**Provisional Application for Patent Cover Sheet**

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c)

### Inventor(s)

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<tr>
<th>Inventor</th>
<th>Given Name</th>
<th>Middle Name</th>
<th>Family Name</th>
<th>City</th>
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</thead>
<tbody>
<tr>
<td>Inventor 1</td>
<td>Seung Woo</td>
<td>Kim</td>
<td>Seoul</td>
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<td></td>
<td>KR</td>
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<tr>
<td>Inventor 2</td>
<td>Sojung</td>
<td>Kim</td>
<td>Seoul</td>
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<td></td>
<td>KR</td>
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<td>Inventor 3</td>
<td>Jin-Soo</td>
<td>Kim</td>
<td>Seoul</td>
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All Inventors Must Be Listed – Additional Inventor Information blocks may be generated within this form by selecting the Add button.

### Title of Invention

TARGETED GENOME ENGINEERING IN CELLS AND ORGANISMS WITH RNA-GUIDED ENDONUCLEASES

### Correspondence Address

Direct all correspondence to (select one):

- The address corresponding to Customer Number
- Firm or Individual Name

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The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

- No.
- Yes, the name of the U.S. Government agency and the Government contract number are:
**Entity Status**

Applicant claims small entity status under 37 CFR 1.27

- ☐ Yes, applicant qualifies for small entity status under 37 CFR 1.27
- ☐ No

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**Signature**

Please see 37 CFR 1.4(d) for the form of the signature.

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<th>Signature</th>
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Title:
Targeted genome engineering in cells and organisms with RNA-guided endonucleases

Inventors: Seung Woo Cho, Sojung Kim, and Jin-Soo Kim

Affiliations:
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Abstract:
We present a novel genome editing technology based on RNA-guided Cas9 endonucleases (RGENs). Cas9 is a sequence-specific endonuclease in type II CRISPR/Cas systems, which confer prokaryotes with adaptive immunity against invading phages and plasmids. Cas9 recognizes and cleaves target DNA sequences complementary to small synthetic guide RNAs embedded in this protein, generating site-specific DNA double-strand breaks in vitro and in human cells, whose spontaneous repair induces targeted genome modifications at high frequencies. Unlike ZFNs and TALENs, which are used widely in research and biotechnology, RGENs are customized without any cloning step, making them a broadly useful, scalable and expeditious platform for genome engineering in cells and organisms.

Summary of the Invention
In some embodiments, the present invention provides compositions and methods for research, clinical and screening applications for genome editing. In some embodiments, the present invention provides nucleic acids encoding RNA-guided Cas9 endonucleases, vectors comprising Cas-9 endonucleases, Cas-9 polypeptides, and uses of such compositions. Additional embodiments are described herein.
We exploited the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas) system (1), an adaptive immune response in bacteria and archaea, to develop a novel genome editing technology based on RNA-guided endonucleases (RGENs). Cas9, an essential protein component in the Type II CRISPR/Cas system, forms an active endonuclease when complexed with two RNAs termed CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA), thereby slicing foreign genetic elements in invading phages or plasmids to protect the host cells. crRNA is transcribed from the CRISPR element in the host genome, which was previously captured from such foreign invaders. Recently, Jinek et al. (2) elegantly demonstrated that a single-chain chimeric RNA produced by fusing an essential portion of crRNA and tracrRNA could replace the two RNAs in the Cas9/RNA complex to form a functional endonuclease, raising the possibility of using this system for genome editing in cells and organisms. Here, we present the first evidence that RGENs can indeed induce site-specific genome modifications in mammalian cells at high frequencies.

We first tested the DNA cleavage activity of Cas9 derived from *Streptococcus pyogenes* in the presence or absence of a chimeric guide RNA *in vitro*. To this end, we used recombinant Cas9 protein that was expressed in and purified from *E. coli* to cleave a predigested or circular plasmid DNA that contained the 23-base pair (bp) human CCR5 target sequence. A Cas9 target sequence consists of a 20-bp DNA sequence complementary to crRNA or a chimeric guide RNA and the trinucleotide (5'-NGG-3') protospacer adjacent motif (PAM) recognized by Cas9 itself (Fig. 1A). Cas9 cleaved the plasmid DNA efficiently at the expected position only in the presence of the synthetic RNA and did not cleave a control plasmid that lacked the target sequence (Fig. 1B).

Next, we used a RFP-GFP reporter to investigate whether the Cas9/guide RNA complex can cleave the target sequence incorporated between the RFP and GFP sequences in mammalian
cells. In this reporter, the GFP sequence is fused to the RFP sequence out-of-frame (3). The active GFP is expressed only when the target sequence is cleaved by site-specific nucleases, which causes frameshifting small insertions or deletions (indels) around the target sequence via error-prone non-homologous end-joining (NHEJ) repair of the double-strand break (DSB). We co-transfected the Cas9-encoding plasmid, the guide RNA, and the RFP-GFP reporter plasmid into human embryonic kidney (HEK) 293T cells, and found that GFP-expressing cells were obtained only when the cells were co-transfected with the Cas9 plasmid and the guide RNA (Fig. 2), demonstrating that RGENs could recognize and cleave the target DNA sequence in cultured human cells.

To test whether RGENs could be used for targeted disruption of endogenous genes in mammalian cells, we analyzed genomic DNA isolated from transfected cells using T7 endonuclease I (T7E1), a mismatch-sensitive endonuclease that specifically recognizes and cleaves heteroduplexes formed by the hybridization of wild-type and mutant DNA sequences (4). We found that mutations were induced only when the cells were co-transfected with both Cas9 and guide RNA (Fig. 3). Mutation frequencies (Indels (%)) in Fig. 3A estimated from the relative DNA band intensities were RNA-dosage dependent, ranging from 1.3% to 5.1%. DNA sequencing analysis of the PCR amplicons corroborated the induction of RGEN-mediated mutations at the endogenous sites. Indels and microhomologies, characteristic of error-prone NHEJ, were observed at the target site. The mutation frequency measured by direct sequencing was 7.3% (= 7 mutant clones/96 clones), on par with those obtained with zinc finger nucleases (ZFNs) or transcription-activator-like effector nucleases (TALENs).

Both ZFNs and TALENs have been successfully developed to disrupt the human CCR5 gene (4-7), which encodes a G-protein-coupled chemokine receptor, an essential co-receptor of HIV infection. A CCR5-specific ZFN is now under clinical investigation in the US for the treatment of AIDS (8). These ZFNs and TALENs, however, have off-target effects, inducing both local
mutations at sites whose sequences are homologous to the on-target sequence (7, 9-11) and genome rearrangements that arise from the repair of two concurrent DSBs induced at on-target and off-target sites (12-13). The most striking off-target sites associated with these CCR5-specific engineered nucleases reside in the CCR2 locus, a close homolog of CCR5, located 15-kbp upstream of CCR5. To avoid off-target mutations in the CCR2 gene and unwanted deletions, inversions, and duplications of the 15-kbp chromosomal segment between the CCR5 on-target and CCR2 off-target sites, we intentionally chose the target site of our CCR5-specific RGEN to recognize a region within the CCR5 sequence that has no apparent homology with the CCR2 sequence.

We investigated whether the CCR5-specific RGEN had off-target effects. To this end, we searched for potential off-target sites in the human genome by identifying sites that are most homologous to the intended 23-bp target sequence. As expected, no such sites were found in the CCR2 gene. Instead, we found four sites, each of which carries 3-base mismatches with the on-target site (Fig. 4A). The T7E1 assays showed that mutations were not detected at these sites (assay sensitivity, ~0.5%), demonstrating exquisite specificities of RGENs (Fig. 4B). Furthermore, we used PCR to detect the induction of chromosomal deletions in cells separately transfected with plasmids encoding the ZFN and RGEN specific to CCR5. Whereas the ZFN induced deletions, the RGEN did not (Fig. 4C). Although we did not detect any off-target effects with RGENs in this study, deep sequencing of candidate sites and whole genome or exome sequencing may reveal off-target mutations induced by RGENs.

Next, we reprogrammed RGENs by replacing the CCR5-specific guide RNA with a newly-synthesized RNA designed to target the human C4BPB gene, which encodes the beta chain of C4b-binding protein, a transcription factor. This RGEN induced mutations at the chromosomal target site in K562 cells at high frequencies (Fig. 3B): Mutation frequencies measured by the T7E1 assay and by direct sequencing were 14% and 8.3% (= 4 mutant clones/48 clones),
respectively. Out of four mutant sequences, two clones contained a single-base or two-base insertion precisely at the cleavage site, a pattern that was also observed at the CCR5 target site. These results indicate that RGENs cleave chromosomal target DNA at expected positions in cells.

ZFNs and TALENs enable targeted mutagenesis in mammalian cells (14-16), model organisms (17-20), plants (21-23), and livestock (24-25), but the mutation frequencies obtained with individual nucleases are widely different from each other. Furthermore, some ZFNs and TALENs fail to show any genome editing activities (26-29). DNA methylation may limit the binding of these engineered nucleases to target sites (30). In addition, it is technically challenging and time-consuming to make custom nucleases. In this regard, RGENs based on Cas9 could provide useful options for genome editing. Compared to ZFNs and TALENs, RGENs can be more readily customized because only the synthetic RNA component is replaced to make a new genome-editing nuclease: No sub-cloning steps are involved to make customized RGENs. Furthermore, the relatively small size of the Cas9 gene (4.2 kbp) as compared to a pair of TALEN genes (~6 kbp) provides an advantage for this system in some applications such as virus-mediated gene delivery. These features will make RGENs scalable, versatile, and convenient tools for genome engineering in cells and organisms.

The specificity of DNA recognition by RGENs is somewhat limited by the requirement for a 5'-GG-3' dinucleotide in the PAM sequence. This motif is recognized by the Cas9 protein but not by the guide RNA. Thus, RGENs can be designed to cleave DNA once per 8 bp (= 4x4/2) on average. This limitation might be relieved by engineering Cas9 or employing Cas9 derived from other species.

Unlike FokI-based ZFNs and TALENs, which produce 4- to 6-base 5' overhangs at cleavage sites, RGENs yield blunt ends rather than cohesive ends (2). Our results show that DSBs with blunt ends can also be readily repaired in mammalian cells. It would be interesting to investigate
how and whether blunt DSB ends would be differentially repaired by endogenous end-joining processes.

Taken together, these findings indicate that RGENs are a new member in the family of genome editing tools that have revolutionized basic and biomedical research but with their own unique features that make them an ideal platform in many applications. We propose that RGENs should find broad utility in research, biotechnology, and medicine in the post-genomic era.

References:


**Figure legends**

Fig. 1. Cas9-catalyzed cleavage of plasmid DNA *in vitro*. (A) Schematic representation of target DNA and chimeric RNA sequences. Red triangles indicate cleavage sites. The PAM sequence recognized by Cas9 is shown in bold. The sequences in the guide RNA derived from crRNA and tracrRNA are shown in red and blue, respectively. (B) *In vitro* cleavage of plasmid DNA by Cas9. An intact circular plasmid or ApaLI-digested plasmid was incubated with Cas9 and guide RNA.

Fig. 2. Cas9-induced mutagenesis at an episomal target site. (A) Schematic overview of cell-based assays using a RFP-GFP reporter. GFP is not expressed from this reporter because the GFP sequence is fused to the RFP sequence out-of-frame. The RFP-GFP fusion protein is expressed only when the target site between the two sequences is cleaved by a site-specific nuclease. (B) Flow cytometry of cells transfected with Cas9. The percentage of cells that express the RFP-GFP fusion protein is indicated.

Fig. 3. RGEN-driven mutations at endogenous chromosomal sites. (A) CCR5 locus. (B) C4BPB locus. (Top) The T7E1 assay was used to detect RGEN-driven mutations. Arrows indicate the expected position of DNA bands cleaved by T7E1. Mutation frequencies (Indels (%)) were calculated by measuring the band intensities. (Bottom) DNA sequences of the CCR5 and C4BPB wild-type (WT) and mutant clones. The region of the target sequence complementary to the guide RNA is shown in red. The PAM sequence is shown in bold. Red triangles indicate the
cleavage site. Bases corresponding to microhomologies are underlined. The column on the right indicates the number of inserted or deleted bases.

**Fig. 4.** RGEN-driven off-target mutations are undetectable. (A) On-target and potential off-target sequences. The human genome was searched *in silico* for potential off-target sites. Four sites were identified, each of which carries 3-base mismatches with the CCR5 on-target site. Mismatched bases are shown in blue. (B) The T7E1 assay was used to investigate whether these sites were mutated in cells transfected with the Cas9/RNA complex. No mutations were detected at these sites. N/A (not applicable), an intergenic site. (C) Cas9 did not induce off-target-associated chromosomal deletions. The CCR5-specific RGEN and ZFN were expressed in human cells. PCR was used to detect the induction of the 15-kb chromosomal deletions in these cells.

**Materials and Methods:**

**Construction of Cas9-encoding plasmids.** The Cas9-coding sequence (4,104 bp), derived from *Streptococcus pyogenes* strain M1 GAS (NC_002737.1), was reconstituted using the human codon usage table and synthesized using oligonucleotides. First, 1-kb DNA segments were assembled using overlapping ~35-mer oligonucleotides and Phusion polymerase (New England Biolabs) and cloned into T-vector (SoIGent). A full-length Cas9 sequence was assembled using four 1-kbp DNA segments by overlap PCR. The Cas9-encoding DNA segment was subcloned into p3s, which was derived from pcDNA3.1 (Invitrogen). In this vector, a peptide tag (NH₂-GGSPPKKRKVYPYDVPDYA-COOH) containing the HA epitope and a nuclear localization signal (NLS) was added to the C-terminus of Cas9. Expression and nuclear localization of the Cas9 protein in HEK 293T cells were confirmed by western blotting using anti-HA antibody (Santa Cruz).

**In vitro DNA cleavage assay.** The Cas9 cassette was subcloned into pET28-b(+) and transformed into BL21(DE3). The expression of Cas9 was induced using 0.5 mM IPTG for 4 h at 25 °C. The Cas9 protein containing the His₆-tag at the C terminus was purified using Ni-NTA agarose resin (Qiagen) and dialyzed against 20 mM HEPES (pH 7.5), 150 mM KCl, 1 mM DTT, and 10% glycerol (2). Purified Cas9 (50 nM) was incubated with super-coiled or pre-digested
plasmid DNA (300 ng) and chimeric RNA (50 nM) in a reaction volume of 20 ul in NEB buffer 3 for 1 h at 37 °C. Digested DNA was analyzed by electrophoresis using 0.8% agarose gels.

**RNA preparation.** RNA was *in vitro* transcribed through run-off reactions using the MEGAscript T7 kit (Ambion) according to the manufacturer's manual. Templates for RNA *in vitro* transcription were generated by annealing two complementary single strand DNAs or by PCR amplification (Table 1). Transcribed RNA was resolved on a 8% denaturing urea-PAGE gel. The gel slice containing RNA was cut out and transferred to probe elution buffer. RNA was recovered in nuclease-free water followed by phenol:chloroform extraction, chloroform extraction, and ethanol precipitation. Purified RNAs were quantified by spectrometry.

**Cell culture.** HEK 293T/17 (ATCC, CRL-11268) cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 100 units/mL penicillin, 100 μg/mL streptomycin, 0.1 mM nonessential amino acids, and 10% fetal bovine serum (FBS). K562 (ATCC, CCL-243) cells were grown in RPMI-1640 with 10% FBS and the penicillin/streptomycin mix (100 U/ml and 100 μg/ml, respectively).

**Genome-editing assay.** To introduce DSBs in mammalian cells using RGENs, 2x10⁶ K562 cells were transfected with 20 μg of Cas9-encoding plasmid using the 4D-Nucleofector, SF Cell Line 4D-Nucleofector X Kit, Program FF-120 (Lonza) according to the manufacturer's protocol. After 24h, 10-40 μg of *in vitro* transcribed chimeric RNA was nucleofected into 1x10⁶ K562 cells. Cells were collected two days after RNA transfection and genomic DNA was isolated. The region including the target site was PCR-amplified using the primers described in Table 1. The amplicons were subjected to the T7E1 assay as described previously (4). For sequencing analysis, PCR products corresponding to genomic modifications were purified and cloned into the T-Blunt vector using the T-Blunt PCR Cloning Kit (SolGent). Cloned products were sequenced using the M13 primer.

**Reporter construct.** The RFP-GFP reporter plasmids used in this study were constructed as described previously (3). Oligonucleotides corresponding to target sites (Table 1) were synthesized (Macrogen) and annealed. The annealed oligonucleotides were ligated into a reporter vector digested with EcoRI and BamHI.

**Episomal reporter assay.** HEK 293T cells were co-transfected with Cas9-encoding plasmid
(0.8 μg) and the RFP-GFP reporter plasmid (0.2 μg) in a 24-well plate using Lipofectamine 2000 (Invitrogen). At 12h post transfection, chimeric RNA (1 μg) prepared by in vitro transcription was transfected using Lipofectamine 2000. At 3d post-transfection, transfected cells were subjected to flow cytometry and cells expressing both RFP and GFP were counted.

Table 1. Oligonucleotides used in this study.

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Fig. 1

A

Chr. 3

CCR5

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3'-TAC...GTTAGCTACTCAAGTATATATAGcccCTGGA-5'

5'-GGGACAAGCCAAUAUAAAGACGCGGAGTT

3'-GCCUUGCCGAAAATGCGCA

guide RNA

Cas9

B

ApaI

pUC on

1.7 kbp

5.4 kbp

KanR

target site

circular

linearized

nicked

linearized

supercircular

5.4 kbp

3.7 kbp

1.7 kbp

Cas9:

guide RNA:

target sequence:

- + - + - + - +
- - + - + - +
- - + - + - +

nicked

linearized

supercircular
Figure 2

(A) Reporter

- Reporter + Cas9

- RGEN-induced DSBs
- NHEJ-mediated frameshift mutations

(B) Reporter only
- Reporter + guide RNA
- Reporter + Cas9
- Reporter + Cas9 + guide RNA
[Fig. 3]

A

**CCR5**

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Indels (%)

|        | 1.3 | 3.9 | 5.1 |

7.3% (7/96) mutated

B

**C4BPB**

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Indels (%)

|        | 14  |

8.3% (4/48) mutated

[Fig. 4]

A

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TGACATCAATTATTATAGATCGG
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**CCR5**

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**ADCY5**

**KCNJ6**

**CntnAP2**

**Chr. 5 N/A**

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(empty vector) RGEN ZFN
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Filed as Small Entity

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| Patent-Appeals-and-Interference:    |          |          |        |                     |
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The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:
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- Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)
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**Multipart Description/PDF files in .zip description**

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**Total Files Size (in bytes):** 2490706

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If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

**National Stage of an International Application under 35 U.S.C. 371**

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

**New International Application Filed with the USPTO as a Receiving Office**

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.
Receipt is acknowledged of this provisional patent application. It will not be examined for patentability and will become abandoned not later than twelve months after its filing date. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections.

Inventor(s)
- Seung Woo Kim, Seoul, KOREA, REPUBLIC OF;
- Sojung Kim, Seoul, KOREA, REPUBLIC OF;
- Jin-Soo Kim, Seoul, KOREA, REPUBLIC OF;

Applicant(s)
- Seung Woo Kim, Seoul, KOREA, REPUBLIC OF;
- Sojung Kim, Seoul, KOREA, REPUBLIC OF;
- Jin-Soo Kim, Seoul, KOREA, REPUBLIC OF;

Power of Attorney:
Tanya Arenson--47391

If Required, Foreign Filing License Granted: 11/16/2012

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is US 61/717,324

Projected Publication Date: None, application is not eligible for pre-grant publication

Non-Publication Request: No

Early Publication Request: No

** SMALL ENTITY **
TARGETED GENOME ENGINEERING IN CELLS AND ORGANISMS WITH RNA-GUIDED ENDONUCLEASES

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process simplifies the filing of patent applications on the same invention in member countries, but does not result in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

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For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, http://www.stopfakes.gov. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

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Title 35, United States Code, Section 184
Title 37, Code of Federal Regulations, 5.11 & 5.15

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---

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial No.: 61/717,324 Filed: October 23, 2012
Applicant: Cho et al.  Confirmation No.: 4637
Title: TARGETED GENOME ENGINEERING IN CELLS AND ORGANISMS WITH RNA-GUIDED ENDONUCLEASES
Atty. Docket: HANOL-32960/US-1/PRO  Examiner: [NOT ASSIGNED]
Art Unit: [NOT ASSIGNED]  Cust. No.: 72960

via EFS-Web
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

REQUEST FOR CORRECTED FILING RECEIPT

Sir/Madam:

Applicant hereby requests a Corrected Filing Receipt. The Filing Receipt dated November 20, 2012 incorrectly indicates the Applicant as Seung Woo Kim (incorrect) as opposed to Seung Woo Cho (correct). Applicant hereby submits a copy of the Filing Receipt highlighting the error, along with a Supplemental Provisional Cover Sheet properly indicating the name of the applicant in the above-captioned application.
No fees are believed due in connection with this filing. Nevertheless, if the Director finds any fees to be due in connection with this or any other communication in this matter, authorization is hereby given to charge deposit account number 50-4302 referencing attorney docket number HANOL-32960/US-1/PRO.

Respectfully submitted,

Date: September 30, 2013

/Tanya A. Arenson/
Tanya A. Arenson
Registration No. 47,391
Casimir Jones S.C.
2275 Deming Way
Suite 310
Middleton, WI 53562
Tel.: 608-662-1277
Fax: 608-662-1276
**Supplemental Provisional Application for Patent Cover Sheet**

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c)

### Inventor(s)

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All Inventors Must Be Listed – Additional Inventor Information blocks may be generated within this form by selecting the Add button.

### Title of Invention

TARGETED GENOME ENGINEERING IN CELLS AND ORGANISMS WITH RNA-GUIDED ENDOMUCLEASES

### Attorney Docket Number (if applicable)

HANOL-32960/US-1/PRO

### Correspondence Address

Direct all correspondence to (select one):

- ☐ The address corresponding to Customer Number
- ☐ Firm or Individual Name

Customer Number

72960

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

☐ No.

☐ Yes, the name of the U.S. Government agency and the Government contract number are:
Entity Status
Applicant claims small entity status under 37 CFR 1.27

☐ Yes, applicant qualifies for small entity status under 37 CFR 1.27
☐ No

Warning
Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

Signature
Please see 37 CFR 1.4(d) for the form of the signature.

Signature /Tanya A. Arenson/  Date (YYYY-MM-DD) 2013-09-30

First Name Tanya A.  Last Name Arenson  Registration Number 47391

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7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.

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CONFIRMATION NO. 4637

FILING RECEIPT

Date Mailed: 11/20/2012

Receipt is acknowledged of this provisional patent application. It will not be examined for patentability and will become abandoned not later than twelve months after its filing date. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections.

Inventor(s)

Seung Woo Kim, Seoul, KOREA, REPUBLIC OF;
Sojung Kim, Seoul, KOREA, REPUBLIC OF;
Jin-Soo Kim, Seoul, KOREA, REPUBLIC OF;

Applicant(s)

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Jin-Soo Kim, Seoul, KOREA, REPUBLIC OF;

Power of Attorney:

Tanya Arenson--47391

If Required, Foreign Filing License Granted: 11/16/2012

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is US 61/717,324

Projected Publication Date: None, application is not eligible for pre-grant publication

Non-Publication Request: No

Early Publication Request: No

** SMALL ENTITY **
TARGETED GENOME ENGINEERING IN CELLS AND ORGANISMS WITH RNA-GUIDED ENDONUCLEASES

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If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

**National Stage of an International Application under 35 U.S.C. 371**

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements, a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

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If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.
United States Patent and Trademark Office
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Non-Publication Request: No

Early Publication Request: No

** SMALL ENTITY **

Title

TARGETED GENOME ENGINEERING IN CELLS AND ORGANISMS WITH RNA-GUIDED ENDONUCLEASES

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications:
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