DNA recombination

Bond between nucleotides: phosphodiester

Restriction endonuclease: cleave DNA on specific sequence

DNA-protein interaction: major groove

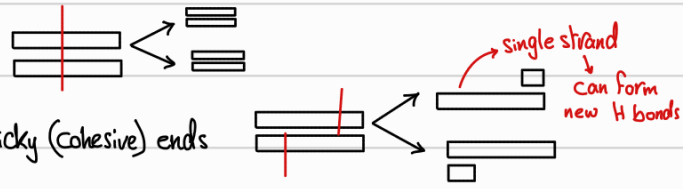
Restriction site: sequence where restriction endonuclease cuts

DNA Ligase: join DNA, ATP dependent

→ 4-8 bp, Palindromic → same sequence on both strands

Cleavage → blunt (sharp) ⇝ cut both strands on the same position

→ staggered (off center) ⇝ on different positions forming sticky (cohesive) ends



DNA recombination ⇝ connect DNA fragment from different sources

→ Consist of the gene of interest and a vector (carrier DNA)

→ Requires Restriction endonuclease + ligase

vector → usually plasmid because it replicates independently

DNA cloning ⇝ DNA recombination → then → insertion into a cell

→ Purposes of cloning: Amplification, Gene expression

Cloning (amplifying) vector

1) own origin of replication (Replicate independently)
2) Antibiotic resistance gene (selectable marker)
3) Restriction site

Expressing vector

- Promoter (initiate transcription)
- Transcription termination sequence
- Shine Dalgarno (SD) sequece (initiate translation)

Challenges: 1) Intron-Exon ⇝ Reverse transcriptase (mature mRNA → cDNA)

2) Many types of RNA ⇝ Poly A tail, Poly T primer

3) Bacteria lacks protein modifications and disulfide bond, causing misfolding and proteins are degraded after synthesis ⇝ Eukaryotic system (yeast)

Recombinant protein: different proteins or domains (→ part of protein (fold, stabilize and function independently)) connected into one protein

Protein tags: allow detection and purification of proteins

→ His tag: 6 His, detected by Nickel and purified using antibodies

→ GFP: 220 A.A, emit green light ⇝ only detection without purification of proteins

Regulation of transcription: 1) Promoter ⇝ initiation of transcription

2) Promoter Proximal Element (PPE)
→ Regulatory sequece near the promoter

3) Enhancer and Silencer ⇝ Orientation and position independent
→ Require binding to Proteins

Analysis of transcription regulation ⇝ we use a reporter gene such as luciferase gene
↳ must be downstream the regulatory sequence

Complete control ⇝ usual promoter (basal activity)
Positive control ⇝ Good promoter (maximum activity)
Negative control ⇝ No promoter (almost zero activity)

Activation ⇝ luciferase signal increases, Inhibition ⇝ Luciferase signal decreases

DNA Library:

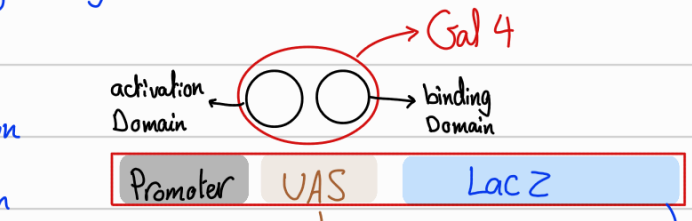
Genomic DNA ⇝ Contain all DNA sequences (coding and non coding)
cDNA ⇝ Only exons (coding = translated and untranslated)

Between individuals ⇝ Both cDNA and genome are different
For the same individual and different tissues ⇝ The same genomic, different cDNA

Detection of protein-protein interaction

1) Immunoprecipitation
uses small beads bound to antibodies for a specific protein
☆ Precipitated proteins interacts with the protein of interest

2) Yeast two-hybrid system
blue → ✓ interaction
white → X interaction



β-galactosidase ↳ break X-Gal emitting blue color

DNA Repair

→ Homology directed repair (homologous chromosome) → accurate
→ Non-Homology end joining repair → X Homologous → Cause indel mutations

CRISPR

⇝ Sequence in bacterial genome, has a role in immunity
↳ Contains parts of viral genome attacked bacteria

Cas9 ⇝ RNA-guided nuclease, gRNA, sgRNA ⇝ guid Cas9 to cut in a specific sequence

Cas9, gRNA system can be used to:

1) Repair mutation → Homology Repair / Bring Deaminase
TA ← ~~CG~~
GC ← ~~AT~~

2) Introduce mutations for researches
3) Introduce new genes
5) dCas9 brings proteins into a sequence, such as → Enzymes / GFP (visualize) / Regulatory proteins

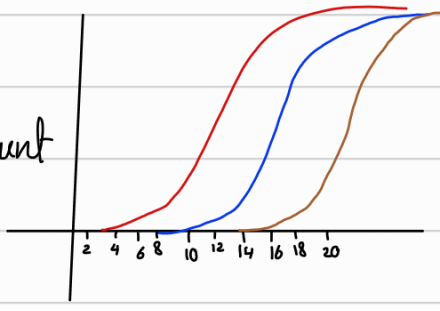
Cas12a → staggered cuts / Cas13a → Cut RNA (ribonuclease)

PCR (Polymerase Chain (many cycles) reaction) ⇝ amplify and purify a specific part of DNA
- Requires: DNA template, Primer, deoxynucleotide, heat stable DNA polymerase → Taq DNA polymerase
- highly sensitive to small amounts of DNA

Steps:
1) Denaturation by Heat
2) Annealing of primer → flank and surround the target sequence
   → The target sequence is flanked completely on the 3 cycle
3) Polymerization

PCR is specific due to
1) Primer specificity
2) high temperatures → prevent non specific pairing

Real Time quantitative PCR (qPCR) ⇝ uses SYBR green
- less threshold (C_T) ⇝ Sooner (earlier) detection ⇝ larger amount



Melting Curve analysis of qPCR ⇝ Ensure specificity
- depends on Tm and light absorbance the produced fragments
- 1 peak → all fragments have the same Tm and Absorbance (identical) → Specific
- many peaks → fragments are not identical → not specific

(the highest peak is for the needed sequence)

Taqman PCR → Ensure specificity
- Most specific, sensetive, Reproducible and multiplexing
- uses Reporter (emit signal) and quencher (inhibit signal)
- The DNA polymerase has 5'→3' exonuclease activity

DNA sequencing

1) Termination methode
   - 4 Reaction mixtures containing DNA template, polymerase, Primer, Deoxynucleotides, dideoxynucleotides ⇝ No OH on 2', 3' C
     - → stop synthesis, can't form phosphodiester bond with next monomer
     - → labeled with radioactive isotope
   - If 2 band on gel electrophoresis has the same position ⇝ Heterozygous gene → mutation → polymorphism

- first eukaryotic genome sequenced → Saccharomyces (yeast)
- first multicellular organism → Nematode Caenohabditis

2) Fluorecent-based DNA sequencing

- all components in the same tube (1 reaction mixture)
- each dideoxynucleotide is labeled by a fluorecent signal with a certain color
- Computer program converts the colors into waves

IF 2 waves present in the same position ⇝ Heterozygous

3) Next generation sequencing ⇝ fastest and most efficient

- DNA is fragmented, DNA adaptors attached to the ends of fragments
- All fragments will have the same adaptor and anneal to the same primer
- we use special nucleotides which must be modified, activated and produce signal to bind the next nucleotide

Analysis of gene expression ⇝ according to the amount of mRNA

All methods require the conversion of mRNA into cDNA (by reverse transcriptase) and double (by DNA polymerase)

1) Quantitative reverse transcriptase

real time qPCR of mRNA

☆ qPCR on cDNA

☆ earlier detection, more cDNA more mRNA, more expression

Housekeeping gene: gene expressed constantly in all cells

→ used to ensure that the sample is taken properly

☆ SARS-Co2 test uses this method detect a viral gene and an Internal Control (human gene)

2) DNA microarray

a slide containing many spots

each spot contains many copies for a specific known gene

cDNA is labeled by radioactive isotope or colored fluorescence for comparison

Upregulated: activated gene

Downregulated: inhibited gene

3) RNA sequencing

☆ Detect the amount and sequence of RNA

☆ Can detect all transcripts

- Known mRNA
- novel mRNA
- splicing variants

Analysis of protein sample ⇝ Polyacrylamide Gel electrophoresis

Analysis of DNA sample ⇝ Agarose Gel electrophoresis