

Restriction Enzyme (RE) Digest

Calculating reaction volumes

Composition of a typical digest reaction: 1. DI H₂O 2. enzyme buffer # 3. BSA*, 4. DNA samples to be digested, 5. RE amount specific to enzyme/desired activity
* not always required.

Add reagents in sequence 1-->2->3->4->5 (esp. 5 last...)

Example reaction:

DI H₂O (43 µl), 10X buffer (5 µl, final dilution to 1 X), pBR322 DNA substrate, (1 µg/1 µl, w/ 1 µl added) ApaI enzyme (10 units/ µl, 1 µl added)

Note: units of enzyme to substrate is 10:1. A unit is a kinetic value of the rate of the enzymes activity and 10:1 is common (but overall digestion is a function of many variables such as T^o, reaction volume, agitation, substrate quality, etc...)

Add reaction components

DI H₂O is RNA and DNAase free. Recall buffer is 10X (or other conc.)

Plasmids are often used as standard for assaying enzyme and enzymes are added to glycerol to help avoid total freezing.

Mix and incubate for 1 hour ApaI palidrome FYI.

Results- Electrophoresis

Gel image discussion: 6 BP palidrome cuts DNA every 3-4 K

8BP cut every _____ (do the math!)

Some general enzyme factoids: Enzymes can cut blunt, or with overhanging (AKA sticky ends) that have 3' or 5' overhang.)

Enzymes stopped with chelating reagent to block out Mg⁺⁺ (cofactor for EN)

Double digestion

Can be done in tandem (if both enzymes will work with a given buffer.)

Question: Explain how double digestion could result in fewer bands than individual enzyme digest?

Or one after another...if enzymes will not work under similar conditions.

Rare Cutting Enzymes: Will give very large fragments (65K) and are used for fragments for viral cloning.

Frequent Cutting Enzymes: Every 3-4 K and are used for fragments for plasmid constructs and subsequent bacterial cloning.

STAR Activity: Undesirable off-target cutting. Occurs at low ionic strength, high pH, organic solvents, high enzyme concentration, high glycerol and other divalent ions.

Try to think about why aforementioned will cause STAR.

Note glycerol is @ 50 % concentration in vial and must be diluted to 10 % or less to avoid STAR activity.

DNA Methylation: Add methyl groups to DNA. Will inhibit EN activity (so that EN will not act on bacteria's own DNA).....