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Building Synthetic Chromosomes from Natural DNA

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Abstract: De novo chromosome synthesis is costly and time-consuming, limiting its use in research and biotechnology. Building synthetic chromosomes from natural components is an unexplored alternative with many potential applications. In this paper, we report CReATiNG (Cloning, Reprogramming, and Assembling Tiled Natural Genomic DNA), a method for constructing synthetic chromosomes from natural components in yeast. CReATiNG entails cloning segments of natural chromosomes and then programmably assembling them into synthetic chromosomes that can replace the native chromosomes in cells. We used CReATiNG to synthetically recombine chromosomes between strains and species, to modify chromosome structure, and to delete many linked, non-adjacent regions totaling 39% of a chromosome. The multiplex deletion experiment revealed that CReATiNG also enables recovery from flaws in synthetic chromosome design via recombination between a synthetic chromosome and its native counterpart. CReATiNG facilitates the application of chromosome synthesis to diverse biological problems.

Aim: To investigate the opinions on building synthetic chromosomes from natural dna.

Objective: To investigate the opinions for understanding genetic function, basic research, biotechnological applications and potential therapeutic applications among the undergraduate dental students based on the gender

To investigate the opinions for understanding genetic function, basic research, biotechnological applications and potential therapeutic applications among the undergraduate dental students based on the year of study

Method: A cross-sectional survey was conducted among 200 dental students, comprising 97 males (48.5%) and 103 females (51.5%). The survey included 15 questions about building synthetic chromosomes from natural dna among undergraduate dental students were analyzed based on gender, age and year of study using chi-square tests to identify statistically significant differences.

Keywords: Genetic engineering, Chromosomes, Synthetic biology, Synthetic chromosomes, natural dna, CReATiNG (Cloning, Reprogramming, Assembling, Tiled, Natural, Genomic DNA), Genes, Plasmids.

INTRODUCTION:

It is now possible to answer fundamental questions in biology by synthesizing chromosomes. For example, a longstanding question has been what is the minimal set of genes required by a living cell3–6? To answer this question, researchers used design-build-test cycles to synthesize a Mycoplasma myocytes chromosome that contains only 473 genes and still produces a free-living bacterium that replicates on lab timescales. Generating this minimal cell involved eliminating 428 (48%) of the genes that are naturally present in M. myocytes. Among the genes remaining in this minimal cell, 83% functioned in the expression and preservation of genetic information, the cell membrane, or cytosolic metabolism, while 17% had unknown functions. Production of this minimal cell demonstrates the potential for using chromosome synthesis to understand defining mechanisms underlying cellular life and its diversity. To date, synthetic chromosomes have exclusively been generated de novo, through the progressive assembly of small synthetic DNA fragments into larger molecules via a combination of in vitro and in vivo techniques. De novo synthesis is powerful because it allows the complete reprogramming of a chromosome's sequence and structure. For example, de novo chromosome synthesis was used to generate an Escherichia coli strain in which all 18,214 instances of three codons were synonymously reprogrammed, resulting in a strain that utilizes only 61 codons18. In another example, the Sc2.0 community is using de novo chromosome synthesis to generate a strain of the model budding yeast Saccharomyces cerevisiae in which all transposable elements have been eliminated and Lox sites have been incorporated between genes, enabling the generation of random chromosome rearrangements by Cre recombinase12.

The substantial amount of DNA fragment synthesis and assembly involved in de novo chromosome synthesis limits its use in biological research. Reductions in labor and reagent costs are needed to enable Biologists to employ chromosome synthesis more widely. Building synthetic chromosomes from cloned segments of natural DNA could be a relatively cheap and fast alternative to de novo chromosome synthesis. Such a method would enable the use of Chromosome synthesis in research that does not require complete Chromosome reprogramming. For example, projects that could be enabled include mapping the genetic basis of trait differences between Individuals and species, probing the structural requirements of chromosomes, and streamlining chromosomes through the systematic Elimination of non-essential genetic elements.

METHODOLOGY:

A. Study design and area: A cross sectional study was carried out at tertiary care teaching hospital Khammam.

B. Study population: The health care students including those of first year to internship dental students who responded to the offline paper print questionnaire survey.

C. Study Instrument: A self-administered questionnaire was designed based on BUILDING SYNTHETIC CHROMOSOMES FROM NATURAL DNA had a total 15 questions. Each participant has to fill their demographic data like Name, age, and year of study.

   Participants have to select one option from the answers provided against questions and the questions were based on building synthetic chromosomes from natural dna.

D. Pilot study: A pilot study was conducted on a group of students to assess the validity and reliability of study

E. Sampling method: The sampling method used is convenience method

F. Inclusion criteria: The students who were interested in study and who are willing to participate

G. Exclusion criteria: students who are not willing to participate are excluded

H. Organizing the study: The study was designed in a paper based version of the self-administered questionnaire of 15 questions focusing on knowledge, awareness.

   Includes the sections of demographic data: Name, Age, Sex and Year of study demographic information and asked to answer all questions by selecting one option from the provided answers.

I. Statistical analysis: Data from the filled questionnaire was conducted in a tabular form in an excel worksheet and evaluated for analysis the analysis was performed by SSPS version 29.

RESULTS: A total of 200 students took part in this with females (51.5) and male of (48.5). Age of the participants ranging from 18-25 years. In this study.

Females were more likely to demonstrate perception in dissection room experiences than male. Significantly second and third years showed greater familiarity with advanced applications than first, fourth years and intern students.

AGE

	N	Minimum	Maximum	Mean	Sta.deviation
Age	200	25	23	24	1.001

Gender

		Frequency	Percentage
Valid	Males	97	48.5
	Females	103	51.5
	Total	200	100

Year of study

		Frequency	Percentage
Valid	1st BDS	49	24.5
	2nd BDS	50	25
	3rd BDS	50	25
	4th BDS	40	20
	Internship	11	5.5
	Total	200	100

Distribution and comparison of responses based on gender:

Item	Response	Males		Females		Chi-square	P value
		n	%	n	%		
Q1	1	85	87.6	87	84.4	0.4284	0.9343
	2	4	4.1	5	4.8		
	3	0	0	0	0		
	4	8	8.2	11	10.6		
Q2	1	81	83.5	84	81.5	1.0851	0.7807
	2	8	8.24	7	6.7		
	3	3	3	3	2.9		
	4	5	5.1	9	8.7		
Q3	1	10	10.3	5	4.8	3.508	0.3197
	2	71	73.1	73	70.8		
	3	8	8.2	12	11.6		
	4	8	8.2	13	12.6		
Q4	1	8	8.2	6	5.8	2.7046	0.4394
	2	4	4.1	10	9.7		
	3	6	6.18	6	5.8		
	4	79	81.4	81	7.7		
Q5	1	8	8.2	7	6.7	1.8239	0.6097
	2	6	6.18	6	5.8		
	3	8	8.2	4	3.8		
	4	76	78.3	85	82.5		
Q6	1	5	5.15	9	8.7	1.6084	0.6575
	2	79	81.4	81	78.6		

	3	6	6.18	8	7.7		
	4	7	7.2	5	4.8		
Q7	1	2	2	8	7.7	4.6282	0.2011
	2	84	86	88	85.4		
	3	5	5.15	4	3.8		
	4	6	6.18	3	2.9		
Q8	1	7	7.2	8	7.7	0.2928	0.9614
	2	9	9.2	8	7.7		
	3	7	7.2	9	8.7		
	4	73	75.2	79	76.6		
Q9	1	6	6.18	7	6.7	1.8221	0.6101
	2	6	6.18	8	7.7		
	3	77	79.3	84	81.5		
	4	8	8.2	4	3.8		
Q10	1	8	8.2	3	2.9	5.1511	0.1611
	2	3	3	8	7.7		
	3	6	6.1	4	3.8		
	4	80	82.4	88	85.4		
Q11	1	7	7.2	4	3.8	1.215	0.7494
	2	6	6.1	5	4.8		
	3	79	81.4	87	84.4		
	4	6	6.1	6	5.8		
Q12	1	87	89.6	87	84.4	2.1076	0.5504
	2	5	5.1	5	4.8		
	3	3	3.0	6	5.8		
	4	2	2.06	5	4.8		
Q13	1	6	6.1	11	10.6	8.0544	0.0449
	2	10	10.3	9	8.7		
	3	66	68	77	74.7		
	4	16	16.4	5	4.8		
Q14	1	5	5.1	8	7.7	1.2023	0.7525
	2	9	9.2	7	6.7		
	3	79	81.4	80	77.6		
	4	5	5.1	7	6.7		
Q15	1	2	2.06	3	2.9	0.3419	0.952
	2	91	93.8	93	90.2		
	3	2	2.06	3	2.9		
	4	3	3.0	3	2.9		

P≤0.05 is statistically significant

Distribution and comparison of responses based on year of study:

Item	Response	1st BDS		2nd BDS		3rd BDS		4th BDS		Intern		Chi-square	P value
		n	%	n	%	n	%	n	%	n	%		
Q1	1	43	87.7	47	94	48	96	34	85	0	0	123.9	0.001*
	2	5	10.2	3	6	1	2	0	0	0	0		
	3	0	0	0	0	0	0	0	0	0	0		
	4	1	2.1	0	0	1	2	6	15	11	100		
Q2	1	45	91.8	36	72	37	74	36	90	11	100	16.6522	0.1632
	2	1	2.1	7	14	7	14	0	0	0	0		

	3	1	2.1	2	4	2	4	1	2.5	0	0		
	4	2	4	5	10	4	8	3	7.5	0	0		
Q3	1	5	10.2	2	4	4	8	0	0	4	36.36	67.413	9.7305
	2	37	75.5	43	86	22	44	40	100	2	18.18		
	3	5	10.2	2	4	11	22	0	0	2	18.18		
	4	2	4.1	3	6	13	26	0	0	3	27.27		
Q4	1	1	2.1	0	0	2	4	7	17.5	4	36.36	94.73	5.9952
	2	0	0	0	0	3	6	7	17.5	4	36.36		
	3	0	0	0	0	2	4	7	17.5	3	27.27		
	4	48	97.9	50	100	43	86	19	47.5	0	0		
Q5	1	1	2.1	3	6	1	2	4	10	4	36.36	70.251	2.8735
	2	0	0	0	0	3	6	6	15	3	27.27		
	3	0	0	0	0	3	6	6	15	3	27.27		
	4	48	97.9	47	94	43	86	24	60	1	9		
Q6	1	1	2.1	0	0	0	0	10	25	3	27.27	129.22	0.001*
	2	48	97.9	50	100	49	98	10	25	3	27.27		
	3	0	0	0	0	1	2	11	27.5	2	18.18		
	4	0	0	0	0	0	0	9	22.5	3	27.27		
Q7	1	0	0	0	0	0	0	6	15	4	36.36	108.68	0.002*
	2	49	100	50	100	50	100	20	50	3	27.27		
	3	0	0	0	0	0	0	8	20	1	9		
	4	0	0	0	0	0	0	6	15	3	27.27		
Q8	1	5	10.2	0	0	0	0	7	17.5	3	27.27	71.75	1.5006
	2	5	10.2	1	2	0	0	9	22.5	2	18.18		
	3	4	8.16	1	2	0	0	8	20	3	27.27		
	4	35	71.4	48	96	50	100	16	40	3	27.27		
Q9	1	0	0	0	0	0	0	10	25	3	27.27	133.22	0.003*
	2	1	2.1	0	0	0	0	10	25	3	27.27		
	3	48	97.9	50	100	50	100	10	25	3	27.27		
	4	0	0	0	0	0	0	10	25	2	18.18		
Q10	1	0	0	0	0	0	0	9	22.5	2	18.18	126.37	0.001*
	2	0	0	0	0	0	0	7	17.5	4	36.36		
	3	0	0	0	0	0	0	6	15	4	36.36		
	4	49	100	50	100	50	100	18	45	1	9		
Q11	1	2	4.1	0	0	0	0	7	17.5	2	18.18	72.91	9.091
	2	2	4.1	0	0	0	0	6	15	3	36.36		
	3	42	85.7	50	100	50	100	21	52.5	3	36.36		
	4	3	6.1	0	0	0	0	6	15	3	36.36		
Q12	1	41	83.6	49	98	50	100	34	85	0	0	88.95	7.8715
	2	4	8.16	0	0	0	0	2	5	4	36.36		
	3	3	6.12	0	0	0	0	2	5	4	36.36		
	4	1	2.1	1	2	0	0	2	5	3	27.27		
Q13	1	2	4.08	2	4	4	8	6	15	3	27.27	34.65	5.3083
	2	4	8.16	2	4	4	8	6	15	3	27.27		
	3	42	85.7	44	88	33	66	21	52.5	3	27.27		
	4	1	2.1	2	4	9	18	7	17.5	2	18.18		
Q14	1	0	0	0	0	0	0	10	25	3	27.27	125.43	0.002*
	2	1	2.1	2	4	0	0	10	25	3	27.27		
	3	48	97.0	48	96	50	100	11	27.5	2	18.18		

	4	0	0	0	0	0	0	9	22.5	3	27.27		
Q15	1	0	0	0	0	3	6	1	2.5	1	9	28.82	0.0042
	2	49	100	50	100	41	82	37	92.5	7	63.62		
	3	0	0	0	0	3	6	1	2.5	1	9		
	4	0	0	0	0	3	6	1	2.5	2	18.18		

P≤0.05 is statistically significant

DISCUSSION: The study assessed about building synthetic chromosomes from natural dna among undergraduate dental students in Khammam city. The demographic analysis revealed that participants were primarily between 20 and 26 years old, with a slight female majority.

CReATiNG makes it possible to build synthetic chromosomes with diverse designs using natural components. Because CReATiNG employs cloned segments of natural chromosomes as opposed to small DNA fragments synthesized de novo, it is substantially cheaper and faster than de novo chromosome synthesis. For example, some of the final chromosomes completed for this paper went from in silicon design to in vivo testing within a month and cost less than five hundred dollars to produce. Although some synthetic chromosome designs will require complete chromosome reprogramming, which is not possible with CReATiNG, many will not. Indeed, we have shown here that CReATiNG can be used to study a variety of fundamental questions in genetics, genomics, and evolution. Moreover, we unexpectedly found an additional benefit of CReATiNG, which is that it can allow cells to recover from unknown design flaws via recombination between a synthetic chromosome and its native counterpart.

CONCLUSION:

The development of CReATiNG (Cloning, Reprogramming and Assembling Tiled Natural Genomic DNA) technology marks a significant breakthrough in synthetic biology. This innovative method enables researchers to construct synthetic chromosomes from natural DNA components, offering a simpler and more cost-effective approach than traditional de novo synthesis methods.

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