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CORE JOBS · INTERVIEW QUESTIONS

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# Recombinant DNA Technology and Tools Interview Questions

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Online, with links to the notes: **iaeacademy.in/study/recombinant-dna-technology/interview-questions**


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## Restriction enzymes, cloning vectors and expression vectors

### 1. Why do most Type II restriction enzymes recognise palindromic sites, and what kinds of ends can they leave?

Most Type II enzymes work as homodimers. Each identical subunit recognises one half of the site and cuts one strand, so the site has twofold symmetry and reads the same 5'→3' on both strands. They cut inside the site. Depending on where the two strand cuts fall, the ends are 5' overhangs, 3' overhangs or blunt.

### 2. A linear DNA of 20,480 bp, random in sequence with 50% GC, is cut with an enzyme that recognises a 6-bp palindromic site. How many fragments are expected?

About 6 fragments. In random DNA with 50% GC, a given 6-bp site occurs on average once every $4^6 = 4096$ bp, so 20,480 bp holds about 5 sites. Cutting a linear molecule at 5 sites gives 6 fragments; a circular molecule would give 5.

**WORKING**

1. Expected spacing of a 6-bp site in random 50% GC DNA: $4^6 = 4096$ bp.
2. Expected number of sites: $20480/4096 = 5$.
3. A linear molecule with $n$ sites gives $n + 1$ fragments: $5 + 1 = 6$.

### 3. What is the difference between a plasmid vector and a BAC vector in insert size and use?

A plasmid carries inserts up to about 10 kb and suits single genes or cDNAs. A BAC, based on the F plasmid and kept at one copy per cell, carries about 100 to 300 kb, so it suits large genomic fragments and genome libraries.

### 4. Why does an expression vector need more elements than a cloning vector?

A cloning vector only has to replicate and carry the insert. To make protein, the vector also needs a promoter for transcription, a ribosome-binding site (a Kozak sequence in eukaryotes), a start codon in frame and a terminator. A tag is often added for purification. Without these, the gene is copied with the vector but not expressed.

## DNA libraries and gene cloning

### 5. How many clones are needed for a 99% probability that a given gene is present in a genomic library of a 4.6 Mb bacterial genome, cloned with an average insert of 23 kb?

About 919 clones. Each clone holds a fraction $f = 23/4600 = 0.005$ of the genome. For probability $P = 0.99$, $N = \ln(1 - P)/\ln(1 - f) = \ln 0.01/\ln 0.995 \approx 919$. The shortcut $N \approx \ln(1 - P)/(-f) \approx 921$ is close because $f$ is small.

**WORKING**

1. Fraction of the genome in one clone: $f = 23/4600 = 0.005$.
2. Use $N = \ln(1 - P)/\ln(1 - f)$ with $P = 0.99$.
3. $\ln(0.01) = -4.605$ and $\ln(0.995) = -0.005013$.
4. $N = 4.605/0.005013 \approx 919$.

### 6. What is the difference between a genomic library and a cDNA library?

A genomic library is made from fragmented genomic DNA, so it holds introns, regulatory regions and non-coding DNA, and is the same in every tissue. A cDNA library is made from poly(A)+ mRNA by reverse transcription, so it holds only expressed, intron-free sequences and depends on tissue and time.

### 7. Why is genomic DNA partially digested, not fully digested, when making a genomic library?

Partial digestion gives large, overlapping fragments that can be sized to the vector's capacity. Complete digestion would cut at every site, giving fragments of a fixed pattern that may split a gene, with no overlaps to join them. Overlaps let the clones cover the genome without gaps.

### 8. How is a DNA library screened by hybridization?

Colonies or plaques are lifted onto a membrane, and the DNA is denatured and fixed. The membrane is incubated with a labelled probe complementary to the gene. Signal marks the positive clones, which are picked from the master plate. This needs some known sequence to design the probe.

## PCR, sequencing, blotting and DNA fingerprinting

### 9. Why does PCR use a heat-stable DNA polymerase?

Each cycle heats the mixture to about 94 to 95 °C to separate the strands. An ordinary polymerase would denature at this temperature, so fresh enzyme would be needed after every cycle. A polymerase from a thermophile, such as Taq, stays active through all cycles.

### 10. A PCR starts with 5 copies of the target and runs for 20 cycles. Taking the product to double in every cycle, how many copies are present at the end?

5,242,880 copies, about $5.2 \times 10^6$. With perfect doubling, $N = N_0 \times 2^n = 5 \times 2^{20} = 5 \times 1{,}048{,}576$. Real reactions give less, because efficiency falls below 100% and the reaction plateaus as primers and dNTPs run down.

**WORKING**

1. Ideal amplification: $N = N_0 \times 2^n$.
2. $2^{20} = 1{,}048{,}576$.
3. $N = 5 \times 1{,}048{,}576 = 5{,}242{,}880$.

### 11. What is the difference between RT-PCR and real-time PCR (qPCR)?

RT-PCR starts from RNA: reverse transcriptase makes cDNA, which is then amplified. Real-time PCR follows the product by fluorescence in every cycle, so it measures how much template was there at the start. The two combine as RT-qPCR to quantify an mRNA.

### 12. How does chain-termination sequencing read a DNA sequence?

A polymerase extends a primer in the presence of normal dNTPs and a small amount of dideoxynucleotides, which lack a 3'-OH and stop the chain. This makes fragments ending at every position of each base. Capillary electrophoresis orders them by size, and a different fluorescent label on each ddNTP gives the base.

## Mutagenesis, CRISPR-Cas, biosensors and interaction tools

### 13. How does CRISPR-Cas9 find and cut its target in DNA?

The guide RNA pairs with about 20 nucleotides of target DNA that lie next to a PAM (NGG for the commonly used Cas9). The HNH domain cuts the strand paired with the guide and the RuvC domain cuts the other strand. The result is a blunt double-strand break a few base pairs from the PAM.

### 14. What is the difference between NHEJ and HDR in repairing a Cas9-induced break?

NHEJ rejoins the ends without a template and often adds or deletes bases, so it is used to disrupt a gene. HDR copies a supplied donor template with matching arms, so it can make a precise change. HDR is mostly active in S and G2 phase and is less efficient than NHEJ.

### 15. Why is the enzyme DpnI used after whole-plasmid PCR mutagenesis?

DpnI cuts GATC sites only when the adenine is methylated, fully or on one strand. The template plasmid, isolated from a dam+ E. coli strain, is methylated, while the PCR product made in vitro is not. Digestion removes the parental template and leaves the mutated copies, so most transformed colonies carry the mutation.

## Microscopy and spectroscopy

### 16. What sets the resolution limit of a light microscope?

Resolution is set by the wavelength and the numerical aperture of the objective: $d = 0.61\lambda/\mathrm{NA}$. A shorter wavelength or a higher NA resolves finer detail. For visible light and an oil objective the limit is about 0.2 µm, and magnifying further adds no detail.

### 17. A DNA solution is diluted 1 in 20 and read in a 1 cm cuvette, giving A260 = 0.24. For double-stranded DNA, A260 of 1.0 corresponds to 50 µg/mL. What is the concentration of the original solution?

About 240 µg/mL, taking the sample as pure double-stranded DNA. For a 1 cm path, concentration is $A_{260} \times 50$ µg/mL, so the diluted sample holds $0.24 \times 50 = 12$ µg/mL. Undoing the 1 in 20 dilution gives $12 \times 20 = 240$ µg/mL.

> **WORKING**
>
> 1. Use $A = \varepsilon c l$ in the form: concentration = $A_{260} \times 50$ µg/mL for a 1 cm path.
> 2. Diluted sample: $0.24 \times 50 = 12$ µg/mL.
> 3. Undo the 1 in 20 dilution: $12 \times 20 = 240$ µg/mL.

### 18. Why can an electron microscope resolve much finer detail than a light microscope?

Electrons accelerated at high voltage have a wavelength of picometres, far below that of visible light, so the diffraction limit is much smaller. In practice a TEM resolves about 0.1 to 0.2 nm, limited by lens aberrations. The sample must be in vacuum, so living material cannot be viewed.

## Electrophoresis and assays

### 19. Why does SDS-PAGE separate proteins by mass?

SDS unfolds the protein and binds it in a roughly constant amount per unit mass, giving every protein a similar negative charge per unit mass. A reducing agent breaks disulfide bonds. The charge-to-mass ratio is then about equal, so the gel sieves by size and smaller proteins move faster.

### 20. A crude extract has 4000 units of an enzyme in 800 mg of protein. After a column step, the pooled fractions have 2400 units in 24 mg of protein. What is the purification fold?

The purification is 20-fold. Specific activity is units per mg of protein: $4000/800 = 5$ U/mg in the crude extract and $2400/24 = 100$ U/mg after the column. Fold purification is their ratio, $100/5 = 20$. The yield of this step is 2400/4000, or 60%.

> **WORKING**
>
> 1. Specific activity of the crude extract: $4000/800 = 5$ U/mg.
> 2. Specific activity after the column: $2400/24 = 100$ U/mg.
> 3. Fold purification = $100/5 = 20$.

### 21. What is the difference between a direct ELISA and a sandwich ELISA?

In a direct ELISA the antigen is coated on the plate and an enzyme-linked antibody detects it, so other proteins in a crude sample compete for the surface. In a sandwich ELISA a capture antibody binds the antigen from the sample and a second, enzyme-linked antibody binds another epitope. Needing two antibodies to bind makes the sandwich format more specific.

## Bioinformatics

### 22. What is the difference between global and local sequence alignment?

Global alignment matches two sequences end to end and suits sequences that are similar over their whole length. Local alignment finds the best-matching segments only, so it suits divergent sequences or ones that share a single domain. Both are computed by dynamic programming.

### 23. Why are substitution matrices such as BLOSUM used to score protein alignments instead of simple identity?

Amino-acid replacements are not equally likely: leucine to isoleucine is tolerated far more often than leucine to aspartate. A matrix scores each pair by observed log-odds, giving positive scores to conservative changes. This lets an alignment detect distant relatives that share few identical residues.

### 24. What does the E-value in a BLAST result mean?

The E-value is the number of hits with an equal or better score that would be expected by chance in a database of that size. A smaller value means a more significant match. It depends on database size, so the same score gives a larger E-value in a larger database.

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