PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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Inventor(s)	:	Daniel E. Bauer, et al.	
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Examiner	:	Nancy J. Leith	
Customer No.	:	198696	
Docket No.	:	BROD-3520US-CON2	
Title	:	FUNCTIONAL GENOMICS USING CRISPR-CAS SYSTEMS FOR SATURATING MUTAGENESIS OF NON-CODING ELEMENTS, COMPOSITIONS, METHODS, LIBRARIES AND APPLICATIONS THEREOF	

AMENDMENT AND RESPONSE

FILED VIA EFS-WEB

Commissioner:

In response to the Final Office Action mailed on April 22, 2025 ("Office Action"), please enter and consider the following amendments and remarks.

Certificate of EFS-WEB Transmission pursuant to 37 C.F.R. § 1.8

I hereby certify that this correspondence is being transmitted via the U.S. Patent and Trademark Office electronic filing system (EFS-Web) to the USPTO, on July 21, 2025.

/Christopher D. Southgate/

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AMENDMENT

In the Claims

Please amend the claims as follows. This listing of claims will replace all prior versions and listings of claims in this application.

1. (**Withdrawn**) A method of screening for genomic sites associated with a change in a phenotype, the method comprising:

(a) introducing a deep scanning mutagenesis guide library comprising a plurality of guide molecules into a population of cells,

wherein each cell of the population contains no more than one guide molecule, and

wherein the library comprises guide molecules, each targeting a non-coding genomic sequence upstream of every PAM sequence within a continuous genomic region;

(b) sorting the cells into at least two groups based on the change in phenotype and

(c) determining the relative representation of the guide molecules present in each group, whereby genomic sites associated with the change in phenotype are determined by the representation of guide molecules present in each group.

2. (**Withdrawn**) The method of claim 1, wherein the continuous genomic region comprises at least 50 kb of genomic DNA.

3. (**Withdrawn**) The method of claim 1, wherein the continuous genomic region comprises one or more transcription enhancer or repressor elements.

4. (**Withdrawn**) The method of claim 1, wherein the continuous genomic region comprises one or more epigenetic modification sites.

5. (**Withdrawn**) The method of claim 1, wherein the change in phenotype is expression of a gene product.

6. (**Withdrawn**) The method of claim 5, wherein the gene product is a transcription factor.

7. (**Withdrawn**) The method of claim 6, wherein the transcription factor is BCL11A.

8. (**Withdrawn**) The method of claim 1, wherein the guide molecules of the guide library target at least 100 genomic non-coding sequences comprising non-overlapping cleavage sites upstream of a PAM sequence for every 1000 base pairs within the at least one continuous genomic region.

9. **(Withdrawn)** The method of claim 1, wherein the population of cells is eukaryotic cells.

10. (**Withdrawn**) The method of claim 9, wherein the eukaryotic cells are hematopoietic stem cells.

11. (**Withdrawn**) The method of claim 1, wherein the guide molecules are Type II CRISPR-Cas guide molecules.

12. (**Withdrawn**) The method of claim 1, further comprising preparing a Type II CRISPR-Cas therapeutic composition comprising one or more guide molecules that target the genomic sites associated with the change in phenotype.

13. (**Withdrawn**) The method of claim 12, further comprising administering the Type II CRISPR-Cas therapeutic composition to a cell *ex vivo* or *in vitro* to generate an engineered cell modified at the genomic site determined to be associated with the change in phenotype.

14. **(Currently amended)** An isolated modified eukaryotic cell obtained by a method comprising

introducing a deep scanning mutagenesis guide library into a population of cells for screening non-coding genomic sites associated with a change in a phenotype, the population of cells comprising a plurality of Type II CRISPR-Cas system guide molecules,

wherein each cell of the population contains a Cas polypeptide and no more than one guide molecule,

wherein each guide molecule targets a non-coding genomic sequence to generate a ~~single-site small~~ an indel mutation within a continuous genomic region,

wherein the indel mutation has a mean deletion size of 11 bp and a mean insertion size of 4 bp that is offset by about 10 bp from the expected cleavage point,

wherein the plurality of guide molecules target at least 100 genomic non-coding sequences comprising non-overlapping cleavage sites upstream of a PAM sequence for every 1000 base pairs within the at least one continuous genomic region;

wherein the plurality of guide molecules are capable of targeting two or more continuous genomic regions that physically interact, the at least one continuous genomic region comprising noncoding sequences,

wherein the guide molecules have a median adjacent genomic cleavage distance between 4 bp and 20 bp, and

sorting the cells into at least two groups based on the change in phenotype; and

determining relative representation of the guide molecules present in each group, whereby genomic sites associated with the change in phenotype are determined by the representation of guide molecules present in each group;

preparing a Type II CRISPR-Cas composition comprising the one or more guide molecules identified in the library that target the non-coding genomic sites determined to be associated with the change in phenotype;

administering the Type II CRISPR-Cas composition to an unmodified eukaryotic cell to obtain the modified eukaryotic cell comprising one or more single-site indel mutations at the non-coding genomic site determined to be associated with the change in phenotype,

wherein the genomic sites associated with the change in phenotype comprise one or more transcription enhancer or repressor elements.

15. (**Original**) The isolated modified eukaryotic cell of claim 14, wherein the change in phenotype is expression of a gene product.

16. **(Previously Presented)** The isolated modified eukaryotic cell of claim 15, wherein the gene product is a transcription factor.

17. (**Original**) The isolated modified eukaryotic cell of claim 16, wherein the transcription factor is BCL11A.

18. (**Canceled**)

19. **(Previously Presented)** The isolated modified eukaryotic cell of claim 14, wherein the modified eukaryotic cell is a hematopoietic stem cell.

20. **(Previously Presented)** The isolated modified eukaryotic cell of claim 14, wherein the CRISPR-Cas composition comprises a *S. pneumoniae, S. pyogenes*, or *S. thermophilus* Cas9.

21. (**Withdrawn**) A method for selecting CRISPR-Cas guide molecules comprising:

introducing a deep scanning mutagenesis guide library comprising a plurality of guide molecules into a population of cells wherein each cell of the population contains no more than one guide molecule and wherein the library comprises guide molecules, each targeting a non-coding genomic sequence upstream of every PAM sequence within a continuous genomic region;

sorting the cells into at least two groups based on a change in phenotype; and

determining relative representation of the guide molecules present in each group, whereby genomic sites associated with the change in phenotype are determined by the representation of guide molecules present in each group;

selecting the CRISPR-Cas guide molecules that target genomic sites associated with the change in phenotype.

22. (**Withdrawn**) The method of claim 21, wherein the change in phenotype is expression of a gene product.

23. (**Withdrawn**) The method of claim 22, wherein the gene product is a transcription factor.

24. (**Withdrawn**) The method of claim 23, wherein the transcription factor is BCL11A.

25. (**Withdrawn**) The method of claim 21, wherein the genomic sites associated with the change in phenotype comprise one or more transcription enhancer or repressor elements.

26. (**Withdrawn**) The method of claim 21, wherein the CRISPR-Cas guide molecule is a Type II guide molecule.

27. (**Withdrawn**) A CRISPR-Cas composition comprising the Type II guide molecule of the method of claim 26, and a Type II Cas polypeptide.

28. (**Withdrawn**) A set of CRISPR-Cas guide molecules, each guide molecule comprising a guide sequence capable of targeting a non-coding genomic sequence within at least one continuous genomic region.

29. (**Withdrawn**) The set of CRISPR-Cas guide molecules of claim 28, wherein the guide molecules target at least 100 genomic non-coding sequences comprising non-overlapping cleavage sites upstream of a PAM sequence for every 1000 base pairs within the at least one continuous genomic region.

30. (**Withdrawn**) The set of CRISPR-Cas guide molecules of claim 28, wherein the guide molecules have a median adjacent genomic cleavage distance between 4 bp and 20 bp.

REMARKS

Status of the Claims

Claims 1–17 and 19–30 are pending, with claims 1, 14, 21, and 28 being independent. Claim 14 is amended. Claim 18 is cancelled without prejudice or disclaimer as to the subject matter recited therein. Claims 1-13 and 21-30 are withdrawn from consideration pursuant to the Restriction Requirement of April 8, 2019.

No new matter has been added.

Claim Rejections under 35 U.S.C. § 112(a), Written Description

In the Office Action, the Examiner rejected claims 14-17 and 19-20 under 35 U.S.C. § 112, first paragraph, as allegedly lacking written description support.

Claim 14 is amended to require *inter alia*:

> . . . wherein each guide molecule targets a non-coding genomic sequence to generate ***an indel mutation offset by about 10 bp from the expected cleavage point, wherein the indel mutation has a mean deletion size of 11 bp and a mean insertion size of 4 bp***, . . (Emphasis added)

Written description support for the claim amendment can be found at the following locations of the corresponding published U.S. Patent Application No. 2024/0102004:

Paragraph [0084]:

> FIG. 37A-37C shows deep-sequencing analysis of insertion-deletion (indel) mutations after genome modification using validation set sgRNAs. a, Mean and standard error of the percent of reads containing an indel mutation for sgRNAs targeting noncoding regions near CUL3 and coding exons of CUL3 (n=24 noncoding sgRNAs, 4 exon-targeting sgRNAs). Cells were selected for lentiviral CRISPR constructs using puromycin for 7 days and then plated in R10+DMSO for a further 4 days. ***b, Average size of insertions (left) and deletions (right) in sgRNAs targeting noncoding regions near CUL3*** and CUL3 exons. c, ***Histograms of indel mutation sizes for 2 sgRNAs that target noncoding regions near CUL3***. Deletions are shown in red and insertions are shown in blue. The larger deletion size (shown in aggregate in b,) can also be seen for these 2 sgRNAs.

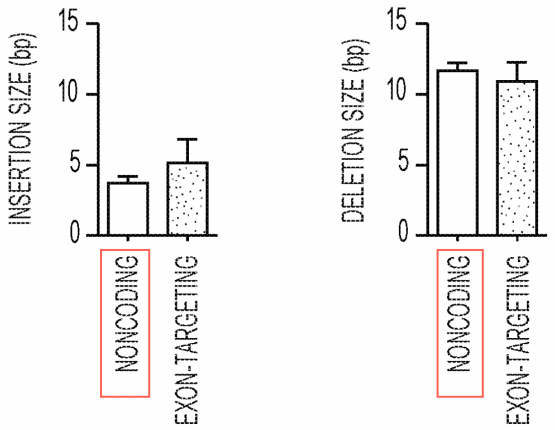

FIG. 37B

Paragraph [0354]:

> Applicants tested the pattern of mutations observed upon treatment of cells with either sgRNA- 1621 or sgRNA-1617 by deep sequencing. Each of these sgRNAs is sufficient to substantially induce HbF in human erythroid cells (FIG. 3i; FIGS. 8a and 23b). Applicants sorted cells exposed to Cas9 and these sgRNAs into HbF-high and HbF-low pools. Applicants determined the indel spectrum in each population by deep sequencing (FIG. 9b). As expected, ***Applicants observed indels clustering around the predicted cleavage positions*** . . . Notably ***both sgRNAs yielded maximal HbF enriching indels not precisely at the expected cleavage position but offset by about 10 bp*** (FIG. 5). (Emphasis added)

Paragraph [0412]:

> Although these properties of enriched sgRNA target sites suggest functionality, Applicants wanted to confirm that mutations in these specific noncoding regions lead to altered drug resistance and to test if these changes were mediated by CUL3. To assay specific sites for noncoding function, ***Applicants individually cloned 25 sgRNAs that had a positive enrichment ratio into lentiviral vectors*** and produced virus (FIG. 33A and table S2). For this validation set, Applicants selected sgRNAs that have at least one other similarly enriched sgRNA within 500 bp. Applicants also attempted to choose these groups of sgRNAs for our validation set from several different genomic regions (e.g. 5′ and 3′ UTRs, promoter, intron, distal 5′ and 3′ regions) in order to understand the relative regulatory ability of noncoding elements across different locations. ***Applicants transduced each lentivirus individually into A375 cells.*** After selection for 7 days, ***Applicants amplified genomic DNA regions surrounding each sgRNA target and found an average of 85% of amplicons contained insertion-deletion (indel) mutations with near complete genome editing at most target sites (mean deletion size=11 bp, mean insertion size=4 bp, n>5000 reads per site)*** (FIG. 37) (table S3). After verifying genome modification at the targeted sites, Applicants measured CUL3 expression using a sensitive ddPCR hydrolysis probe assay. Applicants found that 24 out of the 25 validation

> sgRNAs resulted in decreased CUL3 expression relative to non-targeting sgRNAs (FIG. 33B, left) . . .. (Emphasis added)

Applicants respectfully submit that the rejection does not apply to the claims as presently presented. Withdrawal of the rejection is respectfully requested.

Claim Rejection under 35 U.S.C. § 112(b)

In the Office Action, the Examiner rejected claims 14-17 and 19-20 under 35 U.S.C. § 112(b) as allegedly lacking clarity. Applicant respectfully submits that rejection does not apply to the claims as presently presented. Withdrawal of the rejection is respectfully requested.

Obviousness-Type Double Patenting (OTDP)

The Examiner rejected claims 14-17 and 19-20 of the pending application on the grounds of non-statutory obviousness-type double patenting as being unpatentable over the claims of the following U.S. Patents and pending patent applications in view of Bauer I (Bauer et al., Generation of genomic deletions in mammalian cell lines via CRISPR/Cas9. J Vis Exp. 2015; (95):e52118) and Bauer II (Bauer et al., An erythroid enhancer of BCL11A subject to genetic variation determines fetal hemoglobin level. Science. 2013 Oct 11;342(6155):253-7):

OTDP Rejection 1:	over claims 1-2, 5-9, 12-16, and 19-20 of U.S. Patent No. 8,697,359.
OTDP Rejection 2:	over claims 1-2, 6-9, 11-12, 15-19, 21-24, 26, and 28-29 of U.S. Patent No. 8,771,945.
OTDP Rejection 3:	over claims 1-2, 4-7, 10-11, 13-18, 20-23, 26-27, and 29-30 of U.S. Patent No. 8,795,965.
OTDP Rejection 4:	over claims 1-2, 6-7, 9-10, 13-14, 17-20, 24, and 27-30 of U.S. Patent No. 8,865,406.
OTDP Rejection 5:	over claims 1-2, 7-11, 16-18, 20-23, 26, and 29-30 of U.S. Patent No. 8,871,445.
OTDP Rejection 6:	over claims 1, 3, 6-10, 13, 15, 18-21, 24, and 29-30 of U.S. Patent No. 8,889,356.
OTDP Rejection 7:	over claims 1-28 of U.S. Patent No. 8,889,418.

OTDP Rejection 8: over claims 1-2, 4, 7-9, 11, 14-16, and 18-21 of U.S. Patent No. 8,895,308.

OTDP Rejection 9: over claims 1 and 29-30 of U.S. Patent No. 8,906,616.

OTDP Rejection 10: over claims 1, 3, 7-10, 13-16, 19-22, 25, and 30 of U.S. Patent No. 8,932,814.

OTDP Rejection 11: over claims 1, 4-5, 7-8, 11, 14-15, 17-18, 21, 24-25, and 27-28 of U.S. Patent No. 8,945,839.

OTDP Rejection 12: over claims 1-4, 8, 13-15, 18, 22-23, 26-27, and 42 of U.S. Patent No. 8,993,233.

OTDP Rejection 13: over claims 1, 6, 8-9, 12, 16, 18-19, 22, 25, and 27-28 of U.S. Patent No. 8,999,641.

OTDP Rejection 14: over claims 1, 12-13, and 22-27 of U.S. Patent No. 11,597,949.

OTDP Rejection 15: over claims 1, 14-16, 18, 29-31, 33, 38-41, and 46 of co-pending application number 14/738,398.

OTDP Rejection 16: over claims 13-17 and 19-23 of co-pending application number 14/703,511.

OTDP Rejection 17: over claims 2, 4-6, and 12-18 of co-pending application number 14/704,551.

OTDP Rejection 28: over claims 45 and 54-58 of co-pending application number 14/971,169.

OTDP Rejection 29: over claim 1 of co-pending application number 15/160,710.

OTDP Rejection 30: over claims 1 and 10-12 of co-pending application number 15/230,025.

OTDP Rejection 31: over claims 10-11, 17, 19-21, 24, 29-30, and 37-38 of co-pending application number 15/230,161.

OTDP Rejection 32: over claims 1, 30, and 40 of co-pending application number 15/330,876.

OTDP Rejection 33: over claim 74 of co-pending application number 15/430,260.

OTDP Rejection 34: over claims 1, 3, 8, 10, 16-20, 22-23, 30-31, and 70 of co-pending application number 15/967,464.

OTDP Rejection 35: over claims 58 and 61 of co-pending application number 15/967,495.

OTDP Rejection 36: over claim 52 of co-pending application number 16/177,403.

OTDP Rejection 37: over claims 31, 34, 44, and 47-49 of co-pending application number 16/525,531.

OTDP Rejection 38: over claims 2 and 6-11 of co-pending application number 16/532,442.

OTDP Rejection 39: over claims 47-50 and 63 of co-pending application number 16/535,043.

OTDP Rejection 40: over claims 30 and 51 of co-pending application number 16/906,580.

OTDP Rejection 41: over claims 60, 75-76, 78, and 81 of co-pending application number 16/938,110.

OTDP Rejection 42: over claims 1, 3-6, 8-9, 11, 13-17, 19, 22-26, and 32 of co-pending application number 16/943,234.

OTDP Rejection 43: over claims 1-22 of co-pending application number 17/034,754.

OTDP Rejection 44: over claims 31-60 of co-pending application number 18/109,550.

OTDP Rejection 45: over claims 1-30 of co-pending application number 18/128,122

OTDP Rejection 46: over claims 1-29 of co-pending application number 18/134,317.

OTDP Rejection 47: over claims 1-30 of co-pending application number 18/217,352.

OTDP Rejection 48: over claims 1-30 of co-pending application number 18/217,357.

OTDP Rejection 49: over claims 1-21, and 23-30 of co-pending application number 18/454,343.

OTDP Rejection 50: over claim 1 of co-pending application number 19/028,666.

OTDP Rejection 51: over claim 1 of co-pending application number 19/028,841.

In OTDP rejections 1-51, the Examiner alleges that although the instant claims and the claims of the cited Patent and pending patent applications are not identical, they are not patentably distinct from each other.

The Examiner concedes that the 359, ‘945, ‘965, ‘406, ‘445, ‘356, ‘418, ‘308, ‘616, ‘814, ‘839, ‘233, ‘641, and ‘949 patents and the ‘398, ‘511, ‘551, ‘169, ‘710, ‘025, ‘161, ‘876, ‘260, ‘464, ‘495, ‘403, ‘531, ‘442, ‘043, ‘580, ‘110, ‘234, ‘754, ‘550, ‘122, ‘317, and ‘343 patent application do not claim that the modifications are in a non-coding region. None of the cited documents claims that the non-coding genomic sequence associated with a phenotypic change is a transcription enhancer or repressor as required by the claimed invention.

Nevertheless, the Examiner cites to **Bauer I** stating:

> Bauer I discloses that CRISPR-Cas9 systems can be used to produce genomic deletions and loss-of-function studies, which is interpreted as altering the phenotype of a cell (abstract). ***Bauer I discloses that CRISPR-Cas9 systems can be used to study non-coding elements (abstract).*** Bauer I discloses that the CRISPR-Cas9 can be from *Streptococcus pyogenes* (page 1, second paragraph). ***Bauer I discloses a variety of strategies to produce deletions at a target (Figure 1).***

The Examiner further cites to **Bauer II,** stating:

> Bauer II discloses that the transcription factor BCL11A (a transcriptional repressor) variation determines the level of fetal hemoglobin (abstract). Bauer II discloses that the genetic variation lies in the noncoding sequences (abstract). Bauer II discloses that a BCL11A erythroid deficiency rescues hematologic features of sickle cell disease, which is interpreted as being a hematopoietic stem cell (paragraph bridging pages 253 and 254).

The Examiner concludes it would have been obvious to one with ordinary skill in the art before the effective filing date of the claimed invention to employ the methods of ‘359, ‘945, ‘965, ‘406, ‘445, ‘356, ‘418, ‘308, ‘616, ‘814, ‘839, ‘233, ‘641, and ‘949 patents and the ‘398, ‘511, ‘551, ‘169, ‘710, ‘025, ‘161, ‘876, ‘260, ‘464, ‘495, ‘403, ‘531, ‘442, ‘043, ‘580, ‘110, ‘234, ‘754,

‘550, ‘122, ‘317, ‘343, ‘666 and ‘841 applications, together with the methods of Bauer I to target Bauer II’s BCLIIA transcription repressor’s non-coding regions in order to construct a hematopoietic cell having an altered phenotype. Therefore, the claims are not deemed to be patentably distinct.

The Applicant respectfully disagrees with the Examiner.

1) ***The cited references fail to teach or suggest each and every one of the elements of the claimed invention***

Claim 14 and claims dependent therefrom require *inter alia*:

. . . .wherein ***each cell of the population contains*** a Cas polypeptide and ***no more than one guide molecule***, ...

On the contrary, the Abstract of Bauer I ***explicitly*** states:

> . . . Here we present a simple methodology for CRISPR design, cloning, and delivery for the production of genomic deletions. In addition, we describe techniques for deletion, identification, and characterization. ***This strategy relies on cellular delivery of a pair of chimeric single guide RNAs (sgRNAs) to create two double-strand breaks (DSBs) at a locus*** *in order to delete the intervening DNA segment by non-homologous end joining (NHEJ) repair.* Deletions have potential advantages as compared to single-site small indels given the efficiency of biallelic modification, ease of rapid identification by PCR, predictability of loss-of-function, and utility for the study of non-coding elements. This approach can be used for efficient loss-of-function studies of genes and genetic elements in mammalian cell lines. (Emphasis added)

Bauer I’s method of CRISPR-Cas mutagenesis, therefore, relies on a ***pair*** of sgRNAs that produce two double-strand breaks and delete the intervening DNA. In contrast, the claimed invention requires introducing a deep-scanning mutagenesis guide library into a cell population so that "each cell of the population contains ***no more than one guide molecule"*** that in the presence of CRISPR-Cas produces an indel mutation having a mean deletion size of 11 bp and a mean insertion size of 4 bp.

Paragraph [0208] of the specification further states:

> The term "cleavage site" refers to any site that can be cleaved by a CRISPR enzyme after binding to a target sequence. In general, wild-type S. pyogenes Cas9 (SpCas9) is known to make ***a blunt cut between the 17th and 18th bases in the target sequence*** (3 bp 5' of the PAM) (Nature Protocols November; 8(11):2281-308).

Consequently, the mutation created by each guide molecule targeting a non-coding genomic sequence is a single ***indel mutation*** generated by nonhomologous end joining (NHEJ) repair at a ***single*** double-strand break.

Paragraph [340] of the specification states:

> Applicants hypothesized that composite enhancers may be composed of a functional hierarchy with essential and dispensable constituent components. A functional hierarchy can enable enhancer ***disruption by a single DSB at a critical region followed by nonhomologous end joining (NHEJ) repair with indels***. Indeed single nucleotide changes themselves may substantively modulate enhancer function. Therefore Applicants reasoned that a tiling set of sgRNAs could uncover critical enhancer regions by disruption of essentially *all sequences within an enhancer given the typical* ***indel spectrum of each sgRNA of at least 10 bp***. (Emphasis added)

Paragraph [0342] confirms:

> These results indicate that the large majority of sgRNAs in the library were competent to produce indels.

Having identified candidate indel mutations in non-coding genomic sites associated with a change in phenotype, the claimed invention further requires administering a Type II CRISPR-Cas composition comprising *a candidate guide molecule identified in the library* to an unmodified eukaryotic cell *to obtain a modified eukaryotic cell comprising the indel mutation in the non-coding genomic site* determined to be associated with the change in phenotype.

Bauer I's Abstract further emphasizes:

> ***Deletions have potential advantages as compared to single-site small indels*** given the efficiency of biallelic modification, ease of rapid identification by PCR, predictability of loss-of-function, and utility for the study of non-coding elements.

Thus, a person of ordinary skill in the art would understand Bauer I explicitly teaches away from the claimed invention by pointing out the potential advantages of the disclosed deletion strategy as compared to single-site small indels.

The Examiner appears to infer Bauer I discloses other deletion strategies that do not rely on a pair of single guide RNAs (sgRNAs). Specifically, the Examiner states at page 7 of the Office Action:

"Bauer I discloses a variety of strategies to produce deletions at a target (Figure 1)."

The Applicant respectfully disagrees.

Figure 1 of Bauer I is reproduced below:

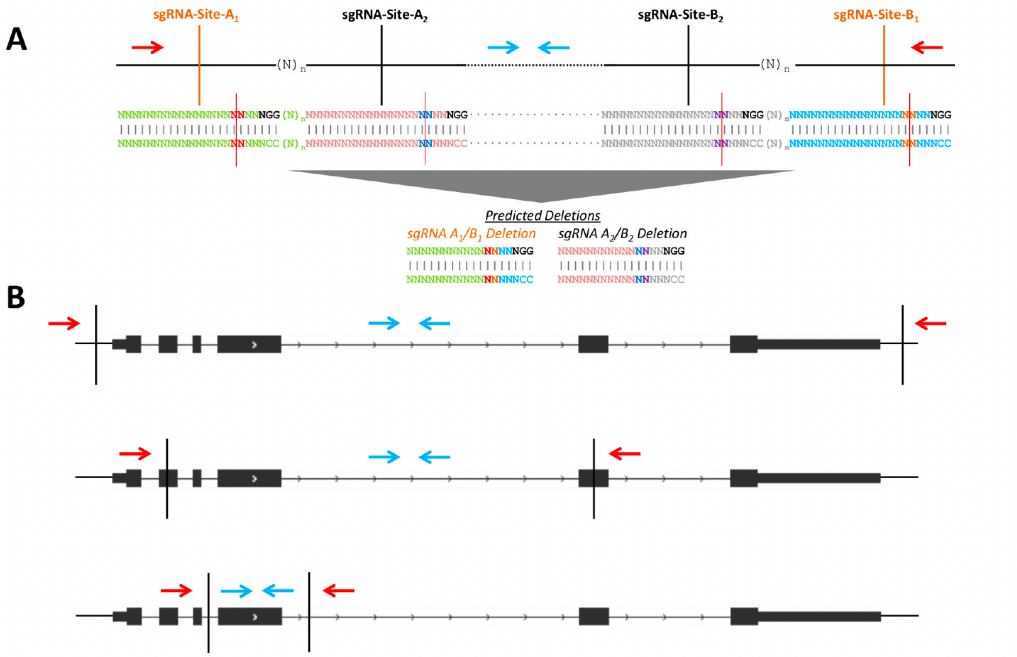

Figure 1. Schematic of possible deletion strategies. (A) Two example sgRNA pairs for genomic deletions (shown in black and orange, respectively). The blue arrows indicate primers to detect the non-deletion amplicon and red arrows indicate primers to detect the deletion amplicon. sgRNA positions 17 and 18 are highlighted in red and blue at sgRNA-Sites-A_1/A_2 and are highlighted in purple and orange at sgRNA-Sites-B_1/B_2 with a red line indicating the predicted Cas9 cleavage between positions 17 and 18. **(B)** CRISPR-directed cleavages are shown as vertical black lines. The blue arrows indicate primers to detect the non-deletion amplicon and red arrows indicate primers to detect the deletion amplicon.

FIG. 1A depicts two sgRNA pairs for genomic deletions. FIG. 1B shows CRISPR-directed cleavages as vertical black lines. It is self-evident that the portrayed deletion strategies all rely on a pair of single guide RNAs (sgRNAs) to create two double-strand breaks (DSBs) at a locus followed by deletion of the intervening DNA.

2) A person of ordinary skill in the art would not have been motivated to generate the Applicant's modified eukaryotic cell using Bauer I's methodology.

To effectively identify regulatory elements within the BCL11A gene, the 2013 Bauer II reference presents a comparison of the distribution of HbF-associated SNPs at BCL11A with DNase I hypersensitive sites (DHSs) at positions +62, +58, and +55. These sites, measured in kilobases from the transcription start site (TSS) of BCL11A, act as major indicators of chromatin state, strongly suggesting their regulatory potential. ChIP-seq analysis revealed histone modifications with an enhancer signature overlapping the trait-associated SNPs at BCL11A intron-

2. The primary erythroid transcription factors (TFs) GATA1 and TAL1 also occupy this enhancer region. ChIP-qPCR confirmed three distinct peaks of GATA1 and TAL1 binding within BCL11A intron-2, each located within an erythroid DHS. Interactions between the BCL11A promoter and fragments across 250 kb of the BCL11A locus using a chromosome conformation capture assay also showed that the strongest promoter interaction occurred in the region of intron-2 with trait-associated SNPs.

Thus, according to at least the Bauer II reference, a person of ordinary skill in the art would have known that the BCL11A enhancer was likely situated near the DNase I hypersensitive sites (DHSs) at positions +62, +58, and +55. The CRISPR-Cas analysis of the BCL11A locus, using the methodology described in Bauer et al., which generates large deletions from 8kb to >1Mb (see Discussion), would yield nothing more than a fundamental analysis of the regulatory elements necessary for BCL11A gene transcription, which was primarily already known in the art.

In contrast, in paragraph [0014] of the specification, the Applicant summarizes the saturation mutagenesis approach as follows:

> . . the present invention provides for a deep scanning mutagenesis library to interrogate phenotypic changes in a population of cells comprising a plurality of CRISPR-Cas system guide RNAs comprising guide sequences that are capable of targeting a plurality of genomic sequences within at least one continuous genomic region, wherein the guide RNAs target at least 100 genomic sequences comprising non-overlapping cleavage sites upstream of a PAM sequence for every 1000 base pairs within the continuous genomic region. Not being bound by a theory, *providing at least 100 guide RNAs, wherein the guide RNAs target at least 100 genomic sequences comprising non-overlapping cleavage sites upstream of a PAM sequence for every 1000 base pairs within a continuous genomic region may result in mutagenesis saturation of the genomic region because cleavage sites for each guide RNA target may differ by about 10 base pairs*. Not being bound by a theory, if each guide RNA results in cleavage of 10 base pairs of the 1000 base pairs, then *the **entire genomic region would be saturated***. . . . The library may comprise guide RNAs wherein the adjacent genomic cleavage distance is ***between 4 bp and 20 bp***. (Emphasis added)

As noted in paragraph [0012] of the specification, the Applicant's saturation mutagenesis method "requires no pre-existing knowledge of the region being screened and enables [the] discovery of both gene-proximal and gene-distal functional elements." Furthermore, paragraph [0363] of the specification highlights a significant advantage of the Applicant's approach: the

"*high-resolution high-throughput pooled tiling sgRNA reveals underlying enhancer sequence requirements approaching* ***nucleotide resolution***." (Emphasis added).

A person of ordinary skill in the art at the time of the Applicant's filing would not have been motivated to adopt the methodology disclosed in Bauer I for analyzing the BCL11A gene regulatory elements in Bauer II. Bauer I's deletion approach, which uses two gRNAs to create large deletions (1 kb to >1 Mb), would be ill-suited to map transcription and repressor elements ***at near-nucleotide resolution***.

Accordingly, Applicant's claimed invention cannot be an obvious variant of the method claims of '359, '945, '965, '406, '445, '356, '418, '308, '616, '814, '839, '233, '641, and '949 patents and the pending claims of '398, '511, '551, '169, '710, '025, '161, '876, '260, '464, '495, '403, '531, '442, '043, '580, '110, '234, '754, '550, '122, '317, and '343 applications, in view of the teachings in Bauer I and II because *inter alia* the references cited by the Examiner fail to teach or suggest a modified eukaryotic cell comprising an indel mutation within a non-coding region that is associated with a change in phenotype, wherein the indel mutation has a mean deletion size of 11 bp and a mean insertion size of 4 bp.

Withdrawal of OTDP rejections 1-51 is respectfully requested.

No Waiver

All of Applicant's arguments and amendments are without prejudice or disclaimer. Applicant may not have addressed each specific rejection of the independent and dependent claims because Applicant submits that the independent claims are allowable, as discussed above. Applicant has not acquiesced to any such rejection and reserves the right to address the patentability of any additional claim features in the future.

CONCLUSION

Applicant submits the foregoing as a complete response to the Office Action. Applicant submits that this Amendment and Response place the application in condition for allowance and respectfully requests such action. If any issues can be resolved with an Examiner's Amendment or a telephone conference, please contact Applicant's representative at 478.292.5119.

No additional fees are believed due for consideration of this paper. However, the Commissioner is hereby authorized to charge any fee deemed necessary for consideration of this paper, including any extension of time fees or additional claim fees, or to credit any overpayment, to Deposit Account Number 50-5193, reference number BROD-3520US-CON2.

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