MUTA-CHROMOPLATE™

A Users Guide

environmental bio-detection products inc.

MUTA-CHROMOPLATE™

Kit Components and Reagents

MUTA-CHROMOPLATE™ Basic Kit

- 12 Sterile 50ml tubes
- 10 Sterile Reagent Boats
- 12 Sterile 96 Well Plates
- 1 Sterile .22ul Filter
- Ziploc and Auto-Clave Bags
- 1 Bacterial Strain (TA100)
  - Other individual strains available upon request
- 1 Positive Control
- Reagents for 12 plates

MUTA-CHROMOPLATE™ Two Strain Kit

- 25 Sterile 50ml tubes
- 20 Sterile Reagent Boats
- 24 Sterile 96 Well Plates
- 2 Sterile .22ul Filters
- Ziploc and Auto-Clave Bags
- 2 Bacterial Strain (TA100 and TA98)
  - Other individual strains available upon request
- 2 Positive Controls
- Reagents for 24 plates

MUTA-CHROMOPLATE™ S9 Activation Enzymes

S-9 Activation Mix Components

- S9A $MgCl_2$ + KCL
- S9B Glucose-6-Phosphate
- S9C NADP
- S9D Phosphate Buffer
- S9E Sterile Distilled Water
- S9F Rat Liver Extract
- 2AA Positive Controls

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- **The Muta-ChromoPlate™ is a 96-well micro-plate version of the *salmonella typhimurium* 'Ames Test,'**
- **Used for detection of mutagenic activity.**

Developed to test mutagenic materials in water (or DMSO) soluble extracts of sediment, air, chemicals, food components, cosmetics, waste waters, potable waters and any other material that can be solubilized or placed into micro suspension in water such that the material being tested can be taken up by the test strain.

The test employs a mutant strain, or several strains, of Salmonella typhimurium, carrying mutation(s) in the operon coding for histidine biosynthesis. When these bacteria are exposed to mutagenic agents, under certain conditions, reverse mutation from amino acid (histidine) auxotrophy to prototrophy occurs allowing growth and turning the purple wells on the microtitre plate to yellow.

- The Muta-ChromoPlate™ kit is generally more sensitive than the Ames pour-plate assay, because it allows testing of higher concentrations of sample (up to 75% v/v).
- The assay procedure is simple and requires minimal training.
- Example Applications
  - Testing of industrial effluents for presence of possible mutagenic compounds
  - Screening of municipal discharges for possible routine presence or spills of mutagenic compounds
  - Screening of surface and/or groundwater for mutagenic residues
  - Screening of potable water supplies for the presence of chemicals with mutagenic potential
  - Screening of water soluble air pollutants for mutagenic agents
  - Evaluation of pure or complex raw mixtures for potential mutagenicity
  - A convenient and easy to use teaching tool for university and college laboratories

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- Currently EBPI offer five different types of Bacterial Strains to meet the OECD's Guideline for Testing of Chemicals.
- TA100
- TA1535
- TA98
- TA97a
- TA102

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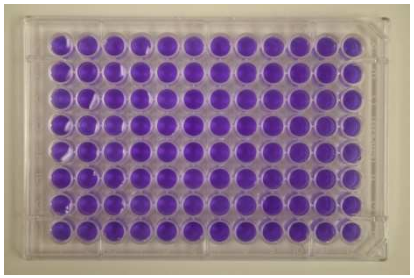
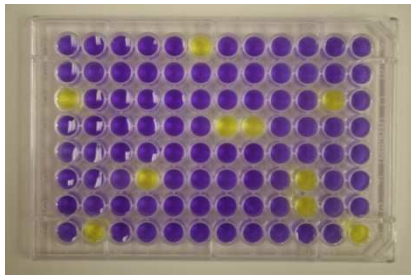
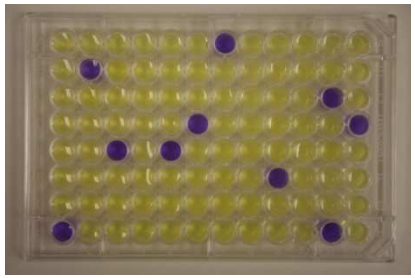
- The Muta-ChromoPlate™ provides a clear colour endpoint.
- Reagents, cultures and other consumable components are supplied ready-to-use in a non-specialized laboratory.



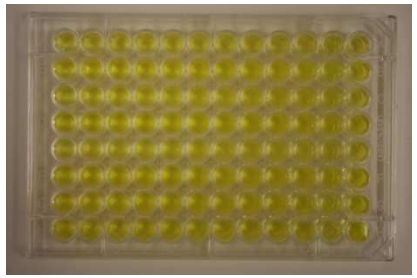
Sterility Check



Background



Strong Mutagen



Positive Control
(Very strong mutagen)

A possible example of the Muta-ChromoPlate™ kit on day 5 of the assay is shown above which includes all controls essential for the assays.

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- No need for cultures

- Quick and easy overnight growth of the bacteria (No need for time consuming dilutions which may lead to contamination).

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Filter Sterilization of the sample for assays

Filter sterilization of the samples is recommended to be preformed prior to starting the assays. This can be done with either the 0.22µm filter unit supplied with the kit, or with a 0.22µm syringe filter (not included unless requested)

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Overnight Growth of the Bacteria

1. Remove the vial of Growth Media from the fridge and remove the vial of Bacteria from the freezer

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- 2. Using aseptic technique open G (Growth Media) and the vial that contains the bacteria. Transfer the contents from vial G to the vial that contains the bacteria.

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- 3. Place the lyophilized stopper back on the vial that now contains the bacteria and growth media and give the vial a quick shake to ensure that the bacteria and growth media are well mixed. Incubate overnight at 37ºC for 16 to 18 hours

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- 4. Visually examine the bacteria grown overnight for turbidity

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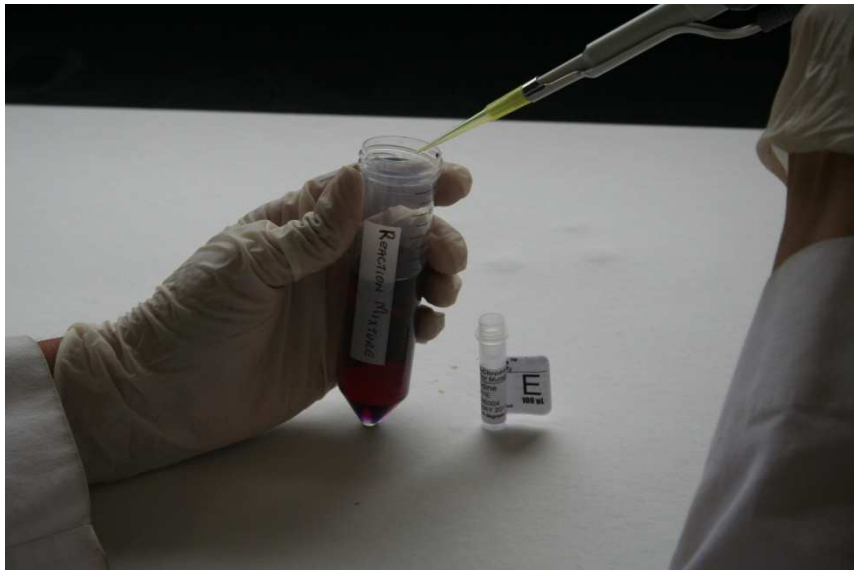
Obtain reagents A through E and dispense as outlined in the protocol into the sterile 50ml tube included in the kit.

Basic Kit (5051)
21.62ml (A) + 4.75ml (B) + 2.38ml (C)+1.19ml (D) + 0.06ml (E) for a total of 30.00ml



Bacterial Strain Kit (B5051)
43.24ml (A) + 9.50ml (B) + 4.76ml (C) +2.38ml (D) + 0.12ml (E) for a total of 60.00ml

NOTE: The Bacterial Strain Kit comes with a 100ml Reaction Mixture Bottle

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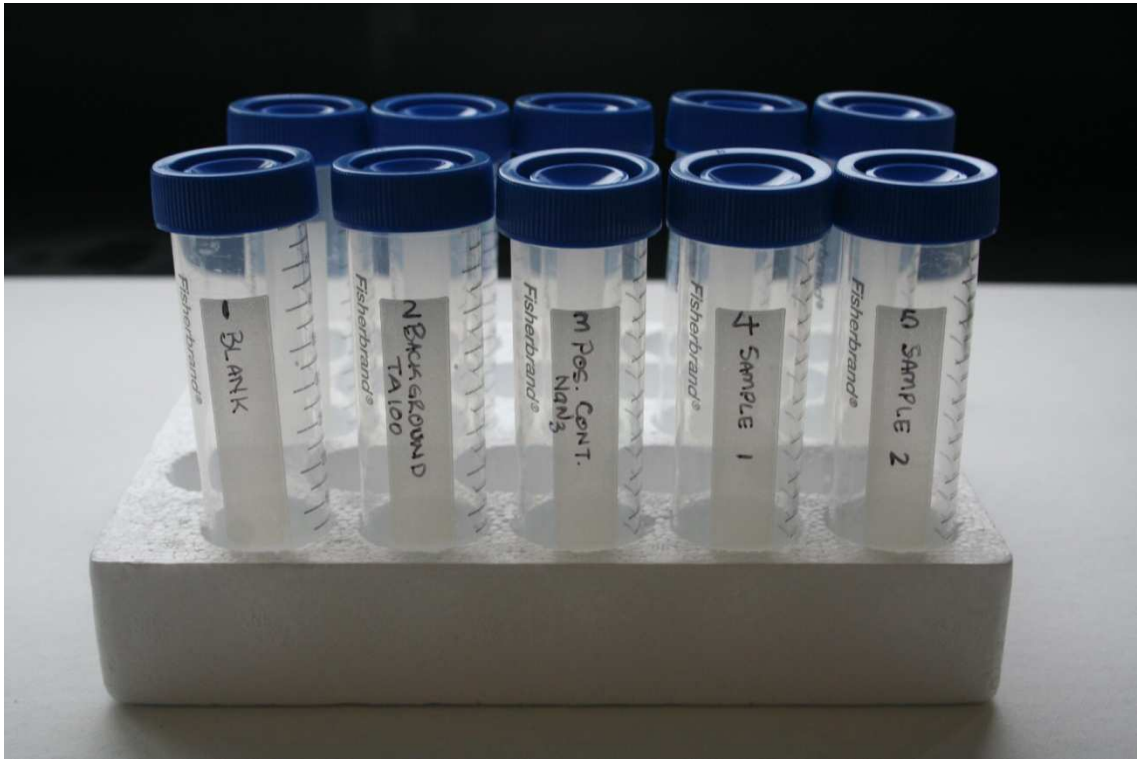

Label the sterile 50ml tubes as well as the sterile 96 well plates

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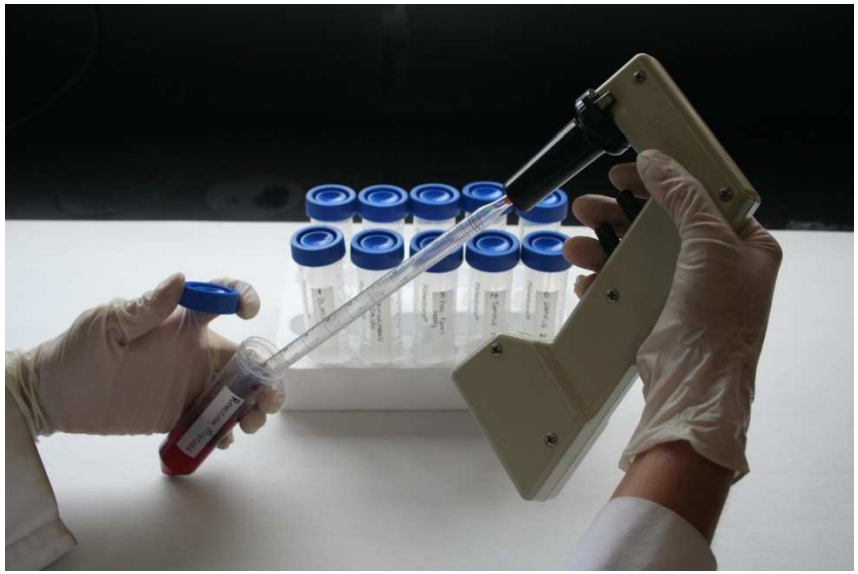
Add 2.5ml of Reaction Mixture to each tube



Add sterile water (included) and (or) sample material to be tested.

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Add 100µl of the Positive Control (included) to the correct tube.

Add 5µl of the bacteria to each tube, except for the blank tube.

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Vortex the tube for 15 seconds to ensure that the contents are mixed well

Pour contents of tube into the sterile reagents boats and dispense 200µl into each well of the sterile 96 well plate

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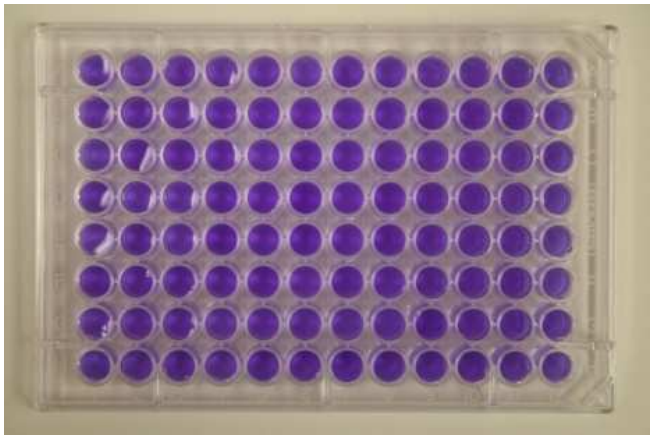
- Once the reagents are mixed and the bacteria and samples for analysis are added the suspension is then dispensed into the 96 well plate. The user will notice that all the wells in the plates will be a purple colour as seen below.



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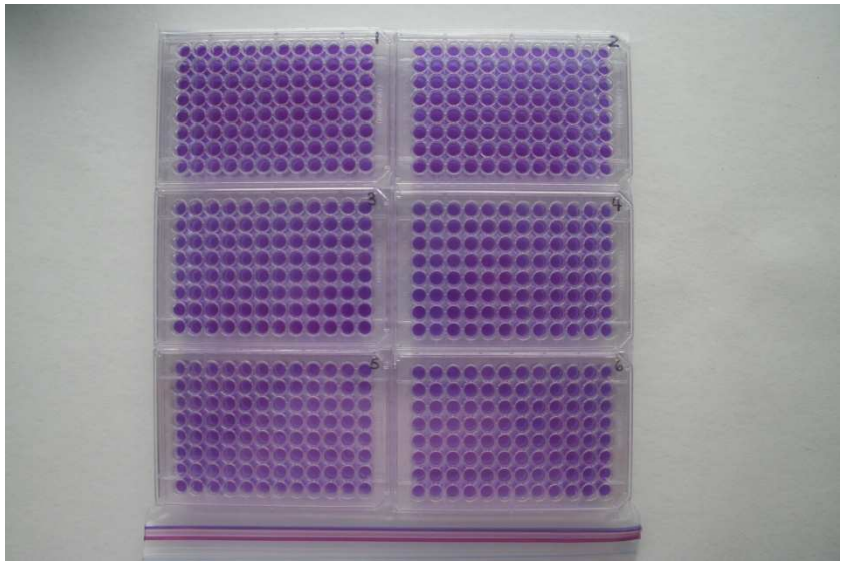
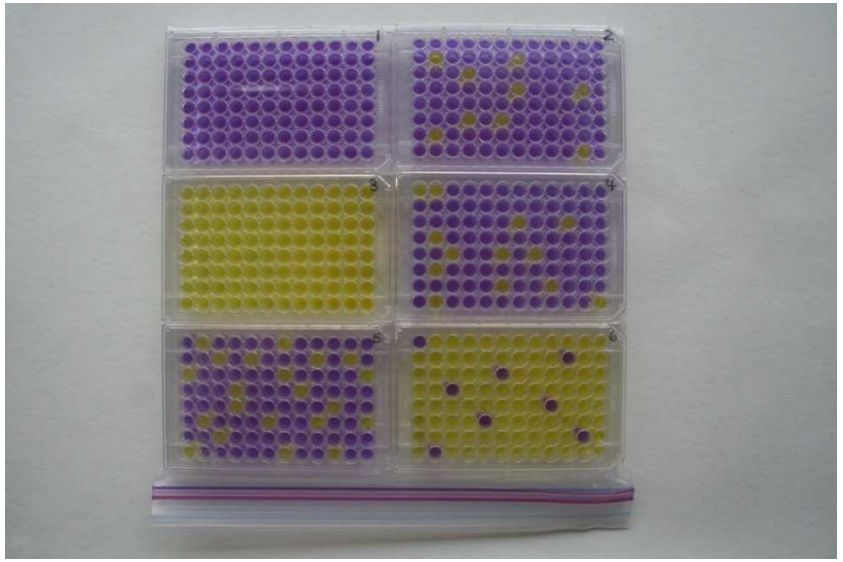
Place the plates into the Ziploc bags and incubate for 5 days at 37°C.



Remove the plates and score



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- Each well of the 96 well plate is considered a colony.
- If the colony reverts back to the natural state, a mutation has occurred.
- If a reverse mutation has occurred, the bacteria in the colony have the ability to synthesize histidine and will continue to grow turning the colour in the well from purple to yellow.
- The Muta-ChromoPlate™ kit (as with the traditional ‘Ames Test’) compares the natural background rate of reverse mutation to a rate of reverse mutation within a sample assay.

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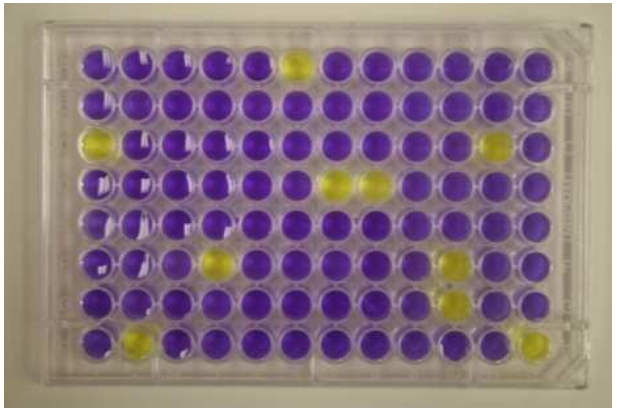
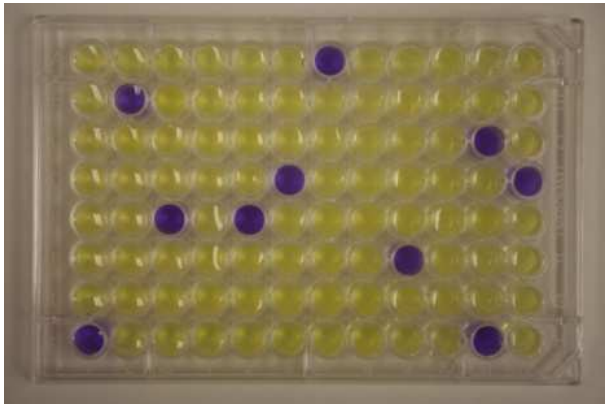
Understanding the results

- For example: There are two plates shown below after 5 days incubation, one is the “Back Ground” plate (left) and one is a “Sample treatment” assay plate (Right).



10 Wells have mutated (TA100 Strain)



86 Wells have mutated (TA100 Strain)

Using Table 2 from the protocol under the "No. Wells Positive in Background Plate" column, locate the number 10 and you will find the statistical significance data for the 96-Well fluctuation test.

No. Wells Positive in Treatment Plate		
0.05	0.01	0.001
19	23	27

The background rate of reverse mutation is compared to the treatment plate of mutation. Since the treatment rate of reverse mutation (86 in our case) is greater than 27, it should be noted that there is a less than a 0.001 chance that 10 and 86 are the same result. Thus suggests that our treatment plate contains a strong mutagenic material producing a very significant difference in reverse mutation rate from that observed in the control.

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Reverse Mutations in Various Bacterial Strains

Base-Pair Substitutions	Positive Controls
TA100	Sodium Azide
TA1535	Sodium Azide
TA102 (Site A-T)	Mitomycin C
Frame-Shift Mutation	
TA98	2-Nitrofluorene
TA97a	9-Aminoacridine

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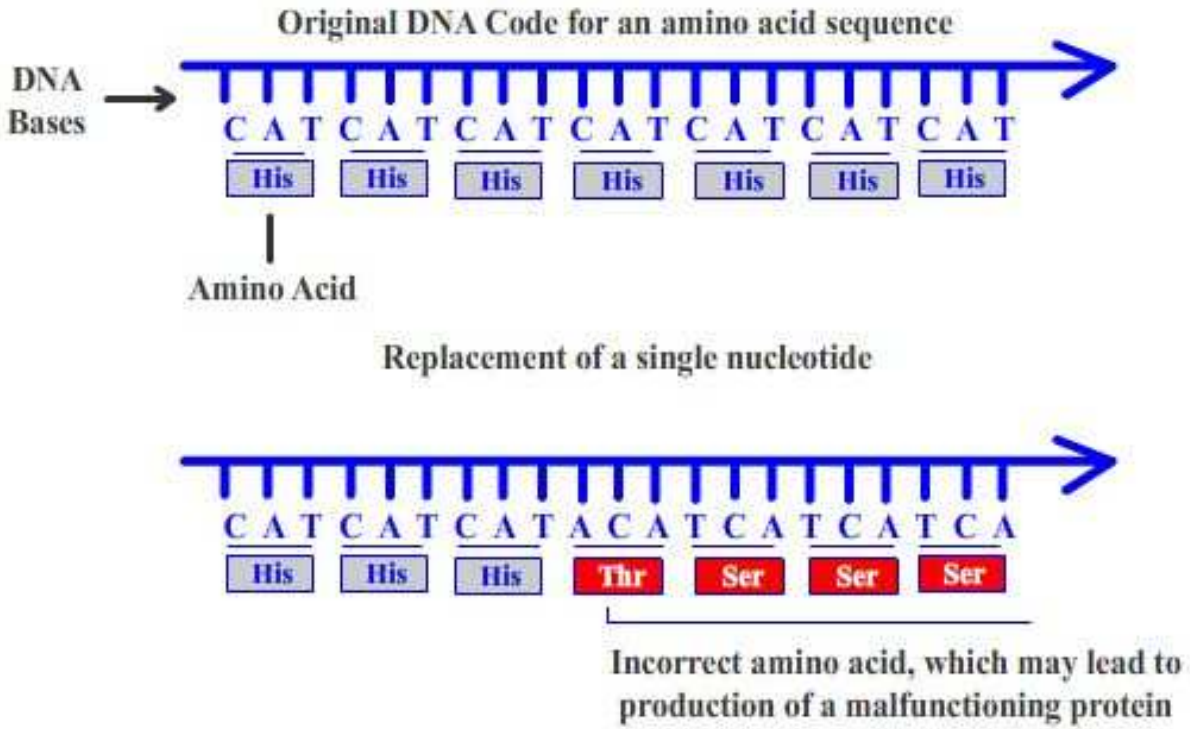
TA100 Base-Pair Substitutions
Insertion Mutation



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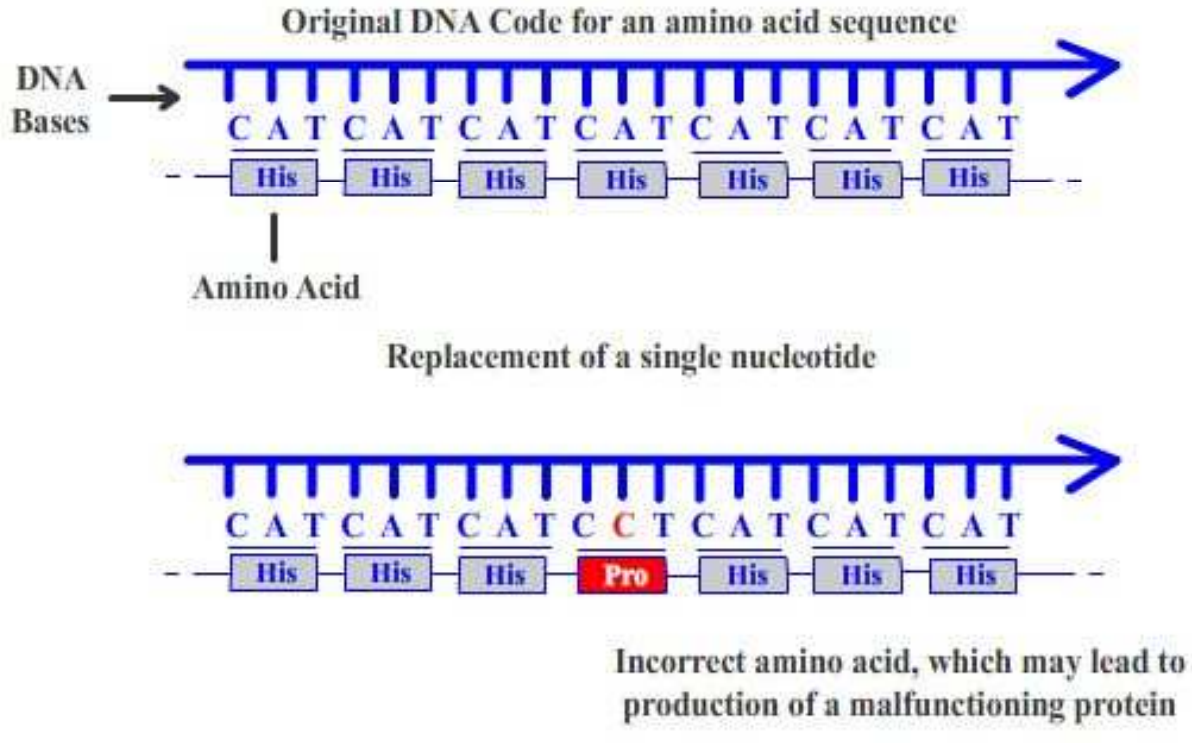
TA100 Base-Pair Substitutions
Missense Mutation



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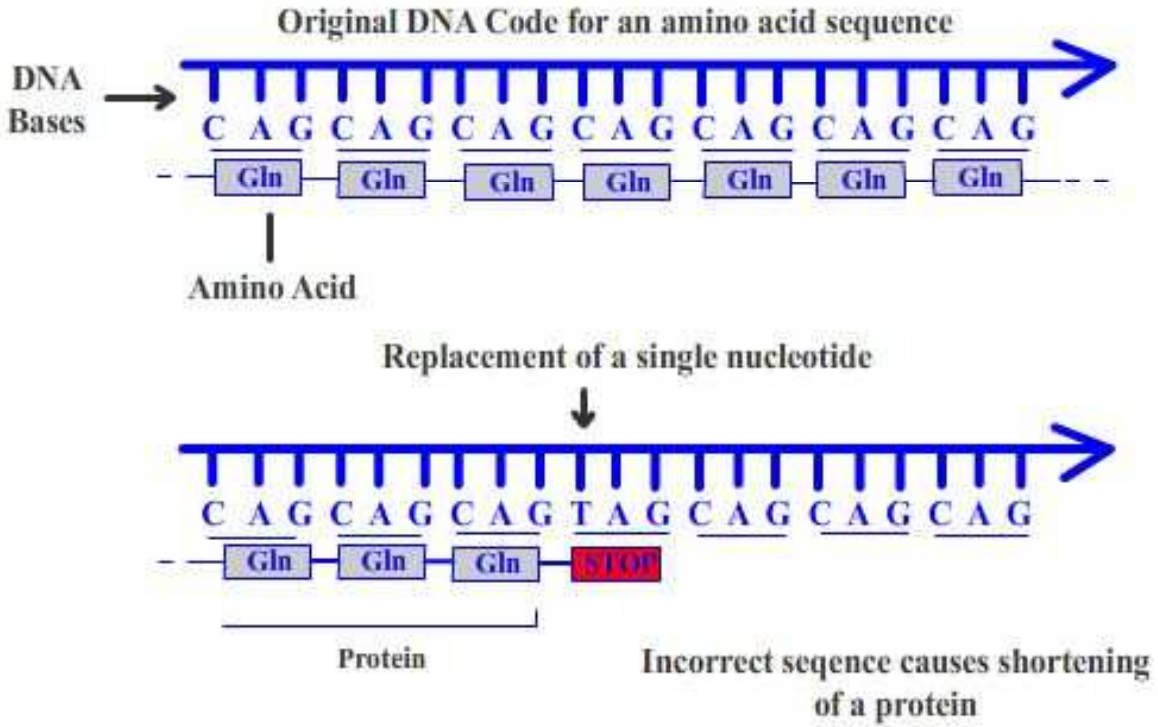
TA100 Base-Pair Substitutions
Nonsense Mutation



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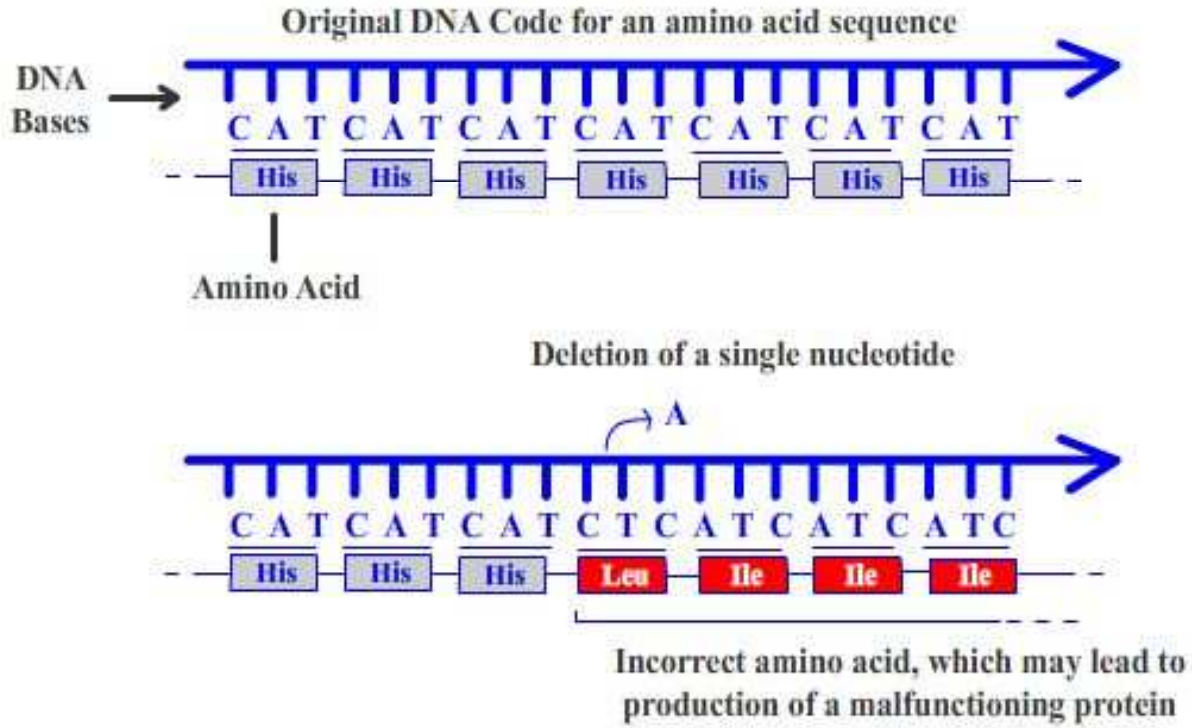
TA100 Base-Pair Substitutions
Deletion Mutation



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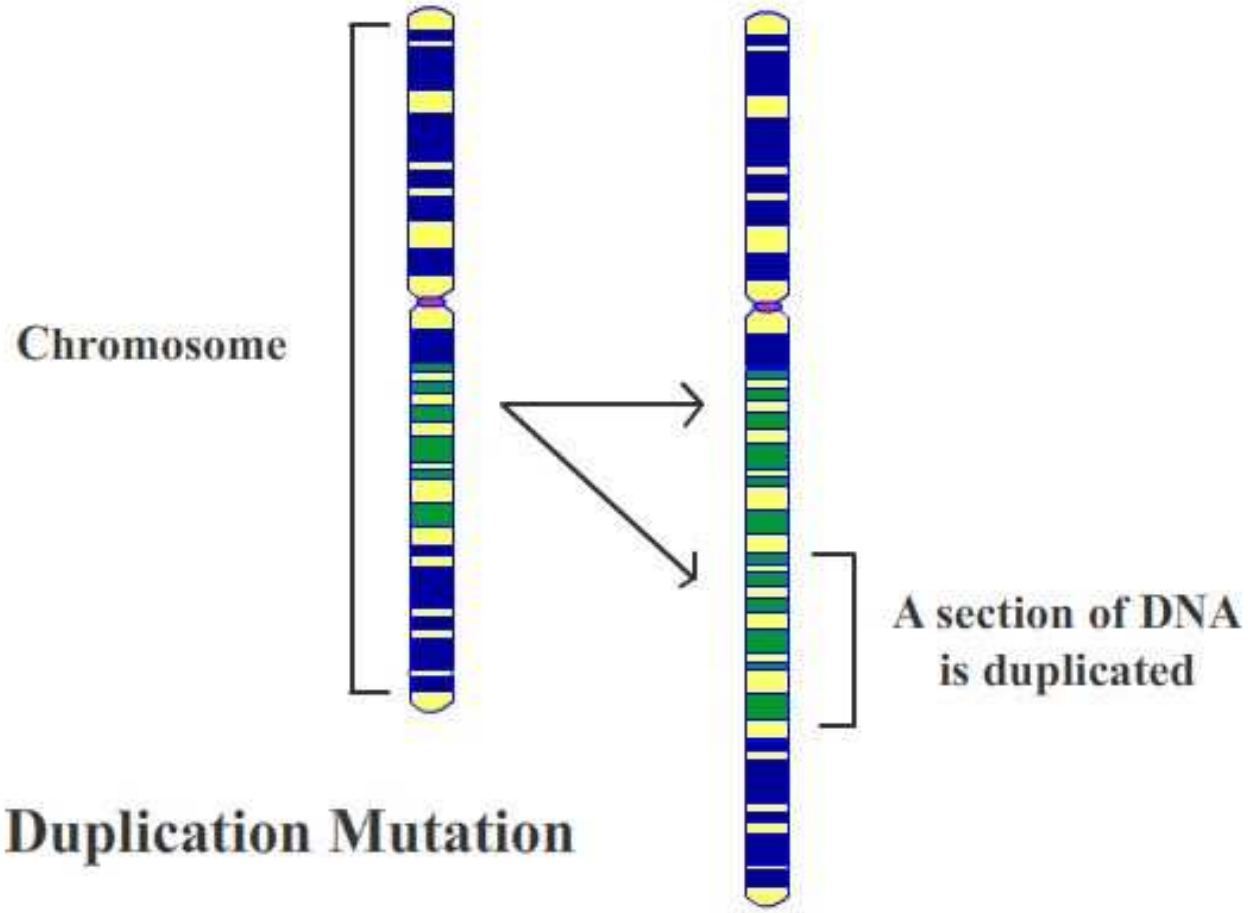

Duplication Mutation

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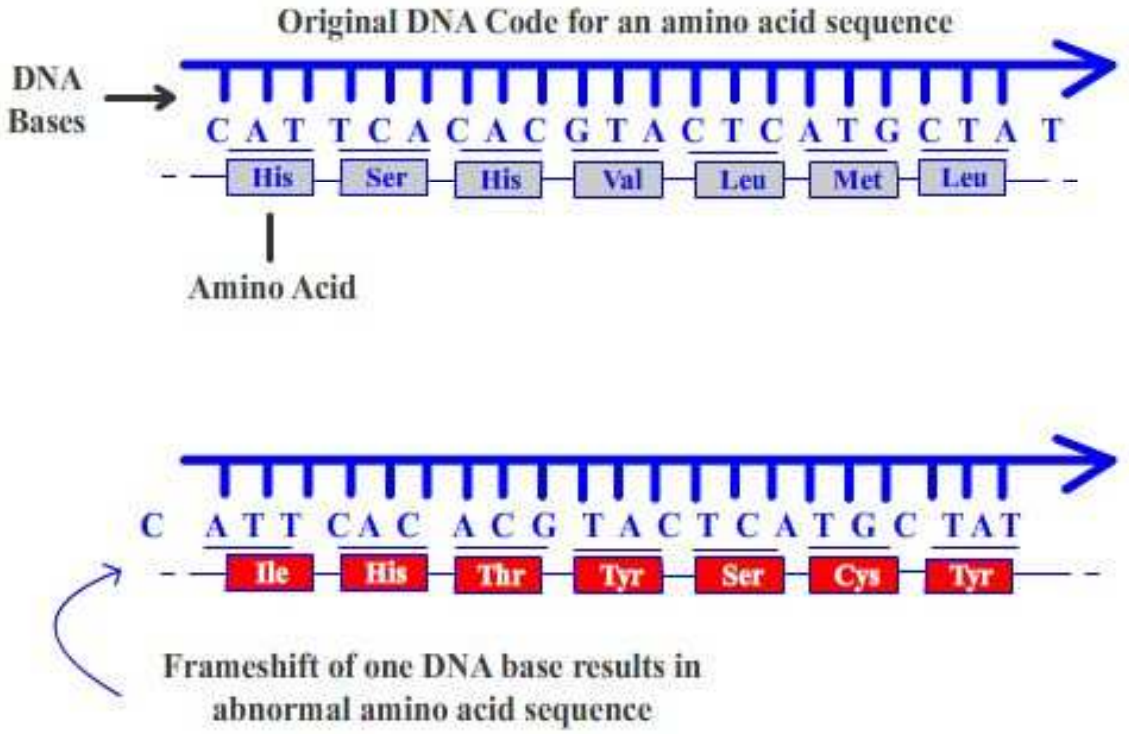
TA98 Frame-Shift Mutation



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- The Muta-ChromoPlate™ kit is available with or without the S9 Activation Enzymes.
- The S9 Activation Enzymes are from the male Sprague-Dawley rat liver – Aroclor 1254 induced.