Annex 2 – Amendments to EP987

Unconditional

1. An *in vitro* or *ex vivo* method for inducing targeted mutagenesis of an endogenous DNA in a eukaryotic cell, comprising introducing into the cell a composition comprising:
a Cas9 protein; and
a guide RNA,
wherein the guide RNA ~~comprises a CRISPR RNA (crRNA) and a transactivating crRNA (tracrRNA),~~ is a single-chain guide RNA (sgRNA) comprising a CRISPR RNA (crRNA) fused to a transactivating crRNA (tracrRNA),
wherein the crRNA comprises a portion that is complementary to a target DNA,
wherein the target DNA is the endogenous DNA of the eukaryotic cell; and
wherein the Cas9 protein is complexed with the guide RNA to form an active endonuclease before being introduced into the eukaryotic cell.

~~2. The method according to claim 1, wherein the guide RNA is:~~
~~i) a dual RNA comprising a crRNA and a tracrRNA; or~~
~~ii) a single-chain guide RNA (sgRNA) comprising a crRNA fused to a tracrRNA.~~

2. ~~3.~~ The method according to claim 1 ~~or claim 2~~, wherein the guide RNA is *in vitro* transcribed RNA.

3. ~~4.~~ The method according to ~~any one of~~ claim~~s~~ 1 ~~to~~ or 2 ~~3~~, wherein the portion of the crRNA that is complementary to the target DNA is 20 nucleotides in length.

4. ~~5.~~ The method according to any one of claims 1 to 3 ~~4~~, wherein the Cas9 protein is derived from *Streptococcus pyogenes.*

5. ~~6.~~ The method according to any one of claims 1 to 4 ~~5~~, wherein the Cas9 protein is expressed in and purified from E. *coli.*

6. ~~7.~~ The method according to any one of claims 1 to 5 ~~6~~, wherein the composition is introduced into the cell by a method selected from microinjection, electroporation, DEAE-dextran treatment, lipofection, nanoparticle-mediated transfection, and protein transduction domain mediated transduction.

7. ~~8.~~ The method according to any one of claims 1 to 6 ~~7~~, wherein the eukaryotic cell is a mammalian cell.

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1. An *in vitro* or *ex vivo* method for inducing targeted mutagenesis of an endogenous DNA in a eukaryotic cell, comprising introducing into the cell a composition comprising:
a Cas9 protein; and
a guide RNA,
wherein the guide RNA ~~comprises a CRISPR RNA (crRNA) and a transactivating crRNA (tracrRNA),~~ is a single-chain guide RNA (sgRNA) comprising a CRISPR RNA (crRNA) fused to a transactivating crRNA (tracrRNA),
wherein the crRNA comprises a portion that is complementary to a target DNA,
wherein the sgRNA and the Cas9 protein are present in a mass ratio ranging from 100:225 to 100:22.5, and
wherein the target DNA is the endogenous DNA of the eukaryotic cell; and
wherein the Cas9 protein is complexed with the guide RNA to form an active endonuclease before being introduced into the eukaryotic cell.

~~2. The method according to claim 1, wherein the guide RNA is:~~
~~i) a dual RNA comprising a crRNA and a tracrRNA; or~~
~~ii) a single-chain guide RNA (sgRNA) comprising a crRNA fused to a tracrRNA.~~

2. ~~3.~~ The method according to claim 1 ~~or claim 2~~, wherein the guide RNA is *in vitro* transcribed RNA.

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6. ~~7.~~ The method according to any one of claims 1 to 5 ~~6~~, wherein the composition is introduced

into the cell by ~~a method selected from microinjection,~~ electroporation~~, DEAE-dextran treatment, lipofection, nanoparticle-mediated transfection, and protein transduction domain mediated transduction~~.

7. ~~8.~~ The method according to any one of claims 1 to 6 ~~7~~, wherein the eukaryotic cell is a mammalian cell.