

Index

A

- AAV. *See* Adeno-associated virus
- Adaptive immunity, CRISPR function
 - in bacteria, 2–3
- Adeno-associated virus (AAV), delivery of
 - CRISPR–Cas in mammalian cells
 - cloning, 59–61
 - gene targeting, 64
 - genome modification, 63
 - materials, 57–59
 - overview, 57
 - recipes, 65–68
 - troubleshooting, 64–65
 - virus production
 - buffer exchange and concentration, 62
 - cell culture, 61
 - iodixanol density gradient centrifugation, 61–62
 - titering, 62

B

- Bacterial lipoprotein (BLP), Cas9 in
 - repression, 7–8
- BLP. *See* Bacterial lipoprotein

C

- Cas
 - Cas9
 - bacteria functions, 7–8
 - crRNA maturation mediation, 4–5
 - deactivated Cas9 for CRISPRa or CRISPRi
 - expression vector preparation, 167
 - lentiviral packaging, 167–168
 - transduction, 168–169
 - guide sequences. *See* Guide RNA
 - nuclease-deficient protein, 10
 - spacer acquisition, 6–7
 - target interference, 5–6
 - target sequence specificity, 10–11
 - tracrRNA interactions, 20–22
 - Cas9–gRNA ortholog characterization
 - applications, 33
 - orthogonality value, 32–33
 - overview, 31–33
 - protospacer adjacent motif characterization for Cas9 orthologs

- high-throughput sequencing
 - and analysis, 37–38
 - materials, 35–36
 - plasmid construction, 36–37
 - sequences by bacteria species, 33
 - validation of sequences, 38
 - putative protein identification, 32
- crRNA interactions, 4
- crystal structures, 2–3
- isoforms in CRISPR systems, 3–4
- nuclease activity, 3
- Cell fate, reprogramming with CRISPRa or CRISPRi, 162
- CFTR. *See* Cystic fibrosis transmembrane regulator
- CRISPRa
 - applications, 170–171
 - cell fate reprogramming, 162
 - deactivated Cas9
 - expression vector preparation, 167
 - lentiviral packaging, 167–168
 - gene expression analysis, 169–170
 - materials, 163–165
 - multiple gene modulation, 161
 - noncoding RNA gene targeting, 161
 - principles, 159–161
 - recipes, 171
 - single guide RNA
 - design, 166
 - generation, 166–167
 - target selection, 165
 - transduction, 168–169
- CRISPR–Cas
 - activation. *See* CRISPRa
 - adeno-associated virus delivery. *See* Adeno-associated virus
 - bacteria functions, 2–3, 7–8
 - Drosophila melanogaster* genome engineering. *See* *Drosophila melanogaster*
 - genetic engineering overview, 8–11, 20–22
 - historical perspective, 1–2, 17
 - human stem cells. *See* Embryonic stem cell; Induced pluripotent stem cell
 - mechanism of action, 2, 17–18, 133–135
 - mouse genome editing. *See* Mouse optimization
 - targeting efficiency, 113–114

- targeting repertoire expansion, 114–115
- zebrafish CRISPR–Cas9 system
 - optimization
 - Cas9 mRNA production and injection of guide RNA complex, 123–125
 - materials, 117–119
 - mutation analysis, 125–130
 - recipes, 130
 - single guide RNA design and generation, 119–123
 - repression. *See* CRISPRi
 - Saccharomyces cerevisiae* genome engineering. *See* *Saccharomyces cerevisiae*
 - types of systems, 2–4, 17–20
- CRISPRi
 - cell fate reprogramming, 162
 - multiple gene modulation, 161
 - noncoding RNA gene targeting, 161
 - principles, 159–161
- crRNA. *See* Guide RNA
- Cystic fibrosis transmembrane regulator (CFTR), mutation repair with CRISPR–Cas, 11

D

- ddPCR. *See* Droplet digital polymerase chain reaction
- Droplet digital polymerase chain reaction (ddPCR)
 - overview, 70
 - single–nucleotide substitution detection in induced pluripotent stem cells
 - challenges, 69–70
 - hydrolysis probe and primer preparation and validation, 74–76
 - materials, 73–74
 - substitution detection in genomic DNA, 76–77
 - troubleshooting, 77
- Drosophila melanogaster*, CRISPR–Cas9 genome engineering
 - delivery overview, 90–91
 - donor construct generation
 - donor construct generation, 95–96
 - homology arm design, 94–95

Drosophila melanogaster, CRISPR–Cas9
genome engineering
(Continued)

materials, 93–94

recipes, 97

troubleshooting, 96–97

editing event detection

high-resolution melt analysis of

indel mutations

data analysis, 100–102

genomic DNA preparation,

99–100

materials, 98–99

polymerase chain reaction, 100

recipes, 104

sequencing, 101, 103

troubleshooting, 103–104

overview, 91

off-target effects, 110–111

overview, 89–90

single guide RNA generation

cloning, 107, 109–110

design, 90, 107–108, 111

materials, 106–107

troubleshooting, 110

E

Embryonic stem cell (ESC), overview of
human genome editing,
149–150

ESC. *See* Embryonic stem cell

F

FACS. *See* Fluorescence-activated cell
sorting

Fluorescence-activated cell sorting
(FACS), stem cell Cas9
transfectants, 156

G

gRNA. *See* Guide RNA

Guide RNA (gRNA)

Cas9 guide sequences

prediction and validation of
sequences

boundary confirmation for

crRNA and tracrRNA, 28

CRISPR repeat and *cas*
prediction in silico, 25–26

materials, 24–25

overview, 24

PAM sequence prediction in
silico, 28–29

tracrRNA prediction in silico, 25,
27–28

troubleshooting, 29–30

tracrRNA interactions, 20–22

Cas9–gRNA ortholog characterization.
See Cas

CRISPRa or CRISPRi single guide RNA
design, 166

generation, 166–167

target selection, 165

Drosophila melanogaster single

guide RNA design, 90,

107–108

human guide RNA design and

generation

induced pluripotent stem cells, 154

overview, 154

mouse single guide RNA

design, 137, 141

generation, 141

screening of CRISPR–Cas9 with single
guide RNA library

negative selection screens, 41

overview, 39–40

positive selection screens, 40–41

principles, 40

virus packaging and cell culture

for screens

data analysis, 54

materials, 49–50

overview, 49

packaging vector preparation,

51–53

recipes, 55

screen cell culture and library

preparation, 53–54

troubleshooting, 54

single guide RNA

large-scale library construction

library amplification and

cloning, 45–46

materials, 43–44

recipes, 47–48

sequence design, 44

transformation, 46

troubleshooting, 47

vector preparation, 45

overview, 20–21, 39

zebrafish single guide RNA

design, 119–120

generation, 120–123

H

HDR. *See* Homology-directed repair

Hepatitis viruses, Cas9 targeting, 11

High-resolution melt analysis (HRMA),
indel mutations in *Drosophila*
melanogaster

data analysis, 100–102

genomic DNA preparation, 99–100

materials, 98–99

polymerase chain reaction, 100

recipes, 104

sequencing, 101, 103

troubleshooting, 103–104

Historical perspective, CRISPR–Cas,
1–2, 17

HIV. *See* Human immunodeficiency virus

Homology-directed repair (HDR)

Cas9 double-strand break repair, 9,
139

efficiency, 147–148

single-nucleotide substitution

detection in induced

pluripotent stem cells

challenges, 69–70

droplet digital polymerase chain
reaction

hydrolysis probe and primer

preparation and validation,

74–76

materials, 73–74

overview, 70

substitution detection in

genomic DNA, 76–77

troubleshooting, 77

HRMA. *See* High-resolution melt analysis

Human immunodeficiency virus (HIV),
Cas9 targeting, 11

I

Induced pluripotent stem cell (iPSC)
genome editing

prospects, 70–71

human CRISPR–Cas editing

colony expansion, 156–157

fluorescence-activated cell

sorting of Cas9 transfectants,
156

guide RNA design and genera-
tion, 154

materials, 153–154

overview, 150–151

recipes, 158

screening, 157

transfection, 154–156

troubleshooting, 157

overview, 149–150

single-nucleotide substitution

detection in induced

pluripotent stem cells

challenges, 69–70

droplet digital polymerase chain
reaction

hydrolysis probe and primer

preparation and validation,

74–76

materials, 73–74

overview, 70

substitution detection in

genomic DNA, 76–77

troubleshooting, 77

Iodixanol density gradient centrifugation.
See Adeno-associated virus
iPSC. See Induced pluripotent stem cell

L

Lentivirus, packaging of deactivated Cas9
for CRISPRa or CRISPRi,
167–168

M

Mouse, CRISPR–Cas9 genome editing
applications
 gene knockout through indel gen-
 eration, 135
 large deletions, 135–136
 large insertions, 136–137
 point mutations, 135
 small insertions, 135
Cas9 mRNA production, 142–143
donor design, 141–142
donor DNA purification, 144
efficiency, 147–148
embryo transfer, 146
genotyping, 146–147
materials, 139–141
mechanisms, 133–135
microinjection
 sample preparation, 144
 technique, 145–146
 zygote preparation, 144–145
prospects, 137
recipes, 148
screening considerations, 137
single guide RNA
 design, 137, 141
 generation, 141
 troubleshooting, 147
Mut–Seq. See Zebrafish

N

NHEJ. See Nonhomologous end joining

Nonhomologous end joining (NHEJ),
Cas9 double-strand break
repair, 9, 134

P

p300, fusion with nuclease-deficient Cas9,
10
PAM. See Protospacer adjacent motif
Polymerase chain reaction. See Droplet
digital polymerase chain
reaction
Protospacer adjacent motif (PAM), 4–6, 9,
22, 113
 characterization for Cas9 orthologs
 high-throughput sequencing and
 analysis, 37–38
 materials, 35–36
 plasmid construction, 36–37
 sequences by bacteria species, 33
 validation of sequences, 38
mutation generation in yeast
 within 20 nucleotides 5' of PAM
 sequence, 83
 within 60 nucleotides 5' of PAM
 sequence, 83–84
sequence prediction in silico, 28–29

R

Repression. See CRISPRi

S

Saccharomyces cerevisiae, CRISPR–Cas9
genome engineering
 competent cell preparation, 81–82
 cotransformation of pCAS and linear
 DNA, 85
 double-stranded DNA repair
 DNA barcode assembly, 83
 error-prone polymerase chain
 reaction for DNA library
 generation, 84–85

mutation generation
 within 20 nucleotides 5' of PAM
 sequence, 83
 within 60 nucleotides 5' of PAM
 sequence, 83–84
principles, 82
synthetic gene construct
 generation for DNA assembly
 in vivo, 84
guide sequence cloning, 80–81
materials, 79–80
overview, 79, 85–86
recipes, 86
Single guide RNA. See Guide RNA
Single-nucleotide substitutions. See
 Homology-directed repair
Spacer acquisition
 Cas nuclease role, 3
 Cas9-dependent CRISPR systems, 6–7
 CRISPR-mediated interference, 2,
 17–18
Stem cells. See Embryonic stem cell;
 Induced pluripotent stem cell

T

tracrRNA. See Guide RNA

Z

Zebrafish, CRISPR–Cas9 system
 optimization
 Cas9
 injection of guide RNA complex,
 125
 mRNA production, 123–125
 materials, 117–119
 mutation analysis
 fragment analysis, 125–127
 Mut–Seq, 127–130
 recipes, 130
 single guide RNA
 design, 119–120
 generation, 120–123

