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(54) Titre : PROCEDES DE DETERMINATION DU SEXE D'EMBRYONS AVIAIRES DANS LES fUFS NON ECLOS ET
MOYENS CORRESPONDANTS
(54) Title: METHODS FOR GENDER DETERMINATION OF AVIAN EMBRYOS IN UNHATCHED EGGS AND MEANS
THEREOF

(57) **Abrégé/Abstract:**

The present invention relates to methods of gender determination and identification in avian subjects. More specifically, the invention provides non-invasive methods using transgenic avian animals that comprise at least one reporter gene integrated into at least one gender chromosome Z or W. The transgenic avian animals of the invention are used for gender determination and selection of embryos in unhatched avian eggs.

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<130> 31036-3019

<150> US 62/262,409

<151> 2015-12-03

<160> 48

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<213> Artificial Sequence

<220>

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<211> 20

<212> DNA

<213> Artificial Sequence

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962

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<223> A "right arm" for the region downstream the Neomycin-resistance

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<223> A "right arm" for the region downstream the polyA site

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Lys Arg Thr Ala Arg Arg Arg Tyr Thr Arg Arg Lys Asn Arg Ile Cys
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Phe Phe His Arg Leu Glu Glu Ser Phe Leu Val Glu Glu Asp Lys Lys
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His Glu Arg His Pro Ile Phe Gly Asn Ile Val Asp Glu Val Ala Tyr
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Ser Thr Asp Lys Ala Asp Leu Arg Leu Ile Tyr Leu Ala Leu Ala His
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Asp Asn Ser Asp Val Asp Lys Leu Phe Ile Gln Leu Val Gln Thr Tyr
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Met Ile Lys Arg Tyr Asp Glu His His Gln Asp Leu Thr Leu Leu Lys
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Gly Thr Glu Glu Leu Leu Val Lys Leu Asn Arg Glu Asp Leu Leu Arg
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Lys Gln Arg Thr Phe Asp Asn Gly Ser Ile Pro His Gln Ile His Leu
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Gly Glu Leu His Ala Ile Leu Arg Arg Gln Glu Asp Phe Tyr Pro Phe
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Leu Lys Asp Asn Arg Glu Lys Ile Glu Lys Ile Leu Thr Phe Arg Ile
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Pro Tyr Tyr Val Gly Pro Leu Ala Arg Gly Asn Ser Arg Phe Ala Trp
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Thr Tyr His Asp Leu Leu Lys Ile Ile Lys Asp Lys Asp Phe Leu Asp
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Ala Asn Arg Asn Phe Met Gln Leu Ile His Asp Asp Ser Leu Thr Phe

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725 730 735

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755 760 765

Thr Thr Gln Lys Gly Gln Lys Asn Ser Arg Glu Arg Met Lys Arg Ile
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Glu Glu Gly Ile Lys Glu Leu Gly Ser Gln Ile Leu Lys Glu His Pro
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820 825 830

Leu Ser Asp Tyr Asp Val Asp His Ile Val Pro Gln Ser Phe Leu Lys
835 840 845

Asp Asp Ser Ile Asp Asn Lys Val Leu Thr Arg Ser Asp Lys Asn Arg
850 855 860

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885 890 895

Phe Asp Asn Leu Thr Lys Ala Glu Arg Gly Gly Leu Ser Glu Leu Asp
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Lys Ala Gly Phe Ile Lys Arg Gln Leu Val Glu Thr Arg Gln Ile Thr

915

920

925

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Lys Lys Tyr Gly Gly Phe Asp Ser Pro Thr Val Ala Tyr Ser Val

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Ser Val Lys Glu Leu Leu Gly Ile Thr Ile Met Glu Arg Ser Ser
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Glu Val Lys Lys Asp Leu Ile Ile Lys Leu Pro Lys Tyr Ser Leu
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Tyr Asn Lys His Arg Asp Lys Pro Ile Arg Glu Gln Ala Glu Asn
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<212> DNA

<213> Artificial Sequence

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<223> Right arm for gRNA8 integration to Chromosome Z

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gaacaagact gatgcattag ttcatgaaa tcaaggctat gtgtcccact ggtttcatta 180

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ccaccgtgtc agaggcagat ggtggtacgg cagtagagat ggaaccttc caccaatatt 360

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<210> 36

<211> 1040

<212> DNA

<213> Artificial Sequence

<220>

<223> Right arm for gRNA9 integration to Chromosome Z

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<400> 36

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 ttagaggttt tccccactgt ttctaaaaat tacagcattc cctgtccctt cttagtata 180
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 ccctggagag attacctgga tgataactgg gggcctcagt gcctcacagc agcaagtccc 660
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 agtgatttcc atgctgccta ccaggttcta agtcagggtca ccctgccagc ttcttccaaa 960
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<210> 37
 <211> 1024
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Left arm for gRNA10 integration to Chromosome Z
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 _1Chromosome Z

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 acagcctggg ctgggctgag gatgggcttg gtgggcagct ggggtgctgcc gtggcagctg 180
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 ccaggtttg ttaagatga aaatgcatgt gggcatacat ttgcacatga gaacacctgc 420
 taatgaaaca cacagagttg tatgtgcagt ctccctggct ggtggcatgt ctgtgggggc 480
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gcag 1024

<210> 38

<211> 1022

<212> DNA

<213> Artificial Sequence

<220>

<223> Right arm for gRNA10 integration to Chromosome Z

TRIM36_PGGT1B_ENSGALG00000008188_ENSGALG00000008197_chrZ_78779927
_1Chromosome Z

<400> 38

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ctcagcagca aataaaccga gattgttctg atcagtaacg agaggctgtg caaaaagctg 120

agaaatgtac agcccttcct gtcaccact acagcccttc atgcagcggc ctgctgcact 180

agggcctgct tggccagagg cagggctgca gcttgagata cgcagcaccc agtaccacag 240

caggctgcca tgtgcttctg tcaggccatg aattgcacaa gttggtatag tttatacag 300

tgtcatgcag aagcacaact cttaccttgg cacactgata tggaaagcaa agtgtaaagg 360

aactgtatgt ttcttggctt gtgagttcca ttggttctcc caggacagca gtgacagctc 420

tagattttct ggttgatctc ttccatctaa gttttactt ctgaatacat ctgtgcagca 480

aagcctgatg cttttttt tccccctccc ctgggatcgt gaaatattat ttcccatct 540

ccataatata gatgatccac tcaaacatgc atcctcagtc gcagtcctca gaagggcctt 600

ttcccacat acacctacac gctacaccag actttattgg tcaccatgcc ttgagggatt 660

ctgcctaaca caggcacaca ggaagggctg ataccagtaa ttcatgctg aggagagaag 720

gggcacttct ttatagtag agccacagaa tcaccaggat tggaaaacac ctccaagatc 780

atccaatcca accaccacc taccaccagt atttccact aaaccatgcc ctttggtacc 840

acatctaaaa gcatcttctg caccttcaca gttcattgc ctttctcagg acgtgctcca 900

gggccttggg gtctttcttg tattgagtgg cccaaaattg aacacaatac tcgaggtgca 960

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cc 1022

<210> 39

<211> 1003

<212> DNA

<213> Artificial Sequence

<220>

<223> Left arm for gRNA11 integration to Chromosome Z

RPS23_ATG10_ENSGALG00000015617_ENSGALG00000027714_chrZ_63364946_-
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aagactctgt aaggacccca atgccatcag ctgtcccccac agcttcagta cccactgcca 120

ccacattttt actgagatag gtagtttgg agccttctc ccatcttga taggggaggg 180

ttaactgctg gcattttgt tcttctctgc aatatgtgtt ttctgcatgc attctcttt 240

cacccaaaat ttaatgtgg acggacttg aggatattct gcatctgccc aatatttct 300

acagcctcac atctactgat ttatgcacag tatcttatat ataatgtata ttatattatg 360

tatattatgc actgaatcac atctactgtg tatgcacagt aactctcct atggtactgt 420

aagcccagaa atcccagata tgcactaca acgtgtctgc tatgtattgc ttctgtgaga 480

cacagatgtg ataacagag gctgtacaca gtagaaatgc atacatatcc tactgtgaaa 540

tctctgaaat ctagccttaa tcttgaaca gaaatgaata tggtagacac ttgatctgat 600

agaattgggt gccagtagca cagctgtaat catccatgat atgaatcaaa ccaagcacag 660

gtaaacaggt gagagaaatc atgaacaatt acatgcaaac ggaccagcta aaatgtgttt 720

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aaataatgtt atctgcacc actgctggtg ttacactatt tcaccaagt ttaccaccag 960

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<210> 40

<211> 1003

<212> DNA

<213> Artificial Sequence

<220>

<223> Right arm for gRNA11 integration to Chromosome Z

RPS23_ATG10_ENSGALG00000015617_ENSGALG00000027714_chrZ_63364946_-
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<400> 40

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taaattagat cttaaaaaca aacaaacaaa caaacaaaaa aactccttgc aaagataatg 180
ttatgtaata ttagttgcaa gaagtaagca aacacacaga actgggagca gaagcaaagc 240
actaagttat taaagcaagt tgcacattt gagttgcatt ttgccactgg tttataaac 300
atgtttagca tgtctggta gaatttggc accaggatgc tttaagatg tctgtctatg 360
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ggaagacgag ccaagccaga cgttatcata gtcactagta aagtgggtct aagcctaatt 480
aagacatgtc agaactatgt gttgtgcaac caaatcctcc aaaagagaaa tcagaggtga 540
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ccaatcatal ctgactgctg gacgcaggag actgaacctt acagaccctg gagaatcact 660
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gagatagatt ccaaacccaa cactgagatt cctctggtga tgcagatgac tgcttactgg 960
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<210> 41
<211> 200
<212> DNA
<213> Artificial Sequence

<220>
<223> For cloning using the gRNA3, Z chromosome, "Left arm"

<400> 41
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gggagatggc aaacatctag agagggacac tgcagggatg ctgcatgaac acctccagcc 120
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caatgtgggc attgccgacc 200

<210> 42
<211> 258
<212> DNA
<213> Artificial Sequence

<220>

<223> For cloning using the gRNA3, Z chromosome, "Right arm"

<400> 42

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gtagctcaga gcatgagata tattgccag gtgtcagtgg acttctggca gtgcatactg 120

cagagagctt tgggtgctgg ccatctggat gatgacaaaa tctcagccac agaccatgtg 180

ggttgaaga gtcctcagga gatcaccttt ccacctctg ctaaagcaca ttcctacag 240

tatgtttcac atgacacc 258

<210> 43

<211> 436

<212> DNA

<213> Artificial Sequence

<220>

<223> For cloning using the gRNA4, Z chromosome, "Left arm"

<400> 43

tcaacagctg tacgaaaagt ccatgcttcc tcattataaa cggaggaaaa aaagttgttt 60

acagctgtaa tgggattata aaaggcaaat ggggatagta cagtggtgag aaccagatct 120

atgaaggaaa agccctaaga aaaagagagt gcacagatac tottaaccat ctaataactg 180

tttctccat cctacagctc agagttaaga ctacagagg actctagtac ttagtaagat 240

gaatacgagc taatagtggc aaaaataatc ccagtgcctc aacactgacc tgggaaaaag 300

gggcatgtat agacctctg atattgtgat gctgtgttg tacacttatt atctacatt 360

tcagaaatta ggttaaactt cagaaaactg aagatctcca gggctttag cagaccctga 420

ccaccagact ggtccc 436

<210> 44

<211> 521

<212> DNA

<213> Artificial Sequence

<220>

<223> For cloning using the gRNA4, Z chromosome, "Right arm"

<400> 44

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tggggcaact tcccctgcc caccttcct tctgtatct tctgaaaagc ttgtagccct 120

caattgtcac gctccagcca tatgaatcat cccaccagct cctgtgttc acaattacct 180

tctagtcttc taattgcacc atggcttcca attcctcctg ttatcaccc acgttgcac 240
cattgatgta gaggcacttc agtcgggcta tcagcaatgt taccacctgt gaggagccct 300
cttgagatct ttaggacaa ttagagggtt tccccactg ttcctaaaa ttacagcatt 360
ccctgtccct tcttagtgat atagaactcc atcccctcc accatcaaac ctagcttaag 420
ctctggtaaa tgatccagcc agtctgcttc ccaaaactct tgctcagttg cattctatct 480
gatgcctaca tgcccaacac cttcaaggcc tgtccaagat c 521

<210> 45
<211> 505
<212> DNA
<213> Artificial Sequence

<220>
<223> For cloning using the gRNA5, Z chromosome, "Left arm"

<400> 45
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gaattgtgga ggaaaccctc ttccacctag ctccaagggt gcacgctcaa tgcttgatg 120
aagattcaag ttctaatga agaactgcat tcaaagacat ctttgagggt gaccagact 180
gcctctggga tgaatccaga acaaacagaa tcttcccagc aacaccttg ttatcatac 240
acgttcagaa atgcagtgcc tgcttggtat tttttaaac tcatcacaga ggaatctctg 300
agtggagcag aggagtgtt cctcttgctt tttcttccc ctttctgta agaaatgcat 360
atgccagttt cccctaaat gtttcaaac tcaaattgt ttctgctgg tgacattctc 420
ctctccagca ctgcctacac cccagctgcg tctgatagga aaagcaccca gtacatgct 480
ggcatggcat ggtcagtgac accca 505

<210> 46
<211> 599
<212> DNA
<213> Artificial Sequence

<220>
<223> For cloning using the gRNA5, Z chromosome, "Right arm"

<400> 46
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tggaatggaa tggaataaca gagggaatgg ggaacaggct gcagtgagaa ggaaaccccc 180

gtgcaccgac caacccgctc ccagtagcct ggtccctacc ttgcccact ccagcagctc 240
 cacgaggtac tcgaagtgcc catgtccggc catccccagc atggcacagc tgtcacctgg 300
 gagcagcggg ggtccggcag gcatcgccgg caccacagc ctgggctggg ctgaggatgg 360
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<210> 47
 <211> 453
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> For cloning using the gRNA6, Z chromosome, "Left arm"

<400> 47
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 atcgggtggc ctgtggagca catcaccctt ggacagtga aacatgtcaa aggggtgttt 180
 ataacagtat agcatatccc ttgaggctg aatttctga gcttatggat atggtagtaa 240
 tgctatacca ggctctctct gcagggtgct cactgaaaca atataccctt tctttctcaa 300
 gaaatgggac tcatgaatag ctaccaggcc ttctgctac tagatactgt agaattacat 360
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 tttccacact gcttttctgc aaatggatta gga 453

<210> 48
 <211> 439
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> For cloning using the gRNA6, Z chromosome, "Right arm"

<400> 48
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 ttgctctgat ctaactctag atcactaaat ggcactgtg caggacactt ttccactgtc 120
 atttggggg gtagaagtgt ggtcagagcc agggaggctt gcagggccca caccagctgt 180

ggccaagca gctgctgtac aatggctgta tggcagtatc gatgtgaact tcctgataat 240
aacagaact cagcagcaaa taaaccaga ttgtctgat cagtaacgag aggctgtgca 300
aaaagctgag aatgtacag cccttctgc tcaccactac agcccttcat gcagcggcct 360
gctgcactag ggctgcttg gccagaggca gggctgcagc ttgagatacg cagcaccag 420
taccagca ggctgcat 439

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CLAIMS:

1. A method of gender determination of avian fertilized unhatched egg, the method comprising the step of:

- (a) providing/obtaining at least one transgenic avian animal comprising at least one exogenous reporter gene integrated into at least one position or location in at least one of gender chromosome Z and W;
- (b) obtaining at least one fertilized egg from said transgenic avian subject, specifically animal or of any cells thereof;
- (c) determining in said egg if at least one detectable signal is detected, wherein detection of said at least one detectable signal indicates the expression of said at least one reporter gene, thereby the presence of said W chromosome or Z chromosome in said avian embryo.

2. The method according to claim 1, wherein said at least one transgenic avian subject comprises at least two different reporter genes, each reporter gene is integrated into at least one locus in one of gender chromosome Z or W.

3. The method according to any one of claims 1 and claim 2, wherein said reporter gene is at least one bioluminescence reporter gene.

4. The method according to claim 3, wherein said reporter gene is luciferase.

5. The method according to claims 1, 3 and 4, wherein said method further comprises the step of providing to said egg of step (b), at least one of substrate and enzyme compatible to said bioluminescence reporter gene, for formation of said detectable signal detected at step (c).

6. The method according to claim 1, wherein said at least one transgenic avian subject, specifically animal is a female avian animal, and wherein said at least one reporter gene is integrated into at least one position of female chromosome Z, thereby detection of a detectable signal indicates that said embryo in said unhatched egg is male.

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7. The method according to claim 1, wherein said at least one transgenic avian subject, specifically animal is a female avian animal, and wherein said at least one reporter gene is integrated into at least one position of female chromosome W, thereby detection of a detectable signal, indicates that said embryo in said unhatched egg is female.

8. The method according to claim 1, wherein said at least one reporter gene is integrated into said gender chromosome of said transgenic avian subject, specifically animal using at least one programmable engineered nuclease (PEN).

9. The method according to claim 8, wherein said PEN is a clustered regularly interspaced short palindromic repeat (CRISPR) type II system.

10. The method according to claim 9, wherein said at least one reporter gene is integrated into said gender chromosome of said transgenic avian animal by homology directed repair (HDR) mediated by at least one CRISPR/CRISPR-associated endonuclease 9 (Cas9) system.

11. The method according to claim 10, wherein said at least one reporter gene is integrated into a gender chromosome of said transgenic avian animal by co-transfecting at least one cell of said avian animal or at least one cell introduced into said avian animal, with:

- (a) at least one first nucleic acid sequence comprising at least one nucleic acid sequence encoding at least one Cas9 protein and at least one nucleic acid sequence encoding at least one guide RNA (gRNA); and
- (b) at least one second nucleic acid sequence comprising at least one said reporter gene.

12. The method according to claim 11, wherein said at least one reporter gene in said second nucleic acid sequence is flanked at 5' and 3' thereof by homologous arms for HDR at the integration site.

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13. The method according to any one of claims 11 and 12, wherein said at least one reporter gene in said second nucleic acid sequence is operably linked to any one of a gender specific promoter, an embryonal specific promoter and an inducible promoter.

14. The method according to any one of claims 11 to 13, wherein said at least one reporter gene is integrated into at least one non-coding region of said gender chromosome.

15. The method according to claim 14, wherein said at least one reporter gene is integrated into at least one site at gender W chromosome locus 1022859-1024215.

16. The method according to claims 11 and 15, wherein said at least one gRNA comprises the nucleic acid sequence as denoted by any one of SEQ ID NO. 1 and 2.

17. The method according to claim 16, wherein said gRNA comprises the nucleic acid sequence as denoted by SEQ ID NO. 1, and wherein said at least one reporter gene comprised within said second nucleic acid sequence is flanked at 5' and 3' thereof by homologous arms comprising the amino acid sequence as denoted by SEQ ID NO. 4 and 5, respectively.

18. The method according to claim 16, wherein said gRNA comprises the nucleic acid sequence as denoted by SEQ ID NO. 2, and wherein said at least one reporter gene comprised within said second nucleic acid sequence is flanked at 5' and 3' thereof by homologous arms comprising the amino acid sequence as denoted by SEQ ID NO. 6 and 7, respectively.

19. An avian transgenic animal comprising at least one exogenous reporter gene integrated into at least one locus in at least one of gender chromosome Z and W.

20. The transgenic animal according to claim 19, wherein said at least one transgenic animal comprises at least two different reporter genes, each reporter gene is integrated into at least one locus in one of gender chromosome Z or W.

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21. The transgenic animal according to any one of claims 19 and claim 20, wherein said reporter gene is at least one bioluminescence reporter gene.
22. The transgenic animal according to claim 21, wherein said reporter gene is luciferase.
23. The transgenic animal according to claim 19, wherein said at least one transgenic avian animal is female, and wherein said at least one reporter gene is integrated into at least one position of female chromosome Z.
24. The transgenic animal according to claim 19, wherein said at least one transgenic avian animal is female, and wherein said at least one reporter gene is integrated into at least one position of female chromosome W.
25. The transgenic animal according to claim 19, wherein said at least one reporter gene is integrated into said gender chromosome of said transgenic animal using at least one PEN.
26. The transgenic animal according to claim 25, wherein said PEN is a CRISPR type II system.
27. The transgenic animal according to claim 26, wherein said at least one reporter gene is integrated into said gender chromosome of said transgenic avian animal by HDR mediated by at least one CRISPR/Cas9 system.
28. The transgenic animal according to claim 27, wherein said at least one reporter gene is integrated into a gender chromosome of said transgenic avian subject, specifically animal by co-transfecting at least one cell of said avian animal or at least one cell introduced into said avian animal:
 - (a) at least one first nucleic acid sequence comprising at least one nucleic acid sequence encoding at least one Cas9 protein and at least one nucleic acid sequence encoding at least one gRNA; and

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(b) at least one second nucleic acid sequence comprising at least one said reporter gene.

29. The transgenic animal according to claim 28, wherein at least one reporter gene in said second nucleic acid sequence is flanked at 5' and 3' thereof by homologous arms for HDR at the integration site.

30. The transgenic animal according to any one of claims 28 and 29, wherein at least one reporter gene in said second nucleic acid sequence is operably linked to any one of a gender specific promoter, an embryonal specific promoter and an inducible promoter.

31. The transgenic animal according to any one of claims 28 to 30, wherein said at least one reporter gene is integrated into at least one non-coding region of said gender chromosome.

32. The transgenic animal according to claim 31, said at least one reporter gene is integrated into at least one site at gender W chromosome locus 1022859-1024215.

33. The transgenic animal according to claims 28 and 32, wherein said at least one gRNA comprises the nucleic acid sequence as denoted by any one of SEQ ID NO. 1 and 2.

34. The transgenic animal according to claim 33, wherein said gRNA comprises the nucleic acid sequence as denoted by SEQ ID NO. 1, and wherein at least one reporter gene comprised within said second nucleic acid sequence is flanked at 5' and 3' thereof by homologous arms comprising the amino acid sequence as denoted by SEQ ID NO. 4 and 5, respectively.

35. The transgenic animal according to claim 33, wherein said gRNA comprises the nucleic acid sequence as denoted by SEQ ID NO. 2, and wherein at least one reporter gene comprised within said second nucleic acid sequence is flanked at 5' and 3' thereof by homologous arms comprising the amino acid sequence as denoted by SEQ ID NO. 6 and 7, respectively.

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36. A cell comprising at least one exogenous reporter gene integrated into at least one locus in at least one of gender chromosome Z and W.

37. The cell according to claim 36, wherein said cell is an avian cell.

38. The cell according to claim 37, wherein said avian cell is a primordial germ cell (PGC).

39. The cell according to any one of claims 36 to 38, wherein said at least one reporter gene is integrated into a gender chromosome of said cell, by co-transfecting said cell with:

(a) at least one first nucleic acid sequence comprising at least one nucleic acid sequence encoding at least one Cas9 protein and at least one nucleic acid sequence encoding at least one guide RNA (gRNA); and

(b) at least one second nucleic acid sequence comprising at least one said reporter gene

40. The cell according to claim 39, wherein said at least one reporter gene in said second nucleic acid sequence is flanked at 5' and 3' thereof by homologous arms for HDR at the integration site.

41. The cell according to any one of claims 39 and 40, wherein said gRNA comprises the nucleic acid sequence as denoted by SEQ ID NO. 1, and wherein said at least one reporter gene comprised within said second nucleic acid sequence is flanked at 5' and 3' thereof by homologous arms comprising the amino acid sequence as denoted by SEQ ID NO. 4 and 5, respectively.

42. The cell according to any one of claims 39 and 40, wherein said gRNA comprises the nucleic acid sequence as denoted by SEQ ID NO. 2, and wherein said at least one reporter gene comprised within said second nucleic acid sequence is flanked at 5' and 3' thereof by homologous arms comprising the amino acid sequence as denoted by SEQ ID NO. 6 and 7, respectively.

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43. A kit comprising:

(a) at least one first nucleic acid sequence comprising at least one nucleic acid sequence encoding at least one Cas9 protein and at least one nucleic acid sequence encoding at least one guide RNA (gRNA); and

(b) at least one second nucleic acid sequence comprising at least one said reporter gene.

44. The kit according to claim 43, wherein said at least one reporter gene in said second nucleic acid sequence is flanked at 5' and 3' thereof by homologous arms for HDR at the integration site.

45. The kit according to any one of claims 43 and 44, wherein said at least one reporter gene in said second nucleic acid sequence is operably linked to any one of a gender specific promoter, an embryonic specific promoter and an inducible promoter.

46. The kit according to any one of claims 43 to 45, wherein said at least one reporter gene is integrated into at least one non-coding region of said gender chromosome, and wherein said at least one gRNA comprises the nucleic acid sequence as denoted by any one of SEQ ID NO. 1 and 2.

47. The kit according to claim 46, wherein said gRNA comprises the nucleic acid sequence as denoted by SEQ ID NO. 1, and wherein said at least one reporter gene comprised within said second nucleic acid sequence is flanked at 5' and 3' thereof by homologous arms comprising the amino acid sequence as denoted by SEQ ID NO. 4 and 5, respectively.

48. The kit according to claim 46, wherein said gRNA comprises the nucleic acid sequence as denoted by SEQ ID NO. 2, and wherein said at least one reporter gene comprised within said second nucleic acid sequence is flanked at 5' and 3' thereof by homologous arms comprising the amino acid sequence as denoted by SEQ ID NO. 6 and 7, respectively.

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49. The kit according to any one of claims 43 to 48, wherein said reporter gene is at least one bioluminescence reporter gene.

50. The kit according to any one of claims 43 to 49, for use in the preparation of a transgenic one transgenic avian animal comprising at least one exogenous reporter gene integrated into at least one locus in at least one of gender chromosome Z and W.

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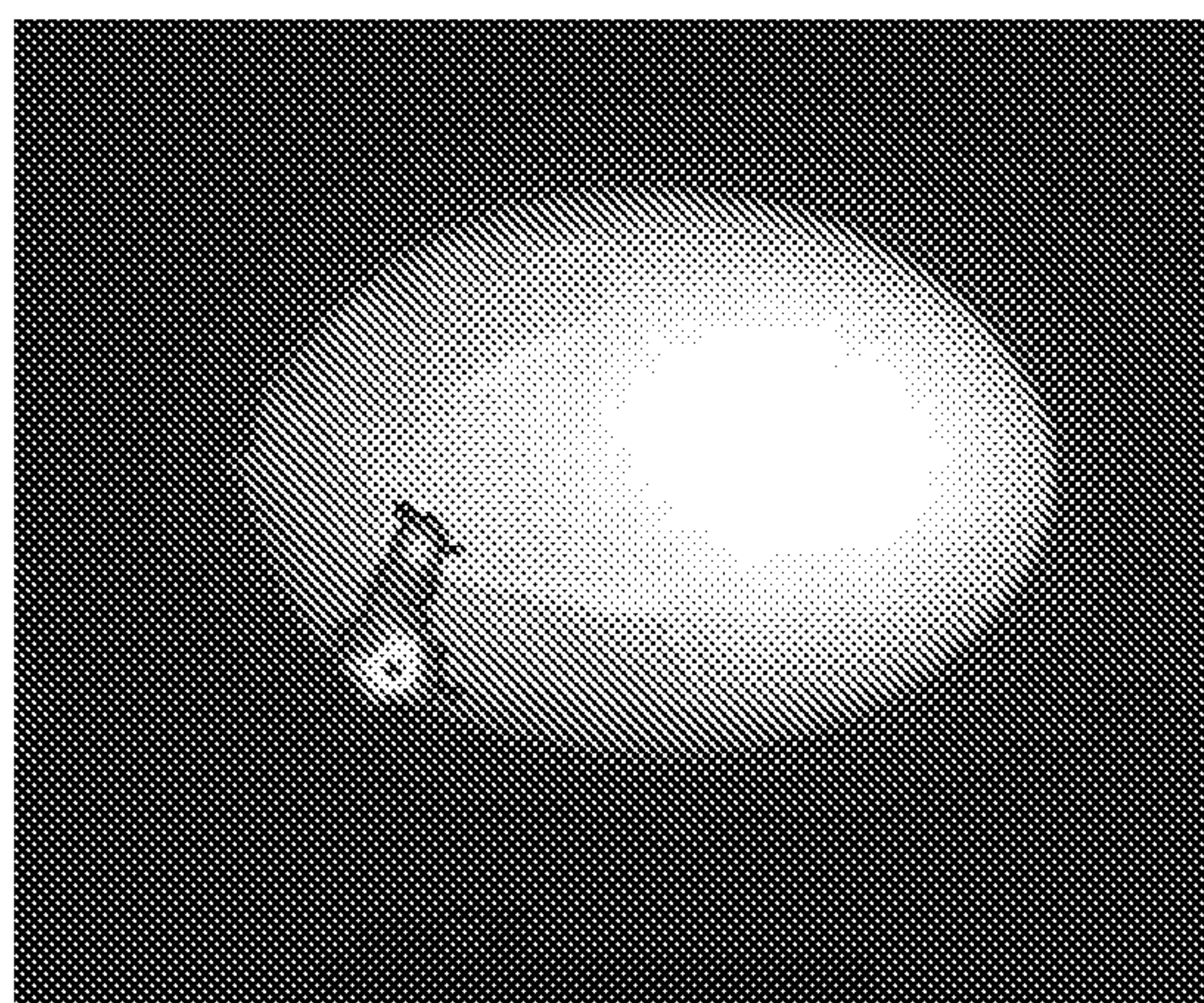


Fig. 1A

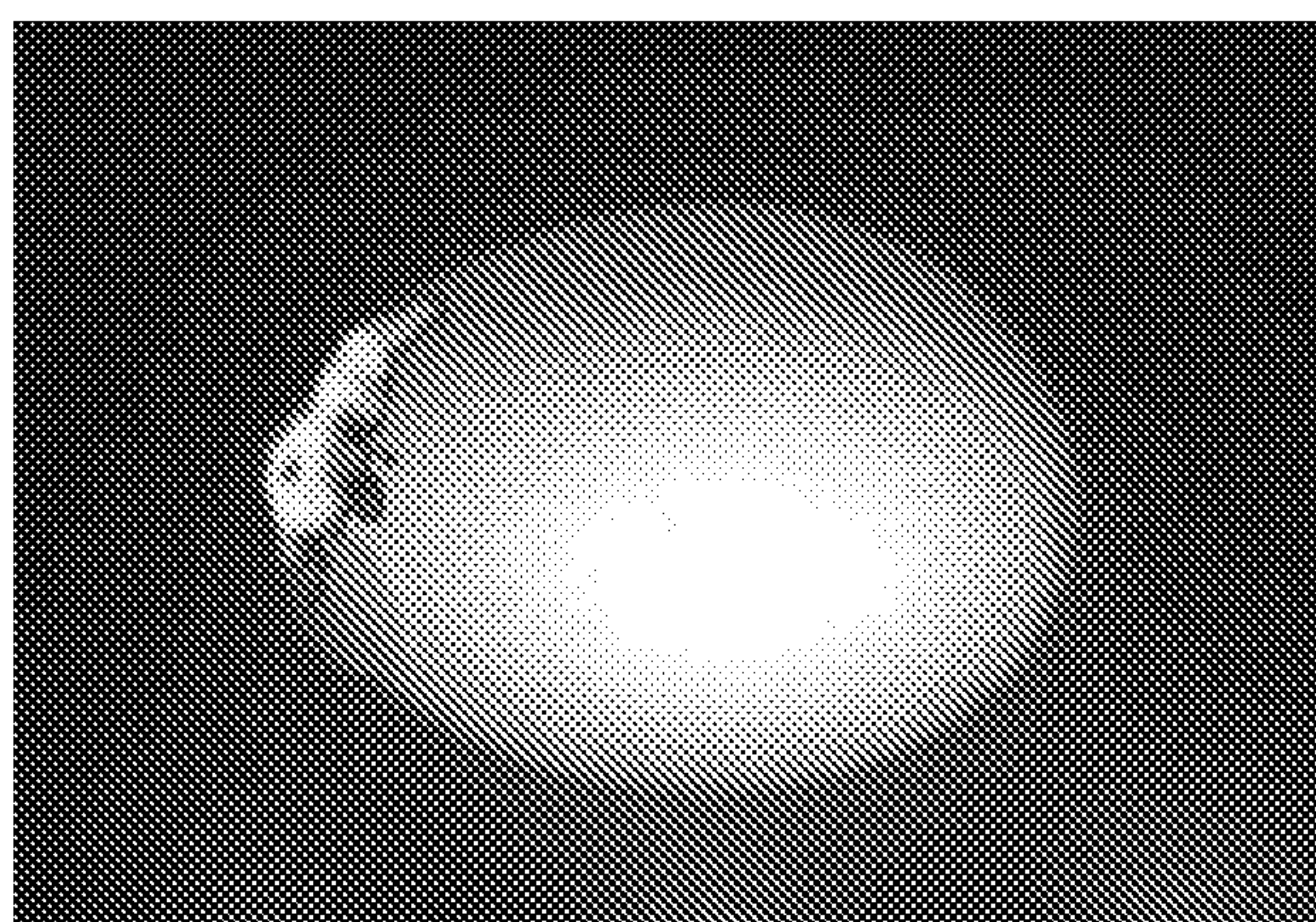


Fig. 1B

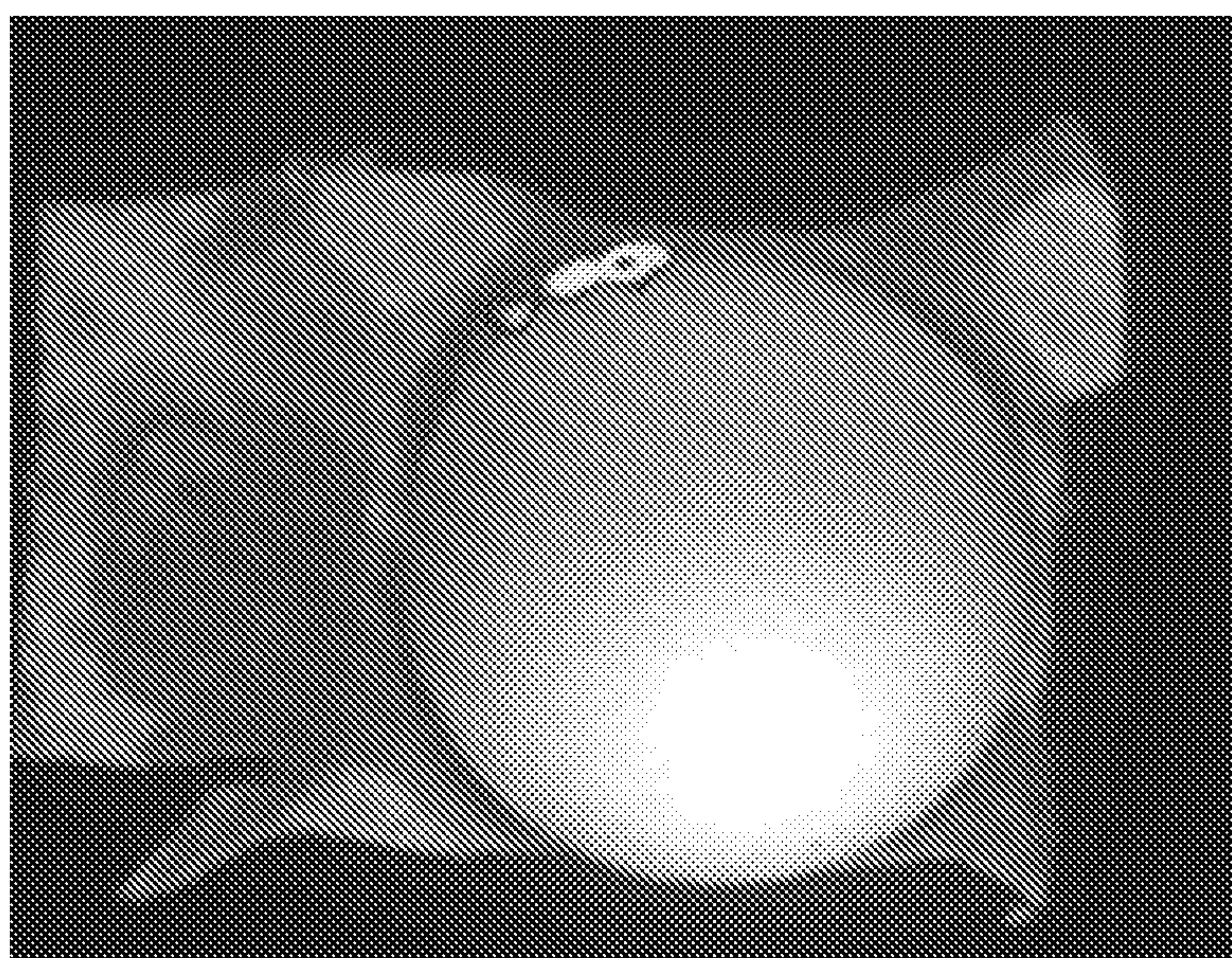


Fig. 2A

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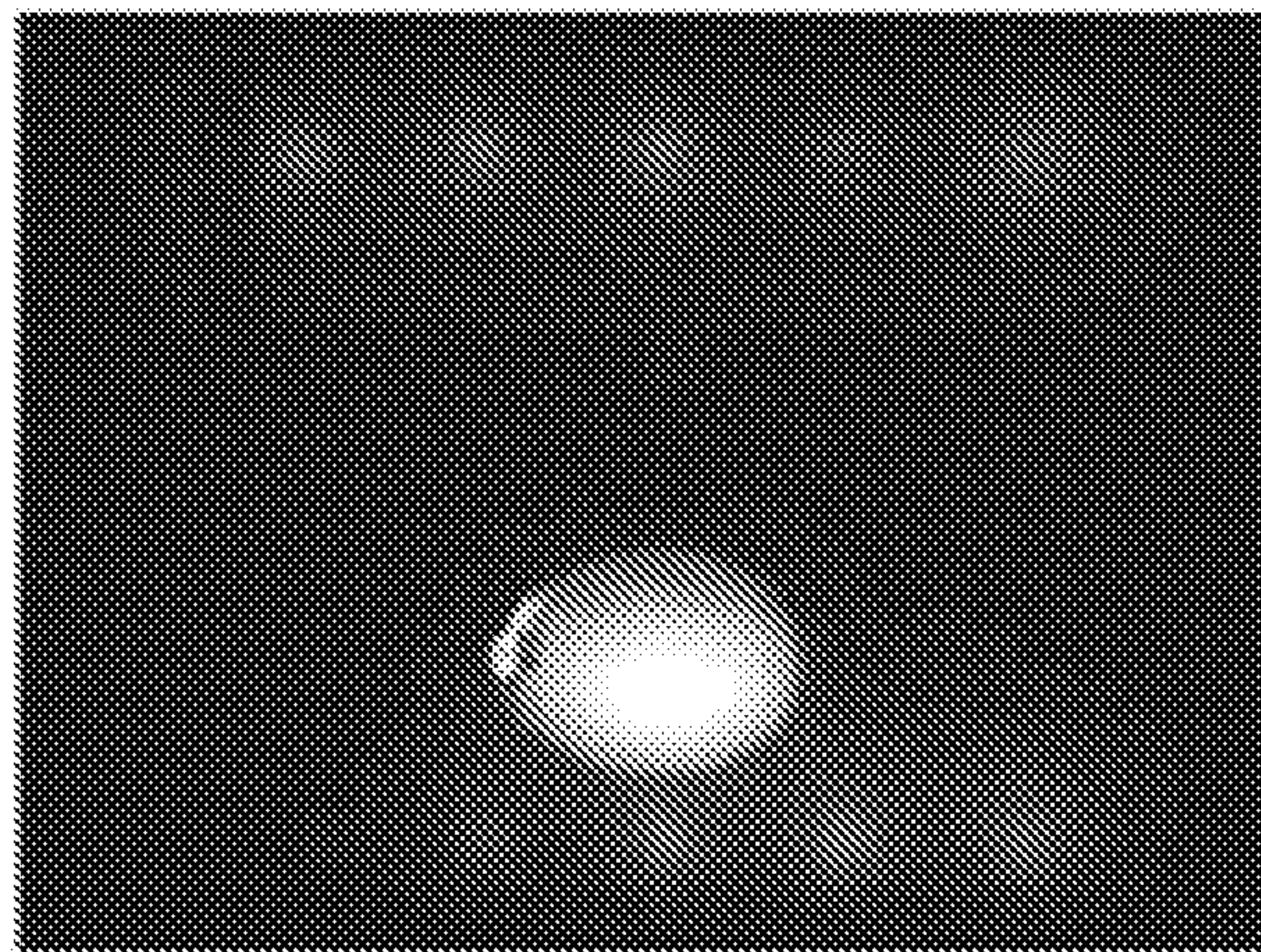


Fig. 2B



Fig. 3A

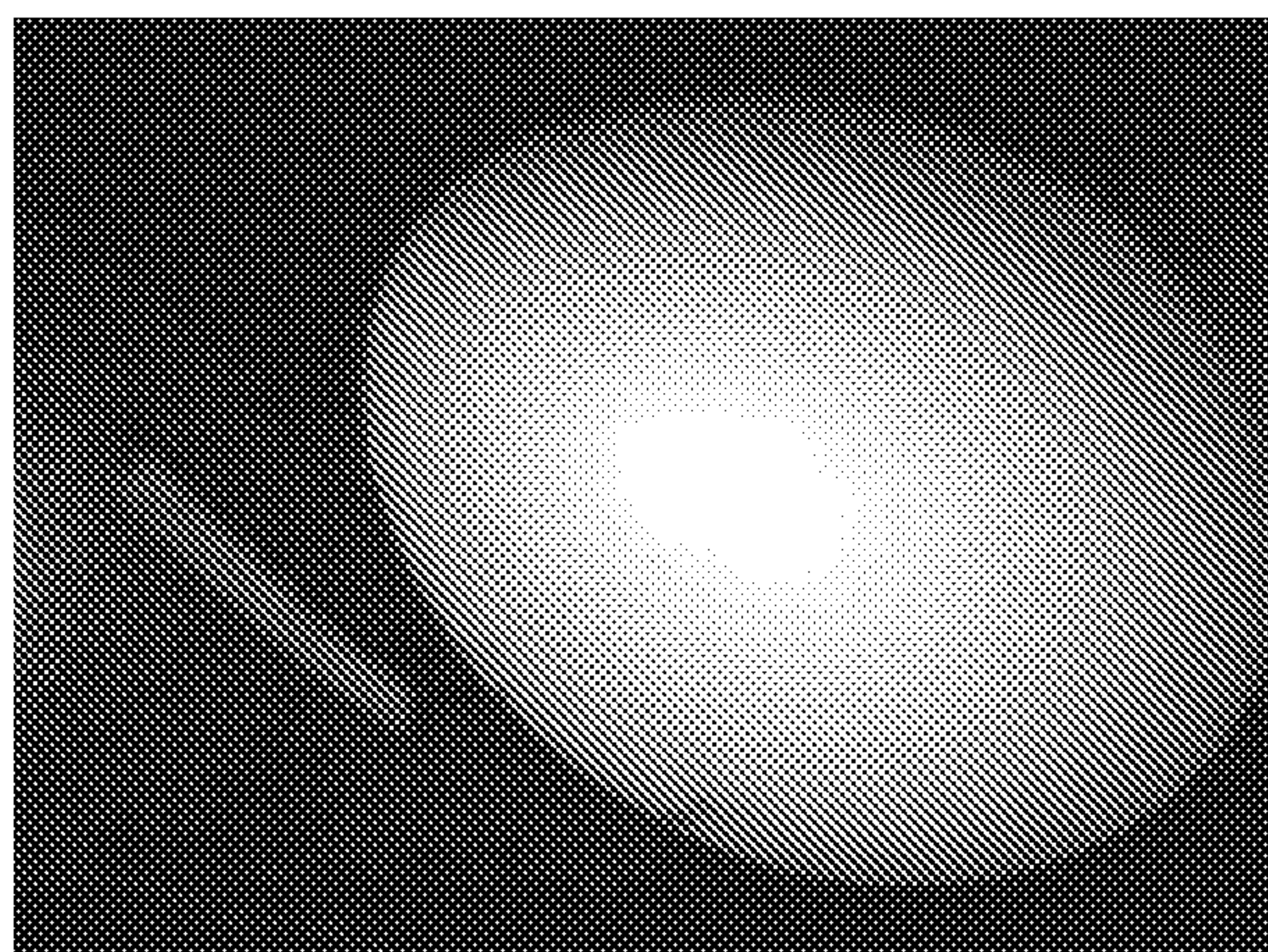


Fig. 3B

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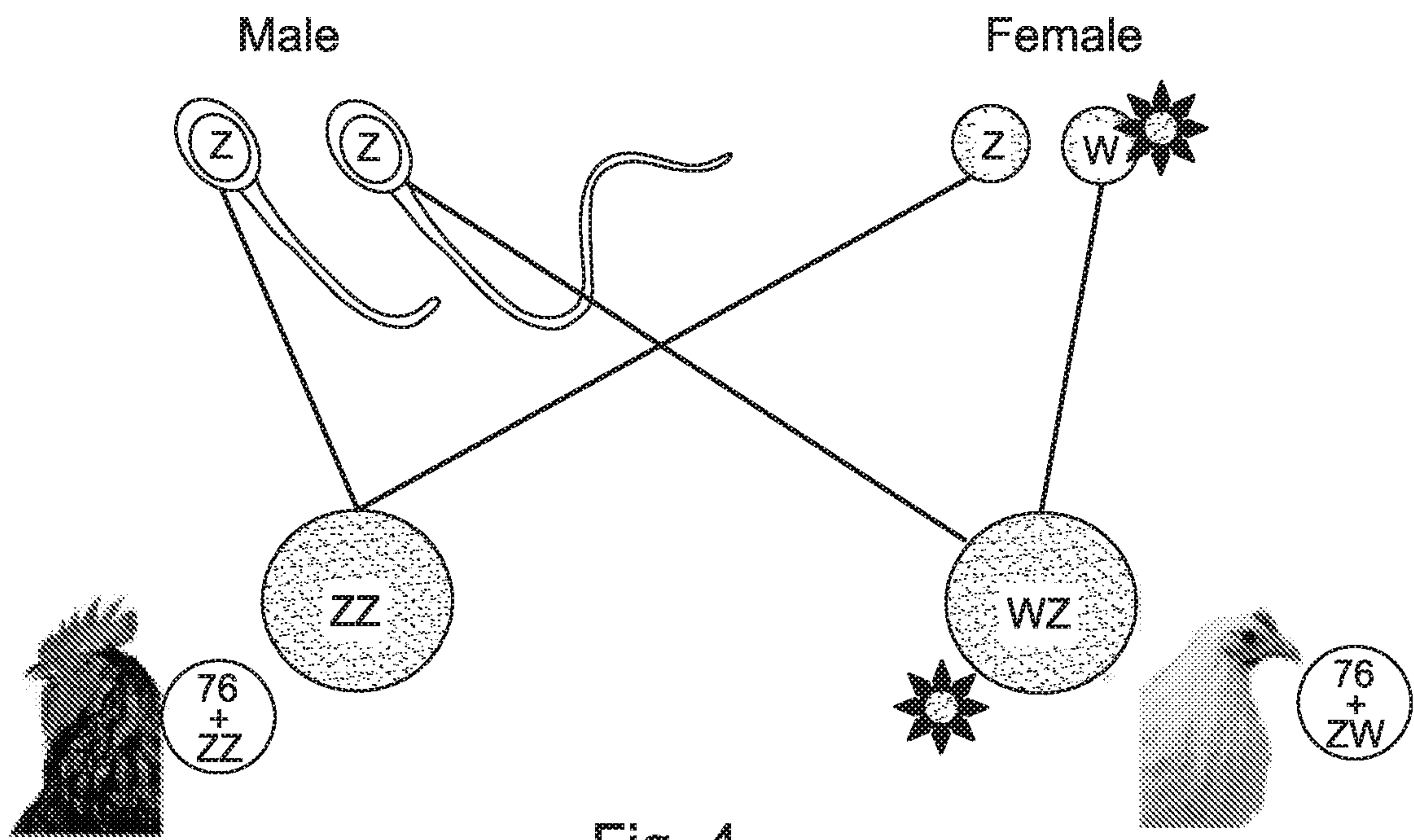


Fig. 4

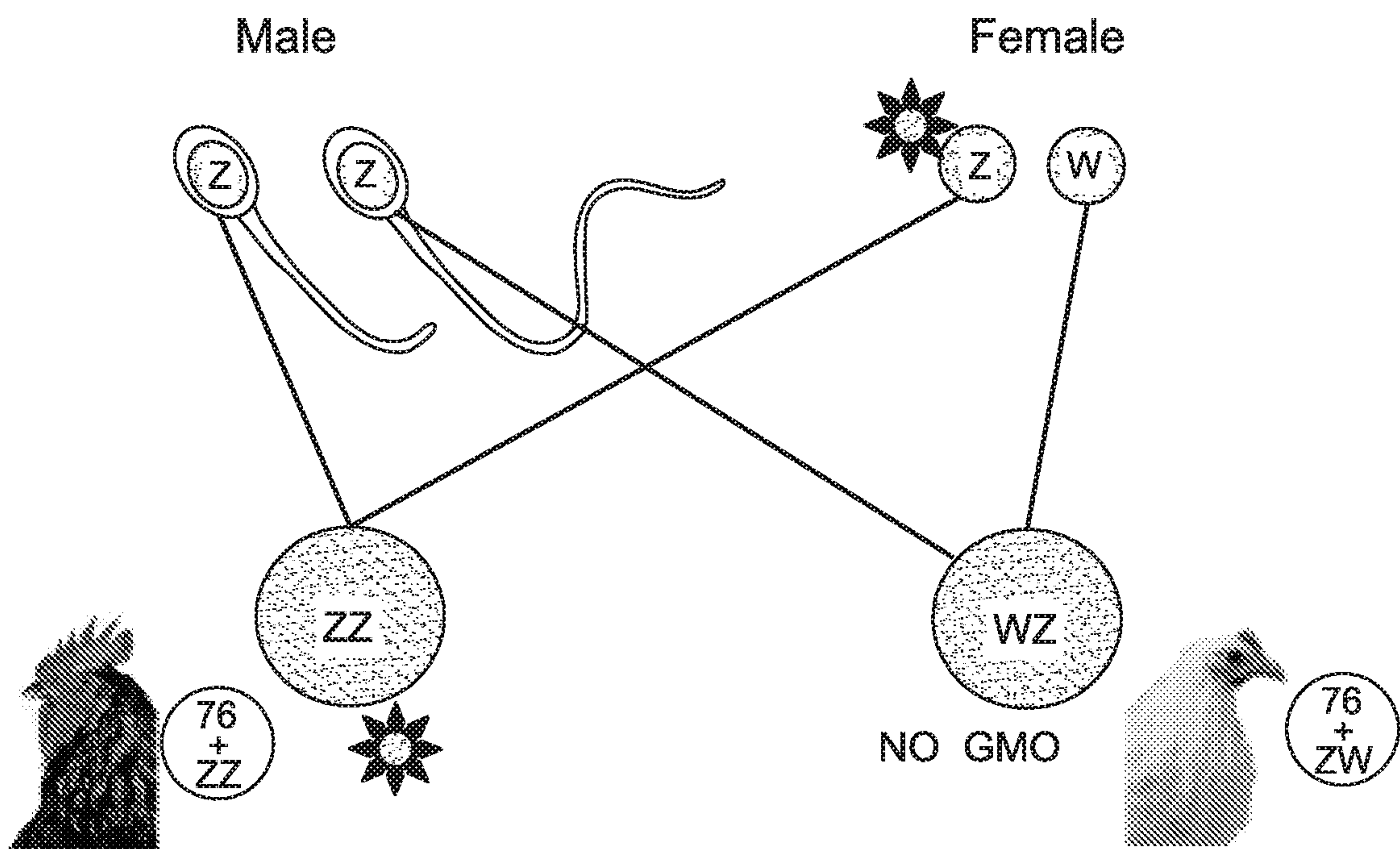


Fig. 5