PCR evidence

Molecular cloning

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Learning outcomes and session plan

Distinguish selection, screening and sequence verification

Interpret colony PCR and restriction-fragment sizes

Read a short Sanger trace and evaluate an ambiguous base

Choose a justified next verification step

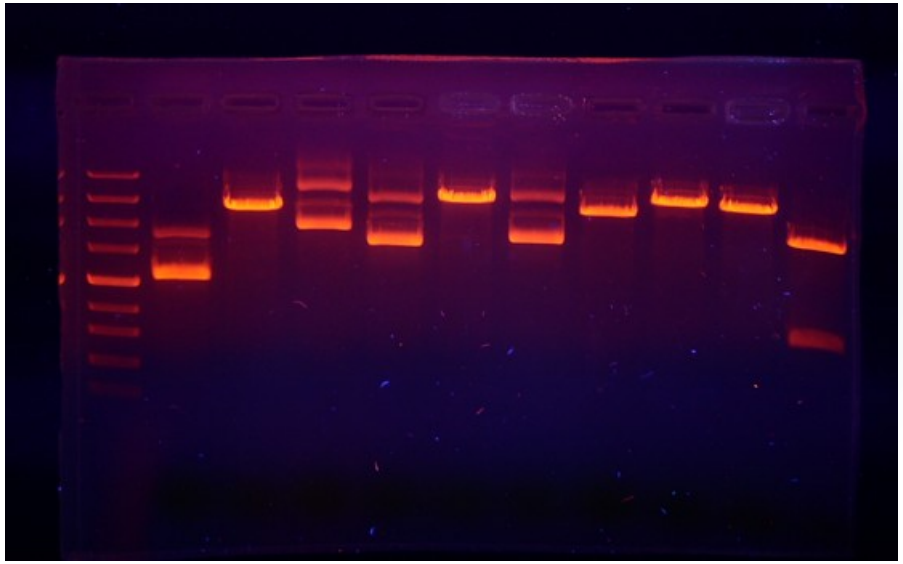
A positive PCR result leaves questions open

Expected-size amplicon: evidence of an amplification product

qPCR signal: evidence interpreted with assay controls

Sequence verification: base order in the region examined

Function requires a separate biological test



Illustrative gel photograph
Band position gives size evidence

The reporter construct

Vector backbone: 3,000 bp

Insert: 900 bp

Expected assembled plasmid: 3,900 bp

Required features: insert identity, orientation and junctions

Selection, screening and verification

A screening example

Colony colour can help select candidates in a suitable blue-white screening system.

The intended sequence still needs verification.

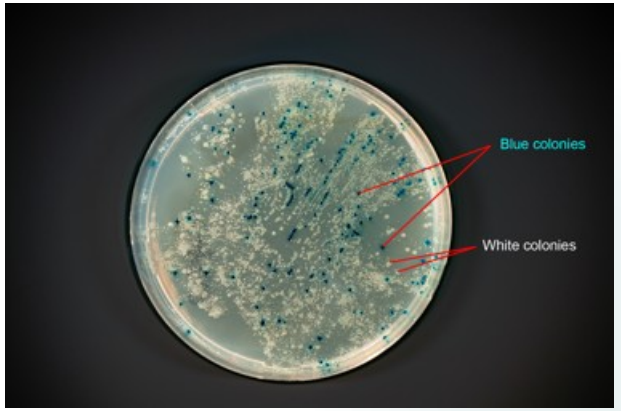


Photo: GoldBio. Screening example.

Stage	Main question	Main limitation
Selection	Which cells grow?	Empty vector may also grow
Screening	Which candidates fit a pattern?	Size does not resolve every base
Verification	Does sequence match the design?	Only reliable covered regions tested

Cloning decisions and controls

Insert and vector ends must be compatible

Restriction enzymes cut defined recognition sites

Ligase joins suitable DNA ends

Transformation introduces DNA into cells

Primer positions determine the question

Primer arrangement	Useful evidence
Both within insert	An internal insert region is present
Vector primers flanking insert	Insert-dependent product size
One vector + one insert primer	A designed junction/orientation test

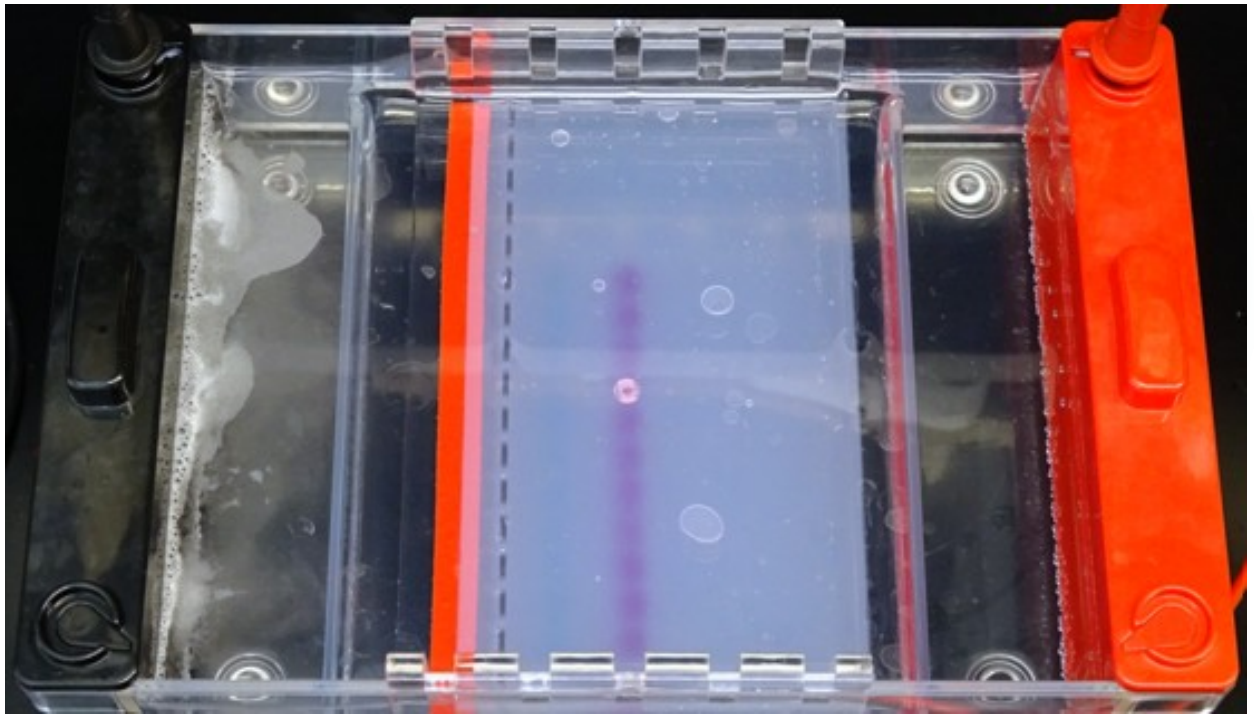
Expected colony-PCR sizes

Empty-vector flanking amplicon: 200 bp

Inserted sequence: 900 bp

Expected recombinant amplicon: 1,100 bp

Prediction assumes intact primer sites and the stated design



Loading samples for gel analysis

Photo: Addgene. Illustrative laboratory step.

Colony-PCR controls

Reaction	Expected result in this model
Known recombinant control	1,100 bp
Empty-vector control	200 bp
No-template control	No band
Unknown colony	Interpret only after controls

Case A: which colonies progress?

Teaching dataset. Products come from flanking vector primers.
Choose candidates for further verification. Explain every rejection or repeat.

Colony	Observed product(s)
A	1,100 bp
B	200 bp
C	No band
D	200 bp and 1,100 bp
E	800 bp

Orientation and junction evidence

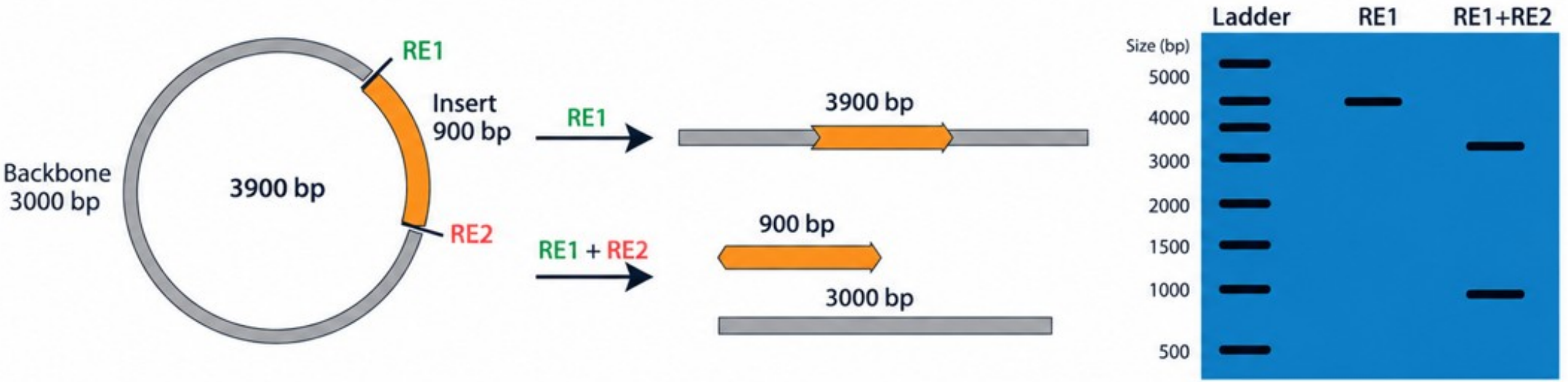
Flanking PCR can give 1,100 bp in either orientation

A vector–insert primer pair can discriminate a designed orientation

Both junctions need appropriate evidence

Absence of amplification needs a working control

Restriction analysis as independent evidence



Teaching example: 3,900 bp plasmid

Defined flanking cuts release 3,000 bp + 900 bp

A single unique cut linearizes a 3,900 bp plasmid

Uncut plasmid forms migrate differently

Expected fragments depend on the actual restriction map

Case B: the verification decision

All digest reactions have a working control
Teaching dataset. Sizes are approximate gel estimates.
Which clone is a candidate for sequencing?

Clone	Digest fragments
P	3,000 bp + 900 bp
Q	3,000 bp only
R	Several unexpected bands
S	3,000 bp + 600 bp