

## USAGE OF ELECTROMAGNETIC FIELD AS A TOOL FOR DENATURATION OF GENOMIC DNA FROM *Saccharomyces cerevisiae*.

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### ABSTRACT

This study reports the effect of the energy of magnetic fields for usage as a denaturing agent of genetic material (DNA) from *Saccharomyces.cerevisiae*. Two different flux densities (0.1 and 0.5 T units) of magnetic field were applied to two different haploid yeast strains (Ura 1 and Gm3) for both 5 minutes and 15 minutes. The absorption spectra of DNA exposed to the magnetic field was analyzed at 230nm,260 nm, and 280 nm, at two different temperatures (35°C and 72°C). Absorbance spectra thus generated was compared to corresponding spectra obtained from *S.cerevisiae* DNA ,not exposed to magnetic field. At 72°C absorbance was much higher in DNA samples exposed to magnetic field after their purification. Much higher impact of magnetic field was observed in the strain having more mutants. The magnetic field increased the energy of hydrogen bonds separated or break its and hard DNA double helix (denaturation). This effect depends on the genetic background.  
**Keywords:** Electromagnetic field ,Yeast ,DNA denaturation.

### INTRODUCTION

The scenario of the world was dramatically changed just after invention of electric energy and as applications of electric energy were discovered, humen being became heavily dependent on it. The dependence rate is in exponential order. Most part of this green planet had been electrified and remains are going to be electrified in near future. Wherever is the electric current, there is Electromagnetic Field (EMF) that has two components.(a) Electric field-screened easily through any conducting material.(b) Magnetic field (MF)-easily penetrates through most material without losing intensity. The horizontal component of MFs depends on their source and distance from source ( Cameron *et al.*,1993).Due to general concerns of the potential hazards related to exposure of MFs, many epidemiological investigations on the linkage between MFs exposure and prevalence of cancers had conducted. The results derived from these investigations are generally subject to continuous debates.. One of the concern is hazardous effect of field exposure on the stability of genetic material. It is important to explore the effect of MFs on the mutagenic mechanism. It was reported that MFs has potential influence on the biosynthesis (Cairo *et al.* ,1998) and activities of transposable elements ,which are known to be responsible to certain stressing environmental factors ( Chow and Tunge,2000).Environmental frequency MF induced significant biological changes in a variety of cells and tissues (Goodman *et al.* ,1995) including induction of the stress response heat shock and several early response gene (eg.c-myc).Both heat shock and MFs induce stress leads the induction of Hsp70 expression. Energy density is a measure of the energy required for MFs-induced stress. Mfs are small

perturbation compared with heat shock. Cellular response are induced at an energy density 14 order magnitude lower than heat shock ( Lin *et al.*,1998).

Several extensive studies shows that frequency power field do not induce any genetic changes or chromosomal aberration however, mutagenic effect and DNA breakage observed (Hungate *et al.*,1979, Reese *et al.*, 1998) . Exposure of extremely low frequency (ELF) MFs may be a possible factor in the development of certain type of cancers especially humen lymphoblastic Leukemia (Sheikh-1986, Savitz *et al.*,1988, Taubes,1993; Pool,1990; Feychiting and Ahlbom-1994) . A variety of Bioeffects that result from exposure of tissue and cells to ELF MFs are reported in literature including change in cell surface properties (Phillips-1986, Paradisi *et al.*, 1993). Altered rate of DNA,RNA and protein synthesis (,Liboff *et al.*,1987, Goodman *et al.*, -1993).

Most molecular assemblies exhibit sufficient diamagnetic anisotropy that DNA became orientated in a magnetic field of moderate strength of 10-20 KG ( Maret *et al.*,1975) . Magnetic fields penetrate to the nucleus without attenuation. DNA conducts electrons through its ? stacked arrays of aromatic bases (McClellan *et al.*,1990) . The rate of electron flow with the stacked bases of DNA is greater than 10<sup>6</sup> per second which is a current density of about 5 x 10<sup>4</sup> amperes/m<sup>2</sup> through the cross sectional area of the DNA as part normal tonic activity, then significant interactions could result from relatively weak magnetic field (Lin *et al.*,1998).

Vulnerable site on the DNA can suffer damage due to electron flow even when an oxidizing agent is attached to the DNA at a distance (Dandliker *et al.*,1997) . MFs interact with moving charges, thus their interaction with conducting electrons in the DNA may result in conformational changes .

Due to the process of urbanization and the fact that electrical appliances are commonly used in our daily life, many living organism, including humen beings , are inevitably subject to frequency electric fields. It is essential and urgent to find out if these fields like other stressing environmental factors, perturbs the stability of genetic systems. ( Chow and Tunge,2000 ) . Many of the electromagnetic field exposure study have methodological limitations. It is necessary to make use of methods that can specifically illustrate the effects of frequency power fields on the separation of dsDNA. In this regard they designed an experiment to address the effect of magnetic field exposure on the stability of dsDNA of *S.cerevisiae*. The magnetic cycle reaction for amplifying nucleic acids that involves separating nucleic acid stands by electromagnetic field (MF). The following are new method for amplifying a specific (ss) target nucleic acid (NA) involves : (a ) incorporating into an (NA) complementary to the target (NA) a solid phase or magnetic primer ,(b) separating the solid phase or magnetic strand and target (NA), (c) incorporating into an (NA) strand a magnetic or solid phase primer, respectively to yield a duplex with one solid phase strand and one magnetic strand , (d) separating the solid phase and magnetic strands by applying an electromagnetic field to dissociate the duplex , (e) allowing magnetic primers complementary to the solid phase strand to anneal to this , and allowing solid phase primers complementary to the magnetic strand to anneal to this , (f)

extending the annealed primers with a suitable DNA-polymerase, and (g) repeating (d) to (f) as necessary to obtain a desired of amplified DNA (Mian , 1995). The non-sterile medium passing through tube was passed through an (MF) and then autoclaved. After 10 hr. of incubation. *S. frigidus* , showed a 27-36 % increase in biomass in the magnetized medium compared with control medium. As well as, showed a significant increase in the growth rate of *B.mucilaginosus* in – magnetized medium. After incubation, the number of cells was 3-fold greater than in the control (within 20 hr. incubation) While, there was no difference between magnetized and control medium cultures (after 44 hr. incubation), (Ergin ,*et al* 1988). The treatment with an MF through medium or its water to be magnetized, increases enzyme activity and growth of yeast , accelerates the cellulose decomposition , and increases hydrolysis rate,(Tessier,1987). The differences in the growth of cells were ascribed to differences in states of nutrition and to the role of water in the realization of magnetic influences ,(Markov,1990). The values and direction of transition moments of the third order space structure of DNA molecule as a whole,(Dovbeshko and Litvinov, 1992 ) . Based on these theoretical prediction this investigation aims inference MF influences on physical energy up on DNA which due to dynamically structures of DNA, (denaturation and and renaturation ).

## MATERIALS AND METHODS

### \* Yeast strains and growth media used :

Two *Saccharomyces cerevisiae* strains, GM3 and Ura 1 ,(Sharaf El-Deen and El-Terra 1999). These strains were haploid phase; GM3 (a/gal 10, trp 1, ura 3, ura 4, met 8, ade 5,7, leu 2, lys 1, ilv 1, aro 1D, can 1, Suc mal cupr.) and Ura 1 (a/ ura1, P+W- C321 R, E 221 R.). Two strains were kindly provided from the Genetic Stock Centre California ,and Genetic Dept. Agric .Fac. Mansoura Univ .Mansoura, Egypt.(Personal connection ) .

Non –selective YPD growth media was used to grow yeast cultures. This media consisted of, yeast extract (1%) ,peptone (1%) and dextrose (2%),(Sherman *et al.*, 1986) . Liquid yeast cultures were grown for approximately three days of incubation at 30°C ,with shaking at 100 rpm. The samples were centrifuge, collected and the wet- cell weigh was 1.1 g . Then applied DNA isolated according to Bendich and Bolton ,1967).

### \* Magnetic field:

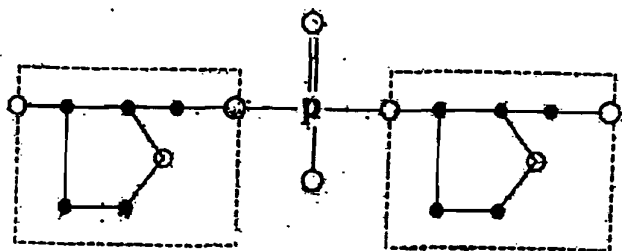
The Magnetic Field (MF) source was, Newport Pagnell, Instrument (Oxford, UK.), The MF applied was 10KG; and high current DC supplied from an Ealing Model supply.. The MF was applied at two different field strengths (H) : 0.1 and 0.5 T, with a current of 0.25 Amps.

**Sample preparation:** The DNA samples were re-suspended in 1.0 ml of 0.1 X SSC and were exposed to MF (0.1 and 0.5 T) for 5 and 15 min. The other side; other samples were exposed through isolation process; after three layers separated in centrifuge tube with chloroform/ octanol, supernatant layer (DNA) were collected , then exposed to MF , snap cooled on ice and

adjusted to 1 M Na Cl. The DNA samples were measured at 230, 260 and 280 nm., using a UV spectrophotometer, Model 2410 PC SHIMADZU .

## RESULTS AND DISCUSSION

Two different strains of *S.cerevisiae* (GM3 and Ura1 ) were subject to two different strengths of magnetic fields ( 0.1 and 0.5 T ). The UV Absorption spectra for both these samples were measured at two different temperatures (35 °C and 72°C ) at three different wavelengths. As a control experiment another batch of the same DNA, isolated from the two *S.cerevisiae* strains GM3 and Ura1 was heated to 100°C in boiling water bath for minutes to achieve DNA denaturation in the traditional manner. UV spectrs obtained from these DNA samples were further compared to UV spectras obtained from DNA that was neither heat treated, nor subject to high magnetic fields. Table 1 shows the differences in the UV absorption spectra between the different DNA samples and conditions used. The absorbance values at 260 nm, were higher for Ura 1 and GM3 treated with MF. This increase in absorbance is a direct indication of DNA denaturation, which was directly shown by observing the absorbance values of DNA that was placed in the boiling water bath. Most investigations were used Thelma denaturation.,(Marmur,1961 ; Mitchel and Morrison, 1982 ). Many investigations were discussed this DNA property,(Hermon *et al.*, 1998 ).They predicted existence charges on the single DNA strand .A single DNA strand can be considered as a One dimension (1 D) array, whose unites "grains" are composed of a sugar and a base attached to it. The grains are connected longitudinally by phosphate bridges which ( Ben-Jacob *et al.*, 1998) called P-bonds, as appear in this model:



**Fig.1 A schematic image of two "grains" in the DNA connected by a P-bond. The dark circles represent carbon atoms and the white circles oxygen atoms.(Ben-Jacob *et al.*,1998).**

Besides the tunneling property of the P-bonds, the DNA also has inductive and capacitive properties . There are three types interactions which contribute to the DNA. The first is a electrical coupling to the environment, or self capacitance. They represented this interaction by a

capacitance from the second subgrain to a common substrate. The second capacitance stems from the properties of the Hydrogen bonds between the bases. The third capacitance is the tunnel junction (P-bond) capacitance.

Combining the chemical bonds to utilize in DNA to logical devices. The DNA molecule has the capability of transmitting information over long distances and in a specific manner (the information is transmitted to /from specific target locations). Possible efficient candidates for such a transmission are solitons and solitary waves. Motivated by studies of topological charge solitons in one-dimensional (1D) arrays of mesoscopic tunnel junctions. The chemical bonds as tunnel elements (Ben-Jacob *et al.*, 1998), possibility of solitonic charge dynamic in DNA molecules. The (1D) arrays of serially coupled normal junctions support collective topological excitations called charge solitons. The propagation of the soliton along the array (due to a bias voltage, for example) is done by tunneling of an electron from the island with the additional charge to an adjacent island, redistribution of the charges in the array, tunneling of another electron to the next adjacent island, charge redistribution, and soon. This explains the fact that the solitons its mass is much smaller than the electron mass.

The charge and dipole solitonic like excitation may exist in both single strand and double strand DNA molecules, (Hermon *et al.*, 1998).

One observes a new kind of physical phenomena arising from the interplay between the bulk and the single electron properties. These phenomena are referred to as coulomb (or single charge) effects (Grabert and Devoret (1992)).

The chemical bonds up on DNA as electromagnetic elements P-bond as tunnel junctions this tunnel junction are the two Oxygen atoms transversely connected to the phosphorus atom. These Oxygen's share three electrons with the phosphorus, giving rise to two sigma bonds. As the pi electron can be share with both Oxygen's, it resembles an electron in a well potential, and occupies the lowest level, (Ben - Jacob *et al.*, 1998). Besides the tunneling property of the P- bonds, the DNA also has inductive and capacitance properties. The units of a ssDNA whose composed of a sugar and a bases attached to it (cell- organisms) can be considered as a 1 D (one dimension). These grains are connected longitudinally by phosphate bridges (There are three types of interactions which contribute to the capacitance properties of the DNA (Amman, *et al.*, 1989 a) The first is the electrical coupling to the environment, so the MF as one of these environmental factors has efficiency on the electrical coupling. The second capacitance stems from the properties of the Hydrogen bonds (H-bond) between the bases. Its can effectively screen additional charge density on either side of the bond by shifting its towards this side. The charge accumulates on the sides of the H-bond, can be viewed as a capacitor. The third capacitance is the tunnel junction (P-bond) capacitance.

Self-capacitance is the smallest of the three types, large than this is the H-bond capacitance and the largest is the P-bond capacitance. When DNA samples measured i.e., at 260 nm., 72°C with MF exposed (before and after DNA isolation). The Ura1 was 0.986 and 1.221 as absorbance units

before and after DNA isolation at 0.1T /5 min. ,respectively. However its absorption were 0.850 and 0.960 at 0.1T/15 min ..respectively.

The GM3 sample not effect at 0.1T/5 min. neither before nor after DNA isolation But absorbance units were increased from 0.900 to 1.416 at 0.1T/15 min.; before isolation and after isolation, respectively. The increase of absorbance was significant at 0.1T/5 min. for Ura1 sample with MF exposed after DNA isolation ,but GM3 not effected with the same condition .The increases were high significant with MF 0.1T/15 min., either Ura1 or GM3 samples. On t he other hand ,the 0.5T/5min. has not significant effect on different samples neither before nor after DNA isolation , but the 0.5T/15 min. has high significant effect through DNA isolation more than after isolation for two used strains , as shown in table (1).

**Table (1) Absorbance of unexposed, MF exposed before isolation ,and MF exposed after isolation for DNA yeast samples.**

MF Exposure	Yeast Strains	Flux Density	Time (min.)	35°C			72°C		
				230nm	260nm	280 nm	230 nm	260 nm	280nm
Unexpo- sed DNA	Ura1	0	0	0.290	0.569	0.331	0.661	0.795	0.443
	GM3	0	0	0.259	0.440	0.259	0.362	0.615	0.386
Exposed DNA Before Isolation	Ura1	0.1T	5	0.530	0.970	0.931	0.541	0.986	0.610
			15	0.340	0.647	0.563	0.711	0.850	0.500
		0.5T	5	0.375	0.850	0.690	0.635	0.820	0.741
			15	0.499	0.944	0.732	0.543	0.761	0.623
	GM3	0.1T	5	0.562	0.900	0.612	0.695	0.975	0.573
			15	0.367	0.810	0.361	0.563	0.900	0.520
		0.5T	5	0.425	0.835	0.483	0.683	1.310	0.770
			15	0.563	1.610	0.809	0.882	1.675	0.845
Exposed DNA After Isolation	Ura1	0.1T	5	0.476	0.968	0.871	0.580	1.221	0.915
			15	0.415	0.860	0.808	0.436	0.960	0.732
		0.5T	5	0.456	0.911	0.900	0.628	1.014	0.931
			15	0.600	1.340	0.845	0.871	1.671	0.960
	GM3	0.1T	5	0.548	0.931	0.946	0.550	0.975	0.815
			15	0.427	0.941	0.985	0.745	1.416	0.608
		0.5T	5	0.574	1.126	1.167	0.480	1.735	0.936
			15	0.860	1.922	0.609	0.731	2.15	0.986

These results showed the magnetic field as a environmental factor to introduce energy into DNA and its influenced on two types the capacitance's properties of the DNA ; the self capacitance or called electrical coupling to the environmental and the Hydrogen bond between the bases . These H – bond is may be added energy on either side of the bond , due to its released and dsDNA to become ssDNA . Moreover , it has found the analysis off variance of absorbency values to all conditions ; different strains , heat degree , flux density and exposure time on a (before isolation) and (after isolation) . In the case ; flux density ( 0.1 – 0.5 T ) and the its interaction with strains were high significant to exposure time . In the (after isolation) case , flux density , its interaction with strains and the interaction between flux density , strains and exposure time were high significant . As well as , that charge and dipole solitons provide the means for communication and control.

After 5 days latter at cold temperature, DNA samples (after isolation ) were measured at 230, 260 and 280 nm., to show continue of MF effects. Table 2 showed that the values which reading at 35°C (room temperature ) were not differ than before their keeping. These have which proven that the MF energy can be continues i.e., for 5 days which tested ,as shown Table (2).

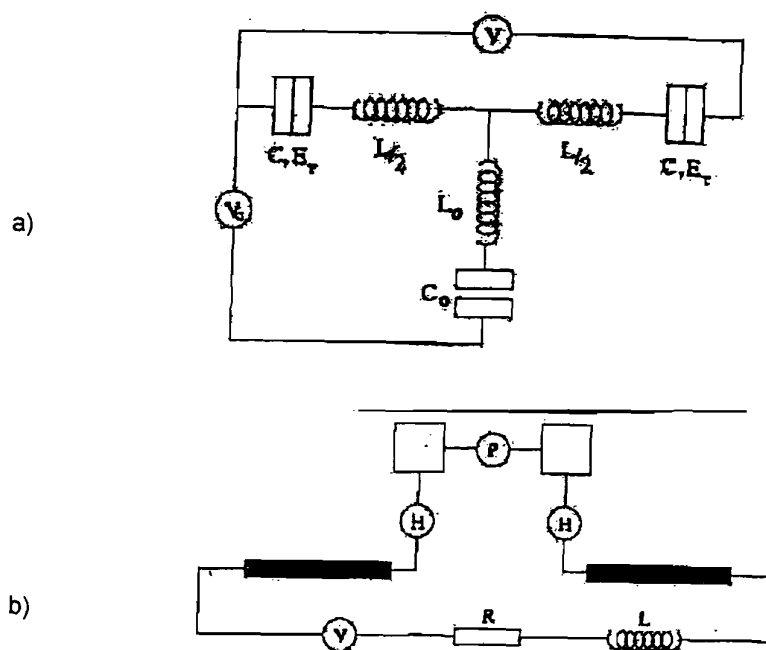
**Table (2) Absorbance of DNA samples, incubated at 35°C for 5 days after magnetic field exposure.**

M F Exposure	Yeast Strain	Flux Density	Time (min.)	230nm	260nm	280nm
Exposed DNA Before Isolation	Ura1	0.1T	5	0.270	0.960	0.933
			15	0.098	0.773	0.757
		0.5T	5	0.375	1.037	0.905
			15	0.407	1.092	1.020
	GM3	0.1T	5	0.350	1.019	1.081
			15	0.410	1.081	1.158
		0.5T	5	0.515	1.198	1.256
			15	0.241	0.908	0.850

The charge and dipole solution dynamics in DNA molecules, and possible role of these excitations in DNA functioning,(Hermon *et al.*, 1998) . They concluded that the enzymes which attach to the DNA at a specific point. At present, it is realized. The genome is not only a " frozen " prescription for the production of proteins but is a dynamic system ( a network of genes ) in which different genes are activated and deactivated , such dynamic system could only function if there exist communication between its different parts , ( Ben- Jacob *et al.*, 1998)

In fact charge solitons can be controlled by applying external voltage and the UV characteristic can be measured with relative ease. One may also measured the magnetic response of circular DNA strands when applying time dependent magnetic flux. For short circular strands at low temperature which expected to absorbance persistent current, ( Murphy *et al.* , 1993 and Gasper and Schuster 1997) .

The thermal energy is smaller than the charging energy . For a typical small junction capacitance of  $10^{-15}$  farad , the temperature should be of the order of 1 Kelvin or lower , ( Hermon *et al.* , 1998) . They suggested that the charge and dipole solitonic like excitation may exist in both single strand and double strand DNA molecules . They presented a – model in which different elements in the DNA were described by their electromagnetic effective properties. This model is described as imaging (Shnirman *et al.*, 1997 and Braun *et al.*, 1998),Fig.2.



**Fig.2** A schematic image of : a) up the equivalent electrical circuit  $V$  and  $V_G$  are the external and the gate voltages, respectively.  $C$  and  $E_T$  are the capacitance and tunneling energy of the P-pond,  $C_0$  is the capacitance of the H-bond, and  $L_0$  and  $L_{1/2}$  are the longitudinal and lateral inductances, respectively.

b) A qubit made of one short DNA strand, attached to two long strands by two H-bonds. The long strands are metal-coated and connected to an external voltage source,  $V$ , via resistance,  $R$ , and inductance,  $L$ .

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## استخدام المجال المغناطيسي كأداة مؤثرة في فك حلزنة الحمض النووي DNA في الخميرة سكارومييسيس سيرفيسيا . شعبان شرف الدين قسم الوراثة الميكروبيية- المركز القومي للبحوث

بناء على وجود طاقة بالمجال المغناطيسي ، قد تم دراسة تأثيرها على المادة الوراثية أو DNA المستخلص من خلايا الخميرة . كنموذج لدراسة هذا التأثير، فقد استخدم سلالتين ذو تركيب وراثية أحادية ونو عدد من طفرات العوز الغذائي، حيث كانت إحداهما أكثر من الأخرى في عدد مواقع هذه الطفرات ، وقد تم تعريض عينات ال DNA للمجال المغناطيسي ( ١ - ٥ ، تسلا ) لمدة (٥-١٥ ق) علي طول موجي ٢٣٠-٢٦٠-٢٨٠ نانوميتر . وكانت المقارنات لقيم الامتصاص عند ٢٦٠ نانوميتر عند درجة [V حوارة ٧٢ م . ففي الجزء الأول من العمل تم قياس عينات ال DNA المستخلصة من السلالتين دون التعرض للمجال المغناطيسي . والجزء الثاني من العمل تم تعريض عينات ال DNA خلال عملية العزل مرة، وبعد إتمام العزل تماما لنفس الجرعات مره أخرى. وقد أوضحت النتائج أن عند ٠.١ تسلا ولمدة ٥ ق كانت الزيادة في قيم الامتصاص من ٠.٩٨٦ الي ٢.٢١١ ، قبل وبعد العزل علي الترتيب في السلالة Ura1 . بينما كانت الزيادة من ٠.٨٥٠ إلى ٠.٩٦٠ عند التعرض لنفس الجرعة وعند مدة ٥ ق لنفس السلالة. وقد تأثرت السلالة GM3 عند ٠.١ تسلا لمدة ١٥ ق فقد زادت قيم الامتصاص من ٠.٩٠٠ إلى ١.٤١٦ قبل وبعد العزل علي الترتيب. بينما كانت الزيادة من ٠.٨٢٠ الي ١.٠١٤ عند تعرض السلالة Ura1 ل ٠.٥ تسلا ولمدة ٥ ق قبل وبعد العزل علي الترتيب بينما كانت الزيادة من ٠.٧٦١ إلى ١.٦٧١ عند التعرض ل ٥ ق لنفس الجرعة ونفس السلالة. أما السلالة GM3 فكانت الزيادة من ١.٣١٠ إلى ١.٧٣٥ عند التعرض ل ٥ تسلا ولمدة ٥ ق ، أما عند الزمن ١٥ ق فقد زادت القيم من ١.٦٧٥ إلى ٢.١٥١ قبل وبعد العزل علي الترتيب . وقد كانت الزيادة معنوية جدا في السلالة GM3 عنها في السلالة Ura1 خاصة عند جرعة ٠.١ تسلا عند الفترة الزمنية ١٥ ق . بحساب الفروق المعنوية لقيم الامتصاص ، فقد كانت عالية جدا بين السلالتين ، وكانت معنوية بين وقت التعرض للمجال المغناطيسي لكل من السلالتين ، حيث كان ذلك في حالة التعرض بعد إتمام فصل ال DNA ، بينما عند التعرض أثناء عملية الفصل كانت الفروق معنوية جدا عند مستوي شدة التعرض للمجال المغناطيسي وبين السلالتين ، وكذلك بين شدة المجال المغناطيسي والسلالتين ، وكذا بين مدة التعرض وبين السلالتين . وبذلك كانت الفروق معنوية جدا بين مدة التعرض وشدة المجال المغناطيسي عند التعرض أثناء العزل عنها بعد إتمام عزل ال DNA . وقد كانت السلالة العالية الطفرات اعلي تأثيرا عند التعرض للمجال المغناطيسي عنها للسلالة الأقل عددا في الطفرات . وعند إعادة قياس قيم الامتصاص علي طول موجي ٢٦٠ نانوميتر للعينات التي تعرضت للمجال المغناطيسي أثناء العزل بعد مدة خمسة أيام علي درجة حرارة الغرفة (٣٥م°) فقد انخفضت قيم الامتصاص وهذا أن دل فأنه يدل علي أن جزء من ال DNA بدأ في الالتحام مرة أخرى ، إلا في حالة السلالة GM3 مازالت قيم الامتصاص عالية في حالة التعرض للمدتين (٥-١٥ ق)، مما يوضح انه من الممكن أن بعض سلالات الخميرة تحتفظ بطاقة المجال المغناطيسي داخل جزيئات ال DNA .