



## NEWSLETTER

### Site Directed Mutagenesis (SDM)



Site directed mutagenesis is a technique for studying protein structure and function.

Changes in our genes (DNA) are called mutations. A mutation in the DNA sequence of an organism, while often harmless, may cause genetic diseases or cancer.

Site directed mutagenesis is a controlled process that deliberately creates specific mutations in a gene in order to study the effects on the associated protein. This mutation in the DNA causes a mutation in mRNA, which leads to a mutated protein. The scientist now can study the mutated protein to see how the change in sequence affects its biological activity.

Gene: a segment of DNA that's transcribed into RNA

Mutation: a change in form, quality or some other characteristic

Genesis: the beginning of a process

Mutagenesis: a permanent transmissible change in the genetic material, usually in a single gene

Transcription: DNA is the template for the synthesis of RNA

Translation: mRNA is the template for the synthesis of protein (this occurs in the ribosome)

Step	Protocol	Thermo Electron Instrument	Disposable
1	<b>Lyse</b> cells	<b>French Press (FA-080A, FA081A)</b>	French press cells (FA-032 standard and FA-003 mini), Rapid Fill Kit (FA-020, FA-021)
2	<b>Purify</b> mRNA from the desired cells	King Fisher (54000500)	Tubes/tip combs (97002090), pipettes, tips
3	Reverse <b>transcribe</b> mRNA to copy DNA (cDNA)	Thermal Cyclers; PCR Sprint, PxE, Px2 or MBS	PCR tubes (HBTC3372N), microplates (HBTC6002N), tape (HBTDXTAPE100), pipettes, tips
4	<b>Amplify</b> the cDNA using appropriate primers		
5	Introduce specific change or <b>mutate</b> the DNA sequence to the PCR product	Thermal Cycler	PCR tubes (HBTC3372N), microplates (HBTC6002N), tape (HBTDXTAPE100), pipettes, tips
6	<b>Analyze</b> the mutated cDNA with gel electrophoresis	Horizontal gel box (MiniCell EC320, MidiCell EC330, Maxicell EC340) Power supply (EC105)	Casting tray, gel tray end dams, combs, pipettes, pipettes, tips
7	<b>Purify</b> PCR product	King Fisher mL (5400050)	Tubes/tip combs (97002090), pipettes, tips
8	<b>Measure</b> spectrophotometrically the DNA concentration	Multiskan Spectrum (51118550)	UV microplates-cat. # 8404
9	<b>Insert</b> the mutated cDNA into a plasmid (a circular piece of DNA)	Water-bath/incubator	
10	<b>Transform</b> competent <i>E. coli</i> cells using electroporation (a technique that enables entry of plasmids into cells)	Electroporator; Celljetc Uno (EPEJ004), Celljetc Pro (EPEJ003), Celljetc Duo (EPEJ004)	Cuvettes (For <i>E. coli</i> , use 2mm electroporation cuvettes, cat. # EPECU102)
11	<b>Grow</b> the transformed <i>E. coli</i> cells overnight. Only those cells containing the plasmid will grow	Orbital shaking incubator (Model 420)	
12	<b>Lyse</b> the culture cells <b>amplify</b> plasmid DNA	French Press (FA-080A, FA081A)	French press cells (FA-032 standard and FA-003 mini), Rapid Fill Kit (FA-020, FA-021)
13	<b>Amplify</b> plasmid DNA	Thermal cycler	PCR test tubes (HBTC3372N),

			microplates (HBTC6002N), tape (HBTDXTAPE100), pipettes, tips
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Step	Protocol	Thermo Electron Instrument	Disposable
14	<b>Analyze</b> PCR product by agarose gel electrophoresis	Horizontal gel box (MiniCell EC320, MidiCell EC330, Maxicell EC340) Power supply (EC105)	Pipettes, tips
15	<b>Sequence</b> PCR product to confirm mutation	Thermal cycler, DNA sequencer	PCR tubes (HBTC3372N), microplates (HBTC6002N), tape (HBTDXTAPE100), pipettes, tips
16	<b>Grow</b> the transformed colonies in broth containing ampicillin. The mutated protein will be expressed.	Orbital shaking incubator (Model 420)	
17	<b>Harvest</b> cells by centrifugation	Centrifuge; Multi/Multi RF (8466,8464-110V)	Rotors, adaptors
18	<b>Lyse</b> cells	French Press (FA-080A, FA081A)	French press cells (FA-032 standard and FA-003 mini), Rapid Fill Kit (FA-020, FA-021)
19	<b>Purify</b> the mutant protein and analyze by vertical gel electrophoresis	Gel Filtration Vertical Gel box (EC175), Power supply (EC1755 / EC1750)	Pipettes, tips
20	<b>Determine</b> protein concentration	Multiskan Spectrum (51118550)	UV microplates # 8404
21	<b>Detect</b> mutant protein for function with ELISA	Fluoroskan Ascent (5210470)	Black microplates , pipettes, tips



## STEP 1: PREPARATION OF CELL LYSATE USING FRENCH PRESS

### Examples of lysis buffers:

Non-denaturing lysis buffer for protein extraction:

- 50 mM Tris-HCl, pH 7.5
- 50 mM NaCl (may vary with applications)
- 1 mM MgCl<sub>2</sub>
- 5 mM DTT
- 1 mM PMSF (phenylmethanesulfonyl fluoride – protease inhibitor)
- 1 mg/ml DNase in H<sub>2</sub>O

2. Lysis buffer for recombinant protein extraction:

- 20 mM Tris-HCl, pH 7.9
- 500 mM NaCl
- 5 mM imidazole

3. Lysis buffer for RNA extraction:

- 140mM NaCl,
- 1.5mM MgCl<sub>2</sub>,
- 10mM Tris-HCl (pH 8.6)
- 0.5% NP4O
- 1mM DTT
- 1000 units/ml RNasein



# Procedure:

- Pellet cells by centrifugation at 200 x g
- Decant supernatant
- Add 200  $\mu$ l of chilled lysis buffer
- Transfer sample to the French press cell
- Apply pressure – 6000 to 9000 PSI
- Collect cell lysate
- Purify cell lysate on the King Fisher instrument

## Advantages of using the French Press...



- 1) Increased lysis efficiency
- 2) Gentle cellular disruption, destroying cell wall yet leaving nucleus and internal proteins in tact.
- 3) Ability to break tough cellular walls such as with Yeast
- 4) More uniform disruption than with Mechanical or Ultrasonic methods
- 5) No clean up of lysis reagents
- 6) No need to pre-heat samples