CBMS331 – Molecular and Medical Biotechnology

Topics;

Toolbox

Genetic Engineering

Biotech pipeline

Protein Secretion

Protein Engineering

Microbes as Factories

Fluorescence Microscopy

Transgenic Plants

Transgenic Animals

Flow Cytometry

Biotech Scale

Microfluidics

Glycomics

Cancer Nanomedicine

Engineering Nanoparticles

Bioinformatics

DNA in Forensics

Biopharmaceuticals

Toolbox

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To make a recombinant protein; genes, promoter, host, vector, cultivation process.

Gene isolation via PCR; Touchdown – cools annealing for best fit. Degenerate – varying primers via conserved motifs. Nested – reamplification via second PCR. RT – rna to cdna. Quantitative – accurate quantification.

New host may not recognise the codons, so modification may be necessary. Identify codons with greatest difference, especially at 3rd position, and figure out how to change them. Orthogonal system is one that doesn't interfere with the host's system.

Reconfiguration checklist;

- Modify or harmonise codon usage
- Assess existing or incorporate new restriction sites for in-frame cloning
- Identify and replace problematic sequence regions (*e.g.* loops and repetitive sequences)
- Identify, and then modify potential PTM[a] sites as required (*e.g.* proteolytic cleavage, N-linked glycosylation)
- Identify and add or replace potential organelle targeting sites (*e.g.* ER retention motif H/KDEL, membrane binding motifs)
- Add a screening or purification tag if desired (*e.g.* GFP, HIS, FLAG[b])
- Fuse to an endogenous highly secreted protein carrier
- Look at the 3D structure if available for potential problem areas (*e.g.* exposed loops)

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Comparison of cloning vectors;

Vector type	Max insert (kb)	Applications	Limitations
Bacterial plasmid (*e.g.* pUC)	6-12	Cloning and sequencing	Small insert size, limited expression of proteins, replication restricted in bacteria, copy number problems
Bacteriophage (linear, *e.g.* lambda)	~25	Gene libraries (genomic and cDNA)	Packaging, host replication
Cosmid (circular)	~35	Cloning of large DNA fragments, genomic and cDNA libraries	Phage packaging, cannot replicate in mammalian cells, restricted protein expression
Bacterial **A**rtificial **C**hromosome (BAC) circular	~300	Cloning of large DNA fragments and genomic libraries	Replication restricted to bacteria, not suitable for protein expression (used for gene cataloguing)
Yeast **A**rtifical **C**hromosome (YAC) circular	~200-1000	Cloning of large DNA fragments and genomic libraries	Restricted to yeast application (centromeres, telomeres)
Ti Vector	Depends on the type of vector	Gene transfer into plants	Main use in plant cells, large size, complicated to manipulate
Shuttle vectors	~25	Can function in two species, *e.g. E. coli* and yeast	Species-specific replication origins (and promoters)

Gateway cloning; allows the gene to be cloned into multiple kinds of backbones without loss of the gene.

Cloning options; using restriction enzymes, in vivo homologous recombination, yeast assembly, Gibson assembly.

A good vector has; effective promoter, secretion signal (optional), flexible multicloning site, terminator, transformation selection marker (antibiotics), transformation into host should be easy.