

INDEX

A

- Activin A.....3, 15, 22, 32, 88
- Activin/nodal signalling98
- Avian. *See* Chicken embryonic stem (cES) cells

B

- Basic fibroblast growth factor (bFGF)15, 22
- Bead graft..... 159–160, 171
- bFGF. *See* Basic fibroblast growth factor (bFGF)
- Bisulfite sequencing analysis.....34
- Blastocysts
 - and assess chimerism29
 - bovine mid-stage115, 116
 - canine..... 71, 74, 75
 - Caprine embryos55, 56
 - cell lineages97
 - cultured, feeder cells.....56
 - embryos77
 - epiblast/ED isolation and culture55–56
 - feeder and isolation, ICM.....55, 56
 - in vitro-produced/in vivo-derived.....90–91, 92
 - injection and embryo aggregation.....85
 - mouse preimplantation70
 - preimplantation 15, 31, 32
 - stage embryos87
 - zona pellucida43, 47
- Blastocyst-stage mouse embryos, EpiSC isolation
 - cryopreservation.....7
 - differentiation to neuroectoderm4, 10–11
 - freezing3
 - global gene expression analysis, RNA sequencing
 - aqueous phase9
 - bioanalyzer.....9
 - Cufflinks/Cuffdiff software packages9–10
 - ESCs.....10
 - HiSeq9
 - phase lock gel tube.....4, 8–9
 - RNeasy Plus Micro.....9
 - and iMEFs.....12
 - mouse strain.....12
 - pluripotency markers (*see* Pluripotency markers, EpiSCs)
 - pluripotent stem cells.....1–2
 - preimplantation
 - culture medium, EpiSC3

- 3.5 dpc4
- harvesting and outgrowth2, 5
- incubator to equilibrate.....5
- pulled glass pipette/30-gauge needle3
- stereomicroscope in sterile hood.....5
- routine culture3
- routine passaging5–7
- SB431542 and dorsomorphin.....12
- thawing.....3, 7
- Blimp1-mVenus and Stella-ECFP (BVSC)179, 181
- Bone morphogenetic protein 4 (BMP4)..... 175, 176, 179
- Bovine
 - derivation..... 115–116, 117, 121
 - equipment and tools113
 - ES cells111–112
 - feeder cell layers (*see* Feeder cells)
 - in vitro* characterization
 - alkaline phosphatase (AP) activity assay117
 - immuno-fluorescence staining.....118–120
 - medium preparation114–115
 - mid-stage blastocysts115, 120
 - molecular markers.....113
 - passage116–117, 118
 - peri-gastrulating (*see* Peri-gastrulating bovine)
 - pluripotency.....112
 - preimplantation embryos (*see* Bovine preimplantation embryos)
 - reagents.....113–114
 - “separate and seed”.....112
- Bovine preimplantation embryos
 - and characterization, neural precursors
 - cell lines205–206, 207
 - derivation (*see* Derivation)
 - embryo culture203
 - in vitro* maturation and fertilization203
 - immunosurgery.....202
 - inductive signals.....200
 - interkinetic nuclear migration.....200
 - media formulation, culture.....202
 - neural rosettes203–205
 - neurulation199
 - ovaries and collection of oocytes202–203
 - production201
 - trypsinization and replating200
- BVSC. *See* Blimp1-mVenus and Stella-ECFP (BVSC)

C

Canine ESCs (cESCs)
 acid Tyrode's solution.....81
 and bFGF stock solution.....80
 body temperature.....80
 cryopreservation.....78–79
 culture medium.....72–73
 derivation, heterogeneity.....71–72
 explant methodology.....74
 feeder-free culture.....78
 and HIFs.....80
 immunodissection (*see* Immunodissection, cESCs)
 initial subculture, explanted.....77
 and iPSCs characterization.....79
 isolation, intact embryo explants outgrowths.....77
 long-term culture.....77–78
 low-oxygen conditions.....80
 and MEF layer.....80
 naïve and primed pluripotent states.....70–71
 pluripotent cell development.....79–80
 preparation.....81
 progressive diseases/gerontological effects.....70

Canine induced pluripotent stem cells (ciPSCs).....72

cBC. *See* Chicken blastodermal cells (cBC)

cESCs. *See* Canine ESCs (cESCs)

Chicken. *See* Chicken embryonic stem (cES) cells

Chicken blastodermal cells (cBC)
 cell preparation and initial plating.....141
 collection.....140–141
 long-term culture, cES.....137

Chicken embryonic stem (cES) cells
 and cBC.....137
 characteristics.....137
 epiblast.....151
 Eyal-Giladi and Kochav (EGK).....151–152
 materials
 cell culture laboratory environment.....139–140
 chemical reagents and antibodies.....139, 149
 culture media.....138
 disposable sterile plastic.....138–139
 dissection material.....139, 149
 ECC medium.....153
 eggs.....138
 manipulation and analysis.....152–153
 methods
 antibody characterization.....144
 bead graft.....159–160
 blastoderm collection.....140–141
 cell preparation and initial plating.....140, 141
 EB formation.....144–145, 149
 electroporation.....146, 149
 epiblast-stage chicken embryos.....154–156
 feeder layer.....165

feeder production.....142
 freezing.....143, 149
 GFP positive.....148, 149
in vitro maintenance.....142–143
in vivo injection.....147–148, 150
 lentiviral infection.....146, 150
 new culture technique.....156–158
 nonmammalian model organism.....170
 pluripotency, Epi-SCs.....168–170
 primary culture, epiblast cells.....167
 recombinant chicken LIF.....164–165, 166
 subculture and maintenance,
 Epi-SCs.....166, 167–168
 subgerminal cavity injection.....160–161
 thawing.....143–144, 149
 transfection.....145–146, 149
 whole-mount RNA in situ
 hybridization.....161–164

Chimera.....147, 148

ciPSCs. *See* Canine induced pluripotent stem cells (ciPSCs)

Colorimetric detection.....125

Cryo-freezing container.....3, 7

Cryopreservation, cESCs.....78–79

Culture medium
 K15F5.....2, 5
 MEF.....2, 5, 7

Cytochemistry and immunofluorescence assay.....33–34

D

Derivation
 bovine ES colony.....115–116, 117
 and characterization, neural precursors
 cell lines.....205–206, 207

EpiLC.....179, 180

neural rosettes.....203–205

NPCs.....186, 191, 194

PGCLC.....179, 180, 182

preimplantation embryos, bovine
 (*see* Bovine preimplantation embryos)

Differentiation
 densities minimize cell death.....27–28
 and heterogeneous Oct4 expression.....12

in vitro
 assays.....28
 gES cells.....63, 64
 low levels.....76, 77
 multi-lineage.....15

to neuroectoderm
 change cell media.....11
 Dorsomorphin.....4
 EpiSCs.....11
 harvest and prepare EpiSC colonies.....10
 incubate cells.....11
 neural culture media.....4

Noggin.....4
 SB4315424
 seed cells10
 pluripotency markers79
 promotion, signaling pathways71
 Digoxigenin-RNA labelled probe.....125
 Dissection
 immuno (*see* Immunodissection, cESCs)
 medium.....2, 4
 scissors and forceps2
 zona pellucida, 30-gauge needles 43, 44

E

EB. *See* Embryoïd body (EB)
 ED. *See* Embryonic discs (ED)
 Electroporation.....146
 Embryo chicken. *See* Chicken embryonic stem (cES)
 Embryoïd body (EB).....144–145
 Embryonic discs (ED).....56
 Embryonic stem cells (ESCs)
 bovine (*see* Bovine)
 BVSC.....179
 canine (*see* Canine ESCs (cESCs))
 characteristics111
 chicken (*see* Chicken embryonic stem (cES) cells)
 chimeric animal productions.....85
 colony picking.....6, 146
 components86
 culture.....47, 179, 180, 182
 development and characterization40
 and express specific pluripotent molecular markers
 freezing46, 143, 194–195
 germline chimeras.....98
 goat (*see* Goat ES (gES) cells)
 and hESCs.....70
 and ICM.....70, 85–86
 and ICM-stage32
 and iPSCs70
 isolation in humans and nonhuman primates85
 IVP and in vivo-derived porcine embryos87
 lines.....39, 40, 51, 66, 87, 92, 93, 111, 112
 medical applications69
 medium.....90, 138, 188
 MEM and serum replacement.....86
 and mESCs70
 mouse and human ES cell lines112
 mouse blastocysts.....97
 Oct-4.....52
 passage number.....66
 passaging116–117, 141
 pluripotency.....51–52, 97
 pluripotent and self-renew indefinitely.....39
 pluripotent cells69, 85
 porcine (*see* Porcine ESCs (pESCs))

preimplantation blastocysts.....15
 and primordial germ cells16
 rabbit (*see* Rabbit embryonic stem (rES) cells)
 SSEA-152
 thawing.....143–144, 195
 types.....51
 Embryos, NPCs. *See* Neural progenitor cells (NPCs)
 Embryo/tissue preparation126
 Epiblast. *See also* Epiblast stem cells (EpiSCs)
 cell culture medium153
 chicken embryos
 bead graft.....159–160
 ex ovo culture technique156
 manipulation and analysis.....152–153
 new culture technique.....156–158
 staging and handing early-stage.....154–156
 subgerminal cavity injection.....160–161
 description151
 ED isolation and culture, feeder55
 and hypoblast.....86, 94
 murine postimplantation70
 Epiblast cell culture medium
 (ECC medium).....153, 167
 Epiblast-like cells (EpiLCs)
 cells harboring176
 derivation.....179
 differentiation medium.....178
 human plasma fibronectin180
 induction, ES cells180
 sensitive to mechanical damage180, 182
 Epiblast stem cells (EpiSCs)
 alkaline phosphatase (AP) assay107
 blastocyst-stage mouse embryos (*see* Blastocyst-stage
 mouse embryos, EpiSC isolation)
 converting mEpiSCs (*see* Mouse embryonic
 stem cells (mESCs))
 culture and colony passages104, 109
 immunocytochemistry (ICC)106
 karyotyping.....107, 109
 OPS.....104, 109
 pluripotency (*see* Pluripotency)
 reversion (*see* Reversion, EpiSCs)
 subculture and maintenance166, 167–168
 thawing.....106
 vitrification105–106, 109
 EpiLCs. *See* Epiblast-like cells (EpiLCs)
 EpiSCs. *See* Epiblast stem cells (EpiSCs)
 ESCs. *See* Embryonic stem cells (ESCs)
 ES-HEPES medium.....89

F

FACS. *See* Fluorescence-activated cell sorting (FACS)
 FCS. *See* Fetal calf serum (FCS)
 Feeder cells. *See also* Mouse embryonic fibroblasts (MEFs)

concentration.....	42, 43
density.....	28
and embryos.....	113
goat.....	57, 58, 59
growth-arrested.....	77
hatched blastocyst.....	56
and ICM.....	55
ICM cells and feeder layer.....	89
LIF/Stat3 signalling.....	23–25
preparation.....	115, 120
preparation, primary MEFs.....	18–20
Fetal calf serum (FCS).....	16, 17, 20, 25, 28
Fluorescence-activated cell sorting (FACS).....	180–181

G

gES cells. <i>See</i> Goat ES (gES) cells	
GFF cells. <i>See</i> Goat fetal fibroblast (GFF) cells	
Goat ES (gES) cells	
blastocysts and expression.....	52
CEeGFP plasmid.....	66
characterization	
alkaline phosphatase.....	58
pluripotency markers Oct-4, Nanog, SSEA-4 and Sox2.....	59, 60
chemicals, materials.....	52
collection	
after culture.....	55, 56
blastocysts.....	55–56
colonies.....	56
DMEM medium.....	55
floating embryos.....	55
cryoprotectants.....	66
GFF (<i>see</i> Goat fetal fibroblast (GFF) cells)	
in vitro differentiation.....	63, 64
karyotyping.....	59–60
passaging.....	56–58
RT-PCR assay (<i>see</i> RT-PCR assay)	
superovulation and embryo collection.....	53
teratoma formation.....	63–65
thawing.....	58
transfection.....	62–63
Goat fetal fibroblast (GFF) cells	
cryopreservation.....	54
deactivation.....	54–55
harvesting.....	53–54

H

hESCs. <i>See</i> Human ESCs (hESCs)	
HIFs. <i>See</i> Hypoxia-inducible factors (HIFs)	
Human ESCs (hESCs).....	31, 32, 70, 71, 186
Hypoxia-inducible factors (HIFs).....	80

I

ICM. <i>See</i> Inner cell mass (ICM)	
<i>In vitro</i> cES genetic modification	
electroporation.....	146, 149
lentiviral infection.....	146, 150
transfection.....	145–146, 149
<i>In vivo</i> injection.....	147–148, 150
iMEFs. <i>See</i> Irradiated MEFs (iMEFs)	
Immunodissection, cESCs	
initial subculture.....	76
inner cell masses	
blastocysts.....	74
embryological immunosurgery.....	74–76
mature batches.....	74
morulae/blastocysts.....	74
progesterone and LH levels.....	74
reagents.....	73
Induced pluripotent stem cells (iPSCs)	
<i>in vitro</i> culture.....	193
NPCs.....	186
porcine.....	187, 192
Inner cell mass (ICM)	
antiserum preparations.....	74–75
blastocysts.....	86
canine blastocyst.....	71
expression levels.....	70
immunological isolation.....	85–86
monolayers, arrested feeder cells.....	76
mouse preimplantation blastocysts.....	70
In situ hybridization	
degradation, cellular RNA.....	130–133
detecting cellular mRNA.....	126
protocol	
post-antibody washes and color development.....	131, 132
post-hybridization washes and anti-DIG antibody.....	132
pre-hybridization and hybridization.....	130–132
RNA	
collect embryos.....	161, 162
DIG / fluorescein-labeled probes.....	161
monitor, color development.....	163
Paraformaldehyde (PFA) and methanol fixed embryos.....	161–162
pluripotency genes.....	162, 164
pre-hybridization.....	163
Proteinase K.....	163
protocols.....	161, 172
serial tissue sections.....	126
troubleshooting	
antisense probe.....	133
probe purification.....	133
probe size, specificity and concentration.....	133

In vitro riboprobe synthesis
 cDNA template 126–128
 transcription 126–128
 iPSCs. *See* Induced pluripotent stem cells (iPSCs)
 Irradiated MEFs (iMEFs)..... 2, 5–7, 10, 12

K

Karyotyping.....59–60

L

Lentiviral infection.....146
 Leukemia inhibitory factor (LIF)
 and bFGF..... 71, 72, 81
 chemical inhibitors32
 and FCS17, 29
 STAT3 and BMP32
 Stat3 signalling
 cell suspension23
 freeze rESCs.....25
 GFP expression24
 incubate23
 MEF medium.....24–25
 reversion experiment.....23–24
 LIF. *See* Leukemia inhibitory factor (LIF)

M

MEFs. *See* Mouse embryonic fibroblasts (MEFs)
 α-MEM-HEPES medium.....89
 mESC-like human induced pluripotent
 stem cells (m-hiPSCs).....32
 mESCs. *See* Mouse embryonic stem cells (mESCs); Murine
 ESCs (mESCs)
 m-hiPSCs. *See* mESC-like human induced pluripotent stem
 cells (m-hiPSCs)
 Mitomycin-C
 avian epiblast and epiblast-derived stem cells
 manipulation..... 153, 165, 168
 chicken embryonic stem cells establishment
 and characterization..... 139, 142
 isolation and culture
 bovine embryonic stem cells 113–115
 pig epiblast stem cells98, 99, 102, 108
 porcine neural progenitor cells 189, 192, 197
 mLIF. *See* Murine leukemia inhibitory factor (mLIF)
 Mouse embryonic fibroblasts (MEFs)
 cells plate94
 derivation.....18
 feeder cells preparation
 derivation.....19
 forceps pair19
 freeze mitomycin C-treated20
 passage.....20
 Reichert’s membrane and placenta19
 tissue culture dish20

feeder layers
 mice87
 preparation.....89–90
 production88
 freezing.....20, 103
 irradiated (*see* Irradiated MEFs (iMEFs))
 g-irradiation..... 76, 77, 78
 layer.....78, 80, 81
 media volume78
 medium
 and cell suspension.....23
 expand primary.....26
 fibroblast cryopreservation103
 fibroblast inactivation102
 pig epiblast stem cells, isolation
 and culture98
 rESCs and feeder cells22
 and spin25
 Sterile 16, 21
 thawing.....103
 trypsin.....24, 102
 and uterine wall21
 mESC medium.....33
 mitomycin C solution26
 mitomycin C treatment20, 102
 and pESC medium.....88
 and spin25

Mouse embryonic stem cells (mESCs)
 bisulfite sequencing analysis34
 cells and cell culture reagents.....33
 chimerism competence32
 cytochemistry and immunofluorescence assay 33–34
 discrepancies32
 and mEpiSCs (*see* mouse EpiSCs (mEpiSCs))
 and m-hiPSCs32
 pluripotent stem cells.....31
 self-renewing signaling pathways31–32
 semiquantitative RT-PCR and real-time PCR.....34

Mouse EpiSCs (mEpiSCs)
 cells and cell culture reagents.....33
 cell types32
 characterization35–36
 culture and passage34
 discrepancies32
 and hESCs.....31
 pluripotent state, small molecules35, 36

Murine epiblast stem cells (mEpiSCs)70–71
 Murine ESCs (mESCs)70, 80
 Murine leukemia inhibitory factor (mLIF)41, 43, 46

N

Naïve mESC-like cells71
 Neural differentiation
 bovine..... 199, 200
 NPCs.....195

Neural precursors
 derivation and characterization.....205–206, 207
 media formulation, bovine.....202

Neural progenitor cells (NPCs)
 antibodies189
 cells/embryos187
 comparative real-time PCR.....196
 consumables.....186–187
 description.....185
 differentiation potential.....195
 freezing.....194–195
 immunocytochemistry.....194, 195–196
 induction.....192, 193
 iPSC and hESC.....186
 isolation, rosette structures
 and expansion.....193–194, 197
 matrigel-coated dishes.....193, 197
 media
 differentiation medium.....188
 embryo flushing solution.....187
 embryo isolation medium/
 fibroblast medium.....187
 ESC medium.....188
 expansion medium.....188
 induction medium.....188
 maturation medium.....188–189
 pluripotent stem cells (*see* Pluripotent stem cells)
 preparation, MS5 stromal cells.....192
 primers.....189, 190
 sequential steps, derivation.....189, 191
 thawing.....195

Neural rosettes
 bovine inner cell.....199–200
 derivation.....203–204
 immunosurgery.....205

Neurons and glia.....185, 189, 190

New culture technique.....156–158

NPCs. *See* Neural progenitor cells (NPCs)

O

Open pulled straws (OPS).....106, 109
 OPS. *See* Open pulled straws (OPS)
Oryctolagus cuniculus.....40

P

PBS. *See* Phosphate-buffered saline (PBS)

Peri-gastrulating bovine
 buffers, reagents.....128–129
 detecting cellular mRNA.....126
 embryo preparation.....126
 equipment.....130
in situ hybridization (*see* *In situ* hybridization)
 RNA probes (*see* RNA probes synthesis)

WISH.....125

pESCs. *See* Porcine ESCs (pESCs)

PGCLCs. *See* PGC-like cells (PGCLCs)

PGC-like cells (PGCLCs)
 characteristics.....176
 derivation.....179, 182
 differentiation medium.....179
 and EpiLCs.....180
 ES cells.....179
 and FACS pattern.....180, 181

PGCs. *See* Primordial gem cells (PGCs)

Phosphate-buffered saline (PBS).....2, 8, 12

Pig epiblast
 artificial insemination
 and embryo collection.....103, 104
 cell lineages.....97
 collection, epiblast isolation and culture.....99–100
 epiblast isolation and initial
 culture.....103–104, 105

EpiSCs (*see* Epiblast stem cells (EpiSCs))
 equipment.....101

ESC.....97–98

FGF2 and activin/nodal signalling.....98

isolation and preparation,
 feeder cells.....98–99

mouse feeder cells preparation
 cryopreservation.....103
 fibroblast isolation
 and culture.....101–102, 108
 inactivation and preparation.....102, 108, 109
 thawing.....103

Pluripotency
 bovine adult fibroblasts.....112
 cultured epiblast cells.....172
 and differentiation.....79
 embryo development.....98
 Epi-SCs.....168–170
 ES cell pluripotency markers.....118–120
 ES cells.....51–52, 112
 ESCs and iPSCs.....80
 extrinsic factors.....112
 gene expression
 EpiSC lines.....28
 rESCs.....29
 and germ cell-associated genes.....16
 goat ES characterization.....59
 hallmarks.....70
 homogeneous expression.....35
 hybridization.....164
 immunostaining, Nanog.....168–170
 intrinsic factors.....112
 LIF.....97
 long-term culture.....70
 maintenance.....172

molecular markers.....113
 molecular mechanisms.....80
 mouse and human ES cells.....172
 mRNA expression analysis,
 Oct4 and Nanog.....168, 169
 self-renewal signaling pathways.....72
 signalling.....98
 Pluripotency markers, EpiSCs
 aspirate cell culture medium.....8
 blocking solution.....3
 DAPI stock solution.....4
 PBS.....3
 primary and secondary antibodies.....4
 Pluripotent cells.....39, 40
 Pluripotent stem cells
 antibodies.....189
 culture
 isolation and culture, epiblast cells.....189–192
 MEF feeder cells.....189
 porcine iPSCs.....189
 cultured mouse blastocysts.....6
 in vitro.....1
 iPSCs.....187
 mouse EpiSCs, human ESCs
 and iPSCs.....2
 to PGCs (*see* Primordial germ cells (PGCs))
 properties.....1–2
 PMEFs. *See* Primary mouse embryonic fibroblast feeder
 cells (PMEFs)
 Porcine ESCs (pESCs)
 cryologic vitrification.....93
 gelatinized dishes preparation.....89
 media and solutions.....88–89
 MEF feeder layers.....87, 89–90
 passaging.....91–93
 pig blastocyst stage embryos.....87
 pig pluripotent primary embryonal
 outgrowth.....90–91, 92
 tissue culture and glassware.....87–88
 warming.....94
 Postimplantation epiblast.....175, 176
 Preimplantation embryo. *See* Bovine preimplantation
 embryos
 Primary mouse embryonic fibroblast feeder cells (PMEFs)
 density.....42, 44
 monolayer.....43
 and rESM.....47
 Primordial germ cells (PGCs)
 epigenetic reprogramming.....176
 EpiLC differentiation.....180, 182
 ES cells culture.....179–180
 FACS sorting.....180–181
 gene expression program.....175–176
 materials.....176–179
 nascent.....176

PGCLC differentiation.....180, 181
 postimplantation epiblast.....175

R

Rabbit embryonic stem (rES) cells
 materials
 cryopreservation.....41
 dissecting embryos.....41
 egg recovery.....40–41
 initial phase, cell derivation.....41
 recovery, cryopreserved.....41
 stable phase, cell derivation.....41
 superovulation.....40
 methods
 cryopreservation.....47
 dissecting embryos.....43, 44
 egg recovery.....42
 embryo explants primary
 disaggregation.....43, 44, 45
 initial phase, derivation.....46
 PMEFs.....42–43
 preparation.....42–43
 recovery, cryopreserved.....48
 secondary passaging.....43, 45
 stable phase of derivation.....46
 superovulation.....42
 pESC lines.....39
 rES cells. *See* Rabbit embryonic stem (rES) cells
 Reversion, EpiSCs
 activin A and bFGF.....15
 animals.....18
 buffers and solutions.....17
 calcium/magnesium-free Tyrode
 Ringer's Saline.....25
 cell culture media.....16–17
 equipment.....18
 FCS.....25
 Feeder cells (*see* Feeder cells)
 MEFs (*see* Mouse embryonic fibroblasts (MEFs))
 mouth pipette.....25, 26–27
 Oct4-DPE-GFP.....25–26
 optimal clump size.....27
 pull borosilicate glass capillaries.....26, 29
 Reverted ESCs (rESCs). *See* Reversion, EpiSCs
 RNA probes synthesis
 cDNA template
 PCR amplification.....126, 127
 primers and annealing temperatures.....126, 127
 in vitro transcription.....126–128
 mEpiSCs and converted cells.....35
 quality and quantity.....59
 RT-PCR assay
 gene expression in goat ES cells.....59, 62
 primer sets.....59, 61

S

Separate and seed112, 116
 Stem cells. *See* Epiblast stem cells (EpiSCs)

T

Teratoma formation
 aspirate medium63
 cells and aliquot cells63
 centrifuge cells63
 histological and indirect immunohistochemical
 analysis.....65
 NOD-SCID mice64
 protocols64–65

Transfection.....145–146
 Transplantation.....186

W

Warming solution.....89
 Whole-mount in situ hybridization (WISH)
 bovine embryos.....125
 digoxigenin-RNA labelled probes125
 heterologous probes and species130, 131
 riboprobe synthesis/ *in situ* hybridization133
 stock solutions129
 WISH. *See* Whole-mount in situ hybridization (WISH)
 Wnt, β -catenin signaling112