بسم الله الرحمن الرحيم

King Saud University

College of Science

Department of Biochemistry

Advances in Applied Biochemistry

(BCH 601)

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BCH 601

Advances in Applied Biochemistry

- Course Symbol & No. : BCH 601
- Credit Hours : 2 (2+0)
- Prerequisite :
- Class schedule : Thursday
  8:00 pm to 10:00 pm.
- Class location : Male campus, 2B2 Bld# 5
- Assessment :
  - Presentation 10%
  - Research paper 10%
  - Reports 10%
  - Scientific activity 10%
  - Mid term exam 20%
  - Final exam 40%

BCH 601course description

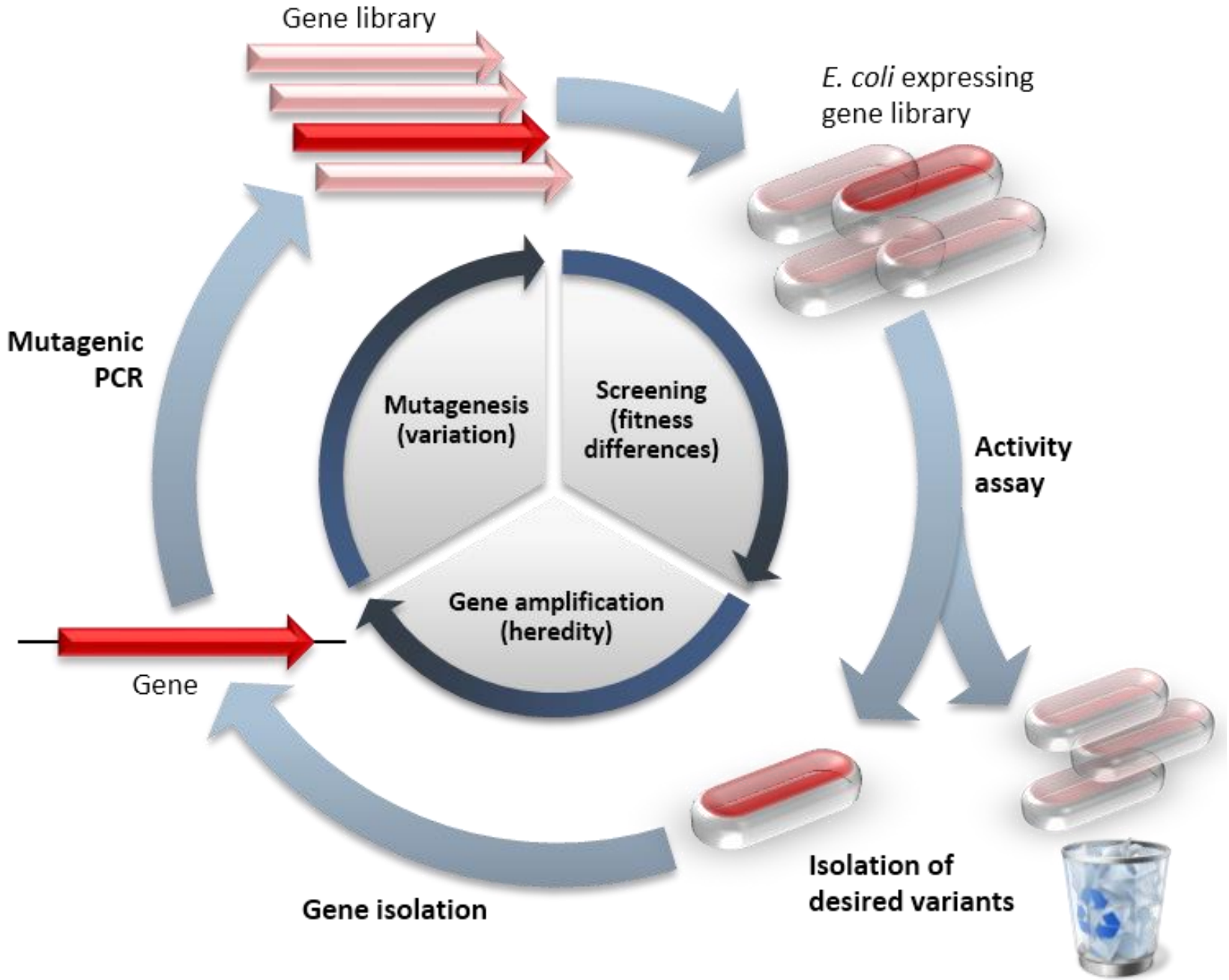
Course Code & No.: BCH601	Name: Advances in Applied Biochemistry	No. of Units (Theoretical & Practical) 2 (2+0)
Methods and techniques associated with biomolecule separation and purification. Biochemical sensor design, biochemical fuel cell applications, bioremediation and biodegradation. Industrial carbohydrate applications. Eicosanoids, heat shock proteins and interferons identification. Applications of enzyme immobilization. Biomolecular electrode technology.		

Course objectives

المفاهيم الأساسية في الكيمياء الحيوية التطبيقية. وتشمل الموضوعات فهم الأساليب والتقنيات المرتبطة بفصل الجزئيات الحيوية وتنقيتها. وصف المبادئ الأساسية لتصميم تقنيات استشعاره بيوكيميائية) ذو أصل جيني (والكيمياء الحيوية للوقود الحيوي ومعاجلته البيولوجية، التحلل البيولوجي والأنتقال الكيميائي الحيوي. أهمية تطبيقات الكربوهيدرات الصناعية. فهم أدوار دهون الأيكوزانويد، وبروتينات الصدمة الحرارية والأنترفيرون والتقنيات الحيوية المرتبطة بنشاط هذه المركبات. شرح أهمية البروتيوم، وتقنية الميكروأراي للبروتينات و التجارب الثنائية الأبعاد لفصل وعزل البروتينات. قياس الطيف الكتلي وأهمية قياس الطيف الكتلي في البروتينات. فهم تقنيات تثبيت الأنزيمات وصناعة الأقطاب ذات الصفة الجزيئية البيولوجية وتطبيقاتها في الصناعة.
الأسس الفيزيائية والكيميائية للمركبات الجزيئية في الكيمياء الحيوية. الجزيئات ودور أنواع الروابط في استقرارها.

Directed evolution (DE) and DNA Shuffling

- Directed evolution is a method used in protein engineering that mimics the process of natural selection.
- It consists of subjecting a gene to iterative rounds of mutagenesis, creating a library of variants, selection through expressing those variants and isolating members with the desired function.
- It can be performed *in vivo*, or *in vitro* .
- Directed evolution is used both for protein engineering as an alternative to rationally designing modified proteins, as well as studies of fundamental evolutionary principles in a controlled, laboratory environment.


Gene library
E. coli expressing gene library
Mutagenic PCR
Mutagenesis (variation)
Screening (fitness differences)
Activity assay
Gene amplification (heredity)
Gene
Gene isolation
Isolation of desired variants

Steps of Directed Evolution

Example directed mutation for production of detoxification enzymes from plant.

- Treatment with heavy metals like nickel (150 μM), zinc (200 μM), and chromium (50 μM).
- Treatment with herbicide mixture (fluazifop-p-butyl (diluted 1:250), atrazine (0.2 mM), and alachlor (0.2 mM) in ethanol solution (20% v/v)
- Treatment with heat stress at 37°C for 24 h.
- Collect samples from different parts (leaves, shoots, and roots).

- Total RNA from different tissues was isolated and checked by agarose electrophoresis for its integrity.
- Total RNA was subjected to DNase treatment with the RNase-free DNase.
- cDNA synthesis was achieved using reverse transcriptase
- Amplification of the GST genes by gradient PCR using degenerate primers.
- The PCR products were analyzed on a 1% (w/w) agarose gel and the corresponding bands were cut out and cleaned and cloned in *E. coli.*
- Plasmids are digested with DNase (0.7 Units) for certain time and the reactions were stopped by EGTA and heate (65°C/ 10 min)
- Agarose gel electrophoresis 2% (w/w) of the DNase products was performed to check for digestion. Random fragments of 50–100 bp obtained after 8–15 min were selected for the shuffling procedure.

- Reassembly of DNA fragments was carried out. using PCR
- PCR reassembly product was used as template in a second PCR with the degenerate primers.
- The product of this reaction was run on a 1% (w/v) agarose gel, excised, and purified. The extracted product was ligated to a suitable plasmid vector.
- The resulting plasmid library was transformed into *E. coli* TOP10 and *E. coli* BL21(DE3) cells.
- Screening of the library and expression of the recombinant enzymes.
- Enzyme purification was carried out using affinity chromatography and the protein purity was judged by SDS PAGE.

Enzyme characterization

- pH optimum
- Substrate specificity
- Effect of inhibitors
- Thermal Stability and Inactivation
- Melting temperatures (T_m)
- CD- analysis
- X-ray crystallography

Advantages of directed evolution

- Rational design of a protein relies on an in-depth knowledge of the protein structure, as well as its catalytic mechanism.
- Specific changes are then made by site-directed mutagenesis in an attempt to change the function of the protein.
- A drawback of this is that even when the structure and mechanism of action of the protein are well known, the change due to mutation is still difficult to predict.
- Therefore, an advantage of DE is that there is no need to understand the mechanism of the desired activity or how mutations would affect it.

Disadvantages of directed evolution

- A restriction of directed evolution is that a high-throughput assay is required in order to measure the effects of a large number of different random mutations. This can require extensive research and development before it can be used for directed evolution.
- Additionally, selecting for improvement in the assayed function simply generates improvements in the assayed function. To understand how these improvements are achieved, the properties of the evolving enzyme have to be measured. Improvement of the assayed activity can be due to improvements in enzyme catalytic activity or enzyme concentration.