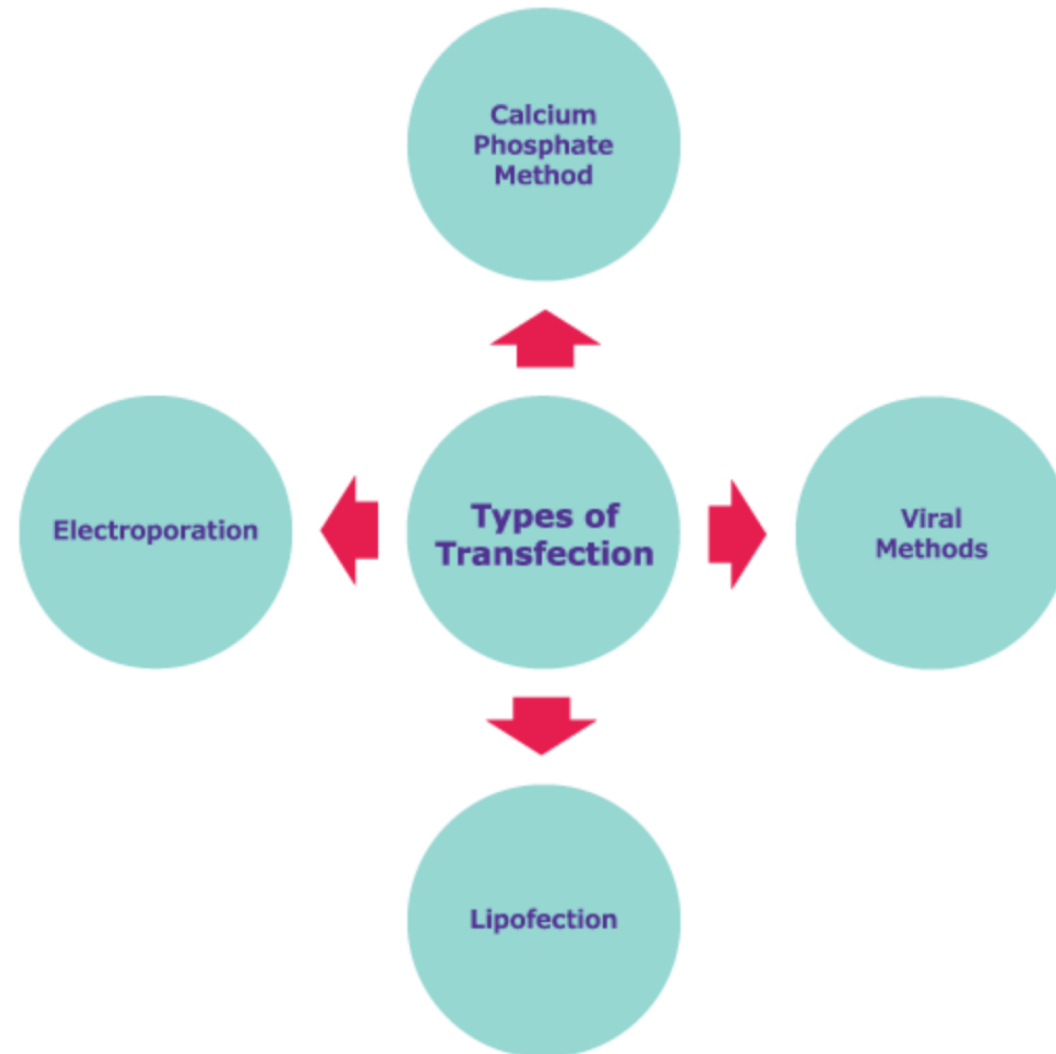


Identification of recombinant phages

- Insertional inactivation of a *lacZ'* gene carried by the phage vector.
- Insertional inactivation of the λ *ci* gene
- Selection using the Spi phenotype
- Selection on the basis of λ genome size

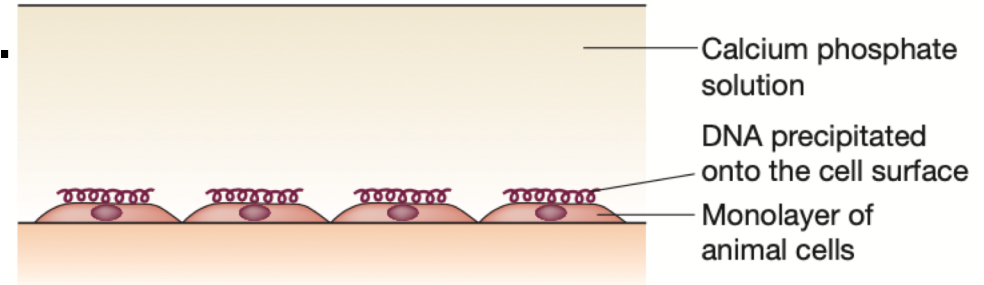


Introduction of DNA into mammalian cells

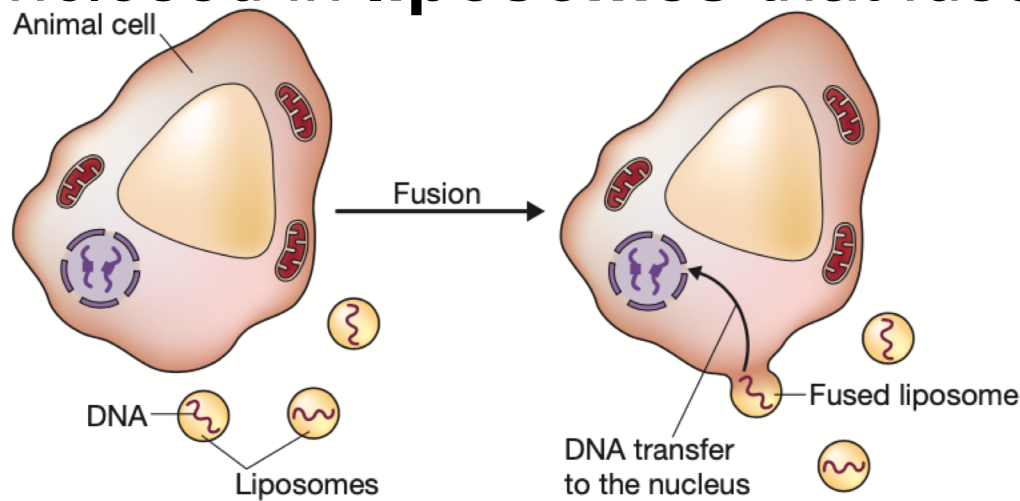


Introduction of DNA into mammalian cells
























- Cultured animal cells, which usually lack cell walls, are easily transformed, especially if the DNA is precipitated onto the cell surface with calcium phosphate.



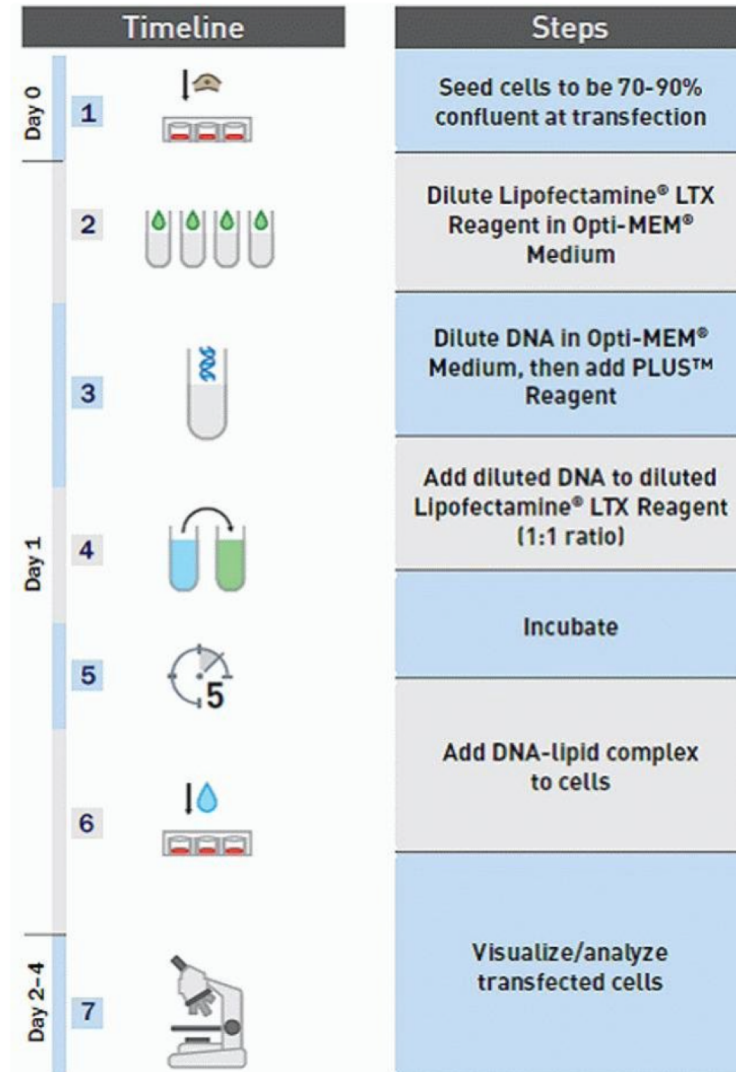
- or enclosed in **liposomes** that fuse with the cell membrane.



Introduction of DNA into mammalian cells

Reagent	DNA	mRNA	RNAi	Co-delivery	CRISPR-Cas9	Cell type(s)	Adherent or suspension
Lipofectamine 2000						Common and easy-to-transfect cell types	Adherent and suspension
Lipofectamine 3000						Workhorse (HeLa) through to hard-to-transfect (cancer cells)	Adherent
Lipofectamine LTX						CHO cells and some primary fibroblast, epithelial and neuronal cells (MEF, HMEC and E18 cells)	Adherent
Lipofectamine RNAiMAX						Established cell lines, hard-to-transfect cells, primary cells, stem cells	Adherent
Lipofectamine MessengerMAX						Neuronal cells, primary cells	Adherent
Lipofectamine CRISPRMAX						Tested in over 20 cell types including iPSC, mESC, N2A, CHO, A549, HCT116, HeLa, HEK293 and several others	Adherent
Neon Electroporation							Suspension
Invivolectamine 3.0						<i>In vivo</i> delivery to liver following tail vein injection	

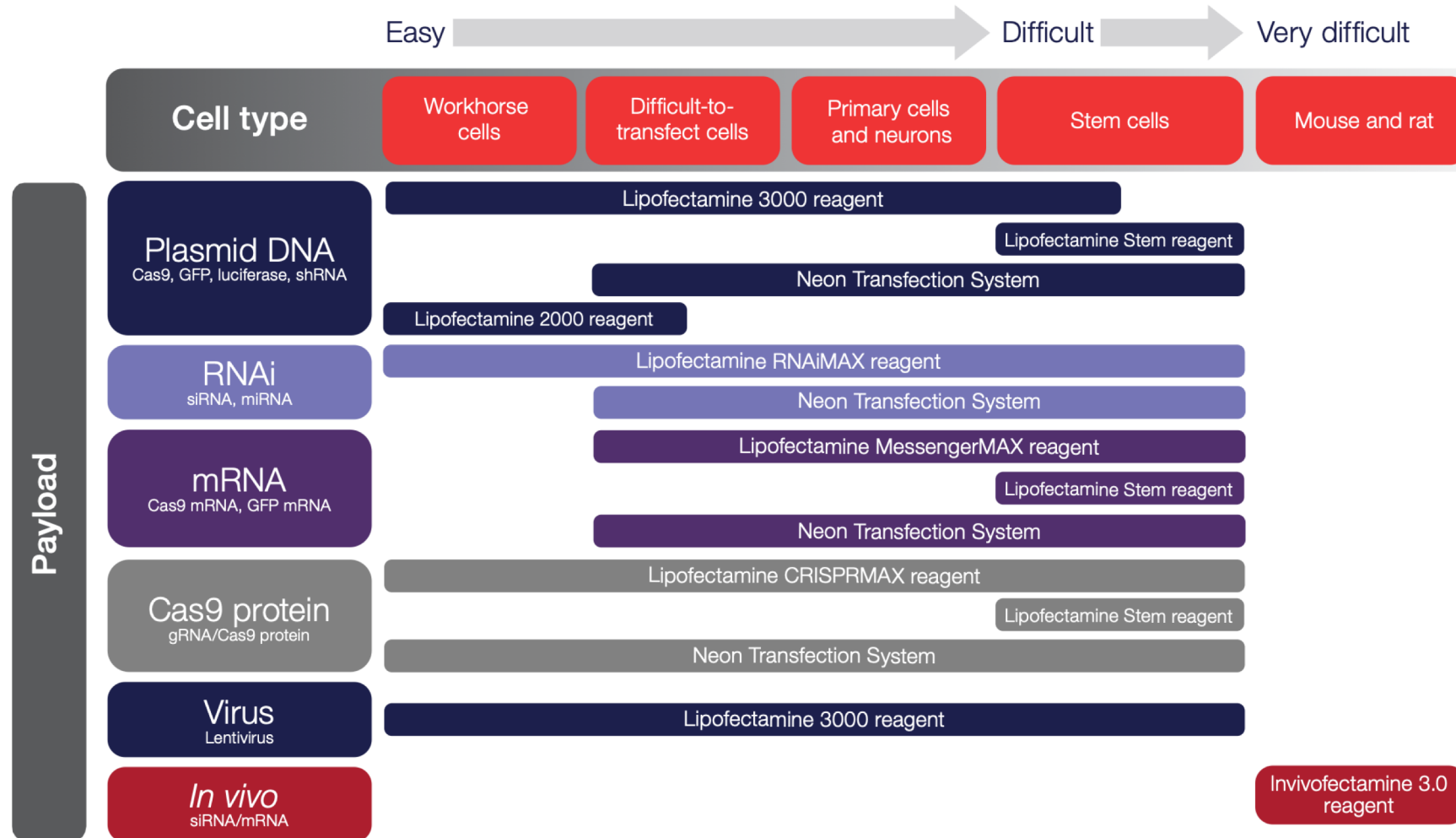
Introduction of DNA into mammalian cells



Lipofectamine® LTX Transfection Workflow

Introduction of DNA into mammalian cells

Decision tree—payload by cell type



Products are listed in order of recommendation for each payload based on effectiveness, ease of use, and cost.

Introduction of DNA into mammalian cells



Introduction of DNA into mammalian cells

Cell type	Species	Cell line
Blood/Immune	Human	BC-1 cells
Blood/Immune	Human	BJAB cells
Blood/Immune	Human	CCRF-CEM cells
Blood/Immune	Human	Dendritic cells
Blood/Immune	Human	HEL 92.1.7
Blood/Immune	Human	HL-60 cells
Blood/Immune	Human	IM-9 cells
Blood/Immune	Human	Jiyoye cells
Blood/Immune	Human	Jurkat cells
Blood/Immune	Human	K-562 cells
Blood/Immune	Human	KG-1 cells
Blood/Immune	Human	LCL cells
Blood/Immune	Human	Macrophage cells
Blood/Immune	Human	NAMALWA cells
Blood/Immune	Human	NK-92
Blood/Immune	Human	PBMC cells
Blood/Immune	Human	Raji cells
Blood/Immune	Human	Ramos cells
Blood/Immune	Human	RPMI8226 cells
Blood/Immune	Human	RS4-11 cells
Blood/Immune	Human	SCID.adh cells
Blood/Immune	Human	SKW6.4 cells
Blood/Immune	Human	T-cells
Blood/Immune	Human	U-937 cells
Blood/Immune	Mouse	BW5147 (T200-A)5.2 cells
Blood/Immune	Mouse	EL4 cells
Blood/Immune	Mouse	M1 cells
Blood/Immune	Mouse	MPC-11 cells
Blood/Immune	Mouse	P815 cells
Blood/Immune	Mouse	Ramos cells
Blood/Immune	Mouse	RAW 264.7 cells
Blood/Immune	Mouse	RBL-2H3 cells

Cell type	Species	Cell line
Connective tissue	Horse	Embryonic dermal fibroblast cells (NBL-6)
Connective tissue	Human	BJ cells
Connective tissue	Human	HOS cells
Connective tissue	Human	HT-1080 cells
Connective tissue	Human	IMR-90 cells
Connective tissue	Human	MH7A cells
Connective tissue	Human	Neonatal dermal fibroblast cells, Gibco
Connective tissue	Human	Saos-2 cells
Connective tissue	Human	U-2 OS cells
Connective tissue	Human	WI-38 cells
Connective tissue	Monkey	COS-7 cells
Connective tissue	Monkey	Vero cells
Connective tissue	Mouse	3T3-L1 cells
Connective tissue	Mouse	Embryonic fibroblast cells (MEF)
Connective tissue	Mouse	L-929 cells
Connective tissue	Mouse	NIH-3T3 cells
Connective tissue	Mouse	PA317 cells
Endothelial cells	Human	Endothelial cells, Gibco
Endothelial cells	Human	HUVEC cells
Endothelial cells	Mouse	b-END.3 cells

Introduction of DNA into mammalian cells

Cell type	Species	Cell line
Epithelial cells	Canine	MDCK cells
Epithelial cells	Hamster	BHK-21 cells
Epithelial cells	Hamster, Chinese	CHO DG44 cells
Epithelial cells	Hamster, Chinese	CHO-K1 cells
Epithelial cells	Human	253J cells
Epithelial cells	Human	293A cells
Epithelial cells	Human	A-431 cells
Epithelial cells	Human	A549 cells
Epithelial cells	Human	ARO cells
Epithelial cells	Human	ARPE-19 cells
Epithelial cells	Human	BEAS-2B cells
Epithelial cells	Human	BT-20 cells
Epithelial cells	Human	BxPC-3 cells
Epithelial cells	Human	C-33 A cells
Epithelial cells	Human	Calu-3 cells
Epithelial cells	Human	ChangX-31 cells
Epithelial cells	Human	COLO 201 cells
Epithelial cells	Human	DU 145 cells
Epithelial cells	Human	FRO cells
Epithelial cells	Human	G-361 cells
Epithelial cells	Human	HCC1937 cells
Epithelial cells	Human	HCT 116 cells
Epithelial cells	Human	HCT15 cells
Epithelial cells	Human	HEK 293 cells
Epithelial cells	Human	HeLa cells (ATCC)
Epithelial cells	Human	Hep G2 cells
Epithelial cells	Human	Hep3B cells
Epithelial cells	Human	HN3 cells
Epithelial cells	Human	Hs-578T cells
Epithelial cells	Human	HT-29 cells

Cell type	Species	Cell line
Epithelial cells	Human	J82 cells
Epithelial cells	Human	LNCaP cells
Epithelial cells	Human	Mammary epithelial cells, Gibco
Epithelial cells	Human	MCF7 cells
Epithelial cells	Human	MCF-ADR cells
Epithelial cells	Human	MDA-MB-231 cells
Epithelial cells	Human	MEWO cells
Epithelial cells	Human	NCI-H23 cells
Epithelial cells	Human	NCI-H69 cells
Epithelial cells	Human	NPA cells
Epithelial cells	Human	PANC-1 cells
Epithelial cells	Human	PC-3 cells
Epithelial cells	Human	RKO cells
Epithelial cells	Human	RT4 cells
Epithelial cells	Human	SK-BR-3 cells
Epithelial cells	Human	SK-HEP-1 cells
Epithelial cells	Human	SK-OV-3 cells
Epithelial cells	Human	SNU-387 cells
Epithelial cells	Human	SW480 cells
Epithelial cells	Human	T24 cells
Epithelial cells	Human	T-47D cells
Epithelial cells	Human	TSU-Pr1 cells
Epithelial cells	Human	WiDr cells
Epithelial cells	Mouse	F9 cells
Epithelial cells	Mouse	P19 cells
Epithelial cells	Rat	GH3 cells
Epithelial cells	Rat	H-4-II-E cells
Epithelial cells	Rat	NRK cells
Epithelial cells	Rat	PC-12 cells

Introduction of DNA into mammalian cells

Cell type	Species	Cell line
iPSC reprogramming	Fibroblast	Fibroblast (iPSC), fibroblast, CD34+
Muscle cells	Human	Aortic smooth muscle cells, Gibco
Muscle cells	Mouse	C2C12 cells
Muscle cells	Rat	Cardiomyocyte cells
Muscle cells	Rat	L6 cells
Neural/Glial cells	Human	SH-SY5Y cells
Neural/Glial cells	Human	SK-N-MC cells
Neural/Glial cells	Human	T98G cells
Neural/Glial cells	Human	U-87 MG cells
Neural/Glial cells	Mouse	Glial cells
Neural/Glial cells	Mouse	GT1-1 cells
Neural/Glial cells	Mouse	GT1-7 cells
Neural/Glial cells	Rat	Astrocyte cells
Neural/Glial cells	Rat	C6 glial cells
Neural/Glial cells	Rat	F-11 cells
Neural/Glial cells	Rat	Glial precursor cells, Gibco
Neural/Glial cells	Rat	HiB5 cells
Neural/Glial cells	Rat	Primary cortical neuron cells, Gibco
Neural/Glial cells	Rat	Primary hippocampal neuron cells, Gibco
Neural/Glial cells	Rat	SCN2.2 cells
Secretory cells	Human	SV40 MES 13 cells
Secretory cells	Human	SW-13 cells
Stem cells	Human	Adipose-derived stem cells (ADSC)
Stem cells	Human	BGO1V embryonic stem cells
Stem cells	Human	H9 embryonic stem cells
Stem cells	Human	Mesenchymal stem cells (hMSC)
Stem cells	Human	Neural stem cells, Gibco
Stem cells	Mouse	Embryonic stem cells
Stem cells	Rat	Neural stem cells, Gibco

Introduction of DNA into mammalian cells

- **Adenoviruses**, up to 8 kb to be cloned.
- **Papillomaviruses**, gives permanently transformed mouse cell line.
- **Adeno-associated virus (AAV)**, integrates into same location on human chromosome 19.
- **Retroviruses**, most commonly used vector in gene therapy.

Gene cloning without a vector

- **Microinjection**

