Index

a
AAT promoter 102
active transcription unit 115
adenoviruses 37
agarose gel electrophoresis (AGE) 74, 78, 81
amber mutation 49
aminoglycoside antibiotic 40
ampicillin resistance gene 15
anaphylaxis 40
antibiotic-free selection system 46–49
antibiotic resistance markers
– host auxotrophy by a function-encoded plasmid 43, 44
– nonviral gene vectors devoid of 43
– operator–repressor titration (ORT) system 44
– protein-based antidote/poison selection systems 44
–– advantages of antidote/poison systems 44
–– RNA-based selection marker 44, 45
–– selection systems devoid of 43
–– suppression of a nonsense mutation 45, 46
antibiotic-resistant pathogens 7
α1-antitrypsin cDNA (AAT) 94
arabinose 76
ARS assays 132
artificial chromosomes (ACs) 126
artificial enhancer/promoter 94
atomic force microscopy (AFM) 79–81
– analyses 80
ATP-dependent nuclease treatment 148
autonomously replicating sequences (ARS)-type vectors 125
autoregulatory principles 144
avian influenza virus H5N1, vaccine against 23
b
Bacillus anthracis 16
Bacillus subtilis 45
bacterial artificial chromosome (BAC) 191–193, 198
base unpairing region (BUR) 107, 122
B-DNA 136
biomolecules, continuous separation 82
biosafe plasmids
– biosafe DNA plasmid vectors
–– development, rationale for 37–39
–– requirements for 40, 41
bla gene 2
bovine papillomavirus (BPV) 126
Burger’s disease 23
canine melanoma vaccine 23
capillary gel electrophoresis 79, 81, 82
cat gene 11
cationic lipids 165
– gene carriers 59
– plasmid DNA complexes 106
CCCTC binding factor 118
cddA-ccdB toxin 8
cell cycle 105, 126
cell death 11
CGE analyses 82, 83
chromatin–DNA interactions 129
chromatin domains 107, 116, 117
chromosomal DNA (chrDNA) 72, 140
chromosome-based expression principles 124
circular DNA molecules 73
circular DNA vector 4
circular GOI molecule 4
cis-diammineplatinum(II) dichloride (cis-DDP) 140
close selection 24, 30
CMV promoter 1, 84, 102, 129
ColE1 plasmid 27, 30
ColE1-type plasmids, replication control 26–28
concatemer formation 178
constitutive expression, of genes 8
coupling targeting ligands 165
CpG-depleted vectors 32
CpG dinucleotides 32, 106
CpG islands 136, 172
CpG methylation 121
cytomegalovirus (CMV) 193, 197
cytotoxicity 37
d

dapD gene 9
  – under control of P_{lac} and lacO 11
  – expression 11
  – mutants 10
dendritic cells 32
diaminopimelic acid (DAP) 9
dielectrophoresis 84, 85
DNA 79, 82
  – based gene transfer 181
  – based therapeutics 110
  – binding protein 78
  – circle 72
  – clinical application 71
  – concentration 81
  – constructs 93, 192
  – demethylation 191
  – diffusion coefficient 207
  – duplex destabilization 125
  – duplex strands 107
  – elements 97, 99, 137
  – injection 192
  – manufacturing process 79
  – methylation 194
  – methyltransferases (5-aza-dC)/histone deacetylases (TSA) 154
  – oligonucleotides 203
  – strand separation 98
  – transfected vector 183
  – transgene 167
  – unpairing elements 122
  – unwinding element 126
  – vectors 93, 206
DNA-C plasmid 13
DNA polymerase I 26
DNA product as medicines 39
  – effect of plasmid size on gene transfer efficiency 41, 42
  – for plasmid quality and purity 39, 40
  – removal of antibiotic resistance markers, positive impact on 41
  – requirements for biosafe plasmids 40, 41
  – specific requirements for use 39
DNA vaccines 1, 12, 17, 37
  – delivery 17, 18
  – and gene therapy vectors 13, 14
dose–response profile 167
double-stranded breaks (DSBs) 190
drug selection marker 135
e
EBV nuclear antigen-1 (EBNA-1) 132
EBV paradigm
  – establishment and maintenance 132–136
  – DNA methylation targets, CpGs role 135
  – L1 transposon system variants 133
  – “molecular glue” complementarity, and replication functions initiator 132, 133
  – pEPIto 135, 136
  – replication-support elements 133, 134
  – selection principles, overcoming need for antibiotics 134, 135
ECM degrading enzymes 209
eGFP. See enhanced green fluorescent protein (eGFP)
electrical parameters' modulation 205
electric pulses 215
electrophoretic effect 84, 215
electroporation 38, 53, 60, 66, 67, 102, 128, 180, 216
electropulsation (EP) 203
  – advantages 204
electrotransfer
  – efficiency 223
  – minicircle mechanism 207
ELISpot 18
endA and recA genes 52
endocytosis 168
endotoxins 39, 86
enhanced green fluorescent protein (eGFP) 129
  – expression 98, 99
  – marker 102, 131, 133, 151
EP. See electropulsation (EP)
epigenetic code, components 121
episomal DNA 194
episomal maintenance
  – minicircle S/MAR vectors
  – generated by Flp recombinase in vitro 98, 99
  – generated using Cre recombinase in Vitro 99, 101
  – minicircle vectors, possible mechanisms promoting 105, 106
  – access to replication machinery by S/MARs 107, 109, 110
Index 

— CpG dinucleotide content reduction 106, 107
— effects of S/MAR in gene expression and vector replication 108
— histone modifications 106
— vector establishment in the correct nuclear compartment 107
— S/MAR minicircles in vivo 102–104
— S/MAR vectors in bovine and murine zygotes 101, 102
episomal replication, of S/MAR minicircle vectors 104, 105
episomal vectors 107, 131, 133, 190
episome 126
Epstein–Barr virus (EBV) 126, 132, 190
Escherichia coli 7, 9, 11, 18, 19, 24, 30, 32, 43–45, 60, 62, 63, 78, 150, 194, 195
euchromatin-associated histone modifications 106
eukaryotic
— base unpairing regions (BURs) 138
— expression cassette 135
— sequences 152
extracellular matrix (ECM) 207, 215
— minicircles vs. plasmids, under gene electrotransfer suboptimal conditions, influence of 215, 216
— B16F1 tumors producing luciferase 223
— cell culture and animals 215, 216
— data analysis 217
— determination of the reporter gene (luciferase) activity 216, 217
— electrotransfer efficiency 216, 223
— minicircle and plasmid 216
— minicircle concentrations adjustments 222, 223
— pericellular matrix limiting accessibility of nucleic acids 223
— in vitro, results 217, 218
— in vivo, results 218–221

f
FAC-sorting 136, 141, 149
factor inhibiting HIF-1 (FIH) 178
fermentations 8, 18, 19, 24, 30, 31
fertilization 191
FISH analysis 98, 109, 140
flow cytometry analysis (FACS) 183, 208
Flp recombinase 101, 141, 150, 151
— target sites 123
Flp recombinase-mediated cassette exchange (Flp RMCE) process 124
fluorescence-activated cell sorting methods 134
force sensor 79
freezing–rethawing cycle 143
functional telomerase holoenzyme 197

β-galactosidase (LacZ) reporter gene 182
β-Gal staining 182
ganciclovir (GANC) 153
gel-filled capillary 88
gene electrotransfer 203, 208, 220
— efficiency 208
gene expression 143
— S/MAR effects 108
gene (cassette) of interest (GOI) 73
gene on duty (GOD) 146
gene silencing 28, 33, 110, 121, 170, 183, 206, 208
gene therapy 4, 7, 37, 115, 165
— advent of novel vector vehicles 115
— approaches 116, 185, 190
— cystic fibrosis 2
— nonviral 61, 177
— vectors 13
gene transfer technology 181
GFP fluorescence 154, 155, 180, 183, 193, 206
β-globin transcription (HBB) unit 147
glyceraldehyde 3-phosphate dehydrogenase promoter (GAPDH) 94
glycosylation 17
GraphPad Prism software 217
growth hormone-releasing hormone (GHRH) 38
— DNA therapy 23

h
hematopoietic system 140
hepatic control region (HCR-1) 105
hepatocyte proliferation 104
hepatocyte-specific promoters 97
herpes simplex virus type 1 (HSV-1) 136
heterochromatin 33
— domains 106
high-voltage pulse (HV) 215
Hirt extraction protocols 149
histone acetyltransferases (HATs) 122, 129
histone deacetylase inhibitors 121, 130, 141
histone deacetylases 154
histone-like proteins 151
histone methyltransferase (HMT) 121
histone modifications 106
hok-sok toxin 8
human telomerase (TERT) 195
Hurler syndrome 97
iBAC-S/MAR vector 136
IDUA enzyme 97
IFN-γ ELISA 18
immunization
– double 16
– and protection against an anthrax challenge 17
– single-dose ORT-VAC oral 18
inactivation/activation cycles 154
induced pluripotent stem (iPS) 41, 153
integration-deficient lentiviral (IDLV) vectors 147
intercalating dye YOYO 82
intracellular proteins 154
in vivo gene delivery system 205, 206
in vivo MC electrotransfer
– muscle 207, 208
– skin 209
– tumor 208, 209
in vivo plasmid stability 15
isopropyl-β-D-thiogalactopyranoside (IPTG) 10
jet injection technology 182, 185
– for in vivo transfer of naked DNA 180–182
kanamycin resistance 1
Kozak sequence 25
lacI gene 9
lacO1–lacO3 conformation 12, 13
lactose operon 9
LacZ encoding plasmid 186
lambda integrase 2
α-liduronidase (IDUA) gene 97
lipopolysaccharides 86
live bacterial vaccines (LBVs) 14
luciferase 42
– production 218–221
– reporter gene 134
lysine 9
lysis 30, 184, 216
lysozyme 18
magnetic nanoparticles (MNPs) 167
magnetite (Fe₃O₄) 167
magnetofection 165, 166, 168, 169, 170
– principles 167, 168
– JT3 cells, representative images 173
mammalian embryos
– minicircles and conventional plasmids
– episomal expression 189–198
– plasmids and minicircles, fate after injection into 192–198
– functional genes expression 195–197
– minicircle/plasmid-mediated expression in 193–195
– properties of minicircles, plasmids, and bacterial artificial chromosomes 191
MC clones 142
mCherry–lacR fusion 130
MC-preparation process 115
MC-treated muscles 208
metabolic burden
– antibiotics and 7, 8
– to bacterial cells 8
microfluidic channels 82
– continuous flow separation in 82–86
MIDGE vectors 43
MINIback plasmid 30
– containing fluorescent reporter gene (EGFP) 33
– efficient gene transfer 32, 33
– improved production processes by 30, 31
– procedure of clone selection 31
minichromosome maintenance protein 109
minicircle-based GFP encoding vector 183
minicircle-based nonviral gene transfer 179
minicircle concentrations, influence of 222
minicircle (MC) DNA 71, 77, 97, 173
– continuous flow separation 84
– efficient tissue-targeted gene delivery implementation 206–209
– vector design 206
– in vitro minicircle electrotransfer 206, 207
– in vivo MC electrotransfer 207–209
– by jet injection, in vivo application 185, 186
– magnetofection 165–173
– cellular uptake 168, 169
– diffusion through cytoplasm 169
– magnetofection principles, overview 167, 168
– transgene expression 169–172
– plasmid DNA electrotransfer, from principle to technical design 204–206
– gene electrotransfer mechanism 204, 205
– preclinical applications 205, 206
– products, quality controls 86
– quality control 86
– tissue-targeted gene electrodelivery 203–209
minicircle electrotransfer 220
  – efficiency 222
minicircle-generating methods 93
minicircle identification sequence 77
minicircle production
  – analytical tools in 71–88
  -- gene transfer for therapy, vaccination, and stem cells 71, 72
  -- minicircle systems 73, 74
  -- plasmids 72, 73
  -- analytics 79–88
  -- finished product control 86–88
  -- in-process control 79–86
  -- cultivation
  -- and harvest, process scheme 75
  -- and induction 74–77
  -- minicircle systems 73, 74
  -- parental plasmid 74
  -- perspectives 88
  -- preparation strategy 77, 78
  -- production for MC-CMV-GFP (MC0904) 73
  -- therapy, vaccination, and stem cells, gene transfer 71, 72
minicircles 1, 2
  -- applications for use of DNA 5
  -- in gene therapy 2
  -- HIF-1-encoding 4
  -- methods 215–217
  -- cell culture and animals 215, 216
  -- data analysis 217
  -- electrotransfer 216
  -- minicircle and plasmid 216
  -- reporter gene (luciferase) activity determination 216, 217
  -- passive episomal maintenance 94, 97
  -- patents 1–6
  -- according to filing date 5
  -- principles of generating minicircle vectors 97, 98
  -- production
  -- in topoisomerase IV-deficient cells 4
    (See also minicircle production)
    -- properties 191
    -- purification of products 3
    -- quality controls of minicircle DNA products 86
    -- by recombination with ΦC31 integrase 3
    -- S/MAR vectors 105 (See also minicircle vectors)
    -- as vectors for gene transfer and 2
    -- in vitro results 217, 218
    -- in vivo results 218–220
  -- vs. plasmids under gene electrotransfer
    suboptimal conditions, efficiency 215–224
  -- minicircle vectors 104
  -- comparative performance analyses 183–185
  -- principles of generating, able to support episomal maintenance 97, 98
  -- utilization 93–110
  -- in vitro performance 184
  -- in vivo application 178–180
minimalistic immunogenically defined gene expression (MIDGE) principle 128
minimalization approaches 137–156
-- oligomerizing S/MAR modules
  -- pMARS and properties 139, 140
  -- replicating minicircles 140–149
  -- from cells to organs 155, 156
  -- clonal behavior 141–143
  -- clonal behavior Bi-MC systems 143, 144
  -- combination of excision and RMCE strategies 151–153
  -- emerging extensions and refinements 149–156
  -- episomal status, proof and persistence 147–149
  -- establishment and maintenance parameters 141
  -- MC size reduction, “in vivo evolution” 144–146
  -- MC withdrawal at Will 153, 154
  -- pronuclear injection and somatic cell nuclear transfer 155
  -- transcriptional termination and polyadenylation, intricate interplay 146, 147
miniplasmid (MP) 1, 73, 123
  -- DNA 77
  -- production 73
  -- specific digesting restriction enzymes 74
  miniplasmid (MP) 1, 73, 123
  -- DNA 77
  -- production 73
  -- specific digesting restriction enzymes 74
  -- mini-UbC-S/MAR vector 101
miniUbC-S/MAR vector 101
minivectors 4
MNP–nucleic acid vector 167
“molecular glue” (MG) 115, 132
Mo-MuLV vectors 119
monocytes 32
MP. See miniplasmid (MP)
Mpt64 protein 17
multiplicities of infections (MOIs) 119
murine secreted alkaline phosphatase gene (mSEAP) 50
mutation 8, 43. See also nonsense mutations
  – lacI 9, 11
  – thyA 48
M18 vector 99, 107, 109, 145, 146, 152
naked transgenes 118
nasopharyngeal tumor xenograft model 178
natural killer cells 32
p-nitroaniline (p-NA) 86
nonhomologous end joining (NHEJ)-related repair activities 116
nonsense mutations 46, 47, 134
- suppression 45, 46
nonviral episomal
- modification 105
- replicating 123–125
nonviral gene therapy 59, 110
- clinical application 177
- minicircle-based vectors for 177–186
- minicircle DNA by jet injection, in vivo application 185, 186
- minicircle technology 177, 178
- minicircle vectors
- -- comparative performance analyses 183–185
- -- in vivo application 178–180
- naked DNA in vivo transfer, jet injection technology 180–182
nonviral gene transfer protocols 181
nonviral in vivo jet injection gene transfer 181
nonviral replicating episomes 149
nonviral vectors 165
- avoiding genomic disturbances 116
nuclear matrix protein 1 (NMP1) 109, 118
nuclear pore 41
nucleic acids 168
- MNP complexes 169
- physical gene transfer 182
- vectors 165
nucleophosmin 118

o
Okazaki fragment 26
oligomerization 63, 139
oligonucleotide 3
operator–repressor interaction 9
operator–repressor titration 7
- mechanism 8–10
- miniplasmid 12, 13
- plasmid stabilization 11
- strain development 9–12
optimal electrotransfer conditions 223
origin recognition complex (ORC) 107, 110, 125
origin recognition element (ORE) 126
OriP-EBNA-1 vector 133
OriP plasmids 132
ORT HIV DNA vaccine 13
ORT-VAC 14
- immunization 16
- miniplasmids 17
- oral delivery of recombinant vaccines 15
- plasmid maintenance 14
- strain 14
- TB DNA vaccine delivery using 18
p
pAAT-Control plasmid 103
PA-HlyA protein 16
parental plasmids (PPs) 73, 75, 123, 137
- analysis 76–78
- structure 76
passive episomal maintenance 105
pCOR plasmids 46
pDNA-based product 23
pDNA-based therapies 24
pDNA electrotransfer 204, 205
pDNA minicircle vector 23
pEPI vector 97, 98
peptide-based delivery of pDNA 23
pFAR4 biosafe miniplasmid 51
pFAR plasmid 46–49
- maps 47
- structures 48
pFAR vectors
- promote efficient expression in mammalian cells 49
- in vitro transfection study 49, 50
- in vivo transfection studies 50, 51
phagosome 18
plasma membrane 207
plasmid DNA (pDNA) 72, 116
- concatemers 62–67
- monomers 62–67
- multimers 62–67
- product analysis 79
- topology and size 60–62
- vectors 203
plasmid-encoded telomerase 196
plasmid episomes, maintenance 147
plasmid recovery approaches 198
plasmids 1, 72
- based gene transfer 203
- based ORT-VAC approach 17
- based vector system 182
- biosafe (See biosafe plasmids)
Index | 233

- copy number 9, 11, 24, 28, 33, 44, 48
- derived vectors 134
- encoded kanamycin resistance gene 31
- functional 194
- quality and purity, requirements for 39, 40
- rescue 147
- retention, in Peyer’s patches 15
- size dependence of luciferase gene expression 42
- stabilization 8
- toxicity of 72
- vector 93, 103
polyadenylation 25, 39, 109, 120, 140, 145–147, 191
polydimethylsiloxane (PDMS) 84
polyethylenimine (PEI) 178
polymerase chain reaction (PCR) 82
population doublings (PDs) 140
positive–negative selection marker 153
postsegregational killing (PSK) systems 8
P_{\text{pagC}} promoter 16
preimplantation embryos, transgene expression in 193
primosome assembly site (PAS) 26, 27
prokaryotic selection markers 177
prolyl hydroxylase (PHD2) 178
promoter CpG methylation 93
promoter–transgene–pA encoding vectors 180
pronuclear injection 189
proof-of-principle experiment 165, 195
proteolytic cascades 208
proton sponge effect 169
PSK genes 8
pUC-ori-based plasmids 24

q
quantitative PCR analyses 104
quantitative real-time PCRs (qRT-PCRs) 87

r
recombinant adeno-associated virus (rAAV) 41
recombinant DNA technologies 189
recombinant protein expression 18, 19
recombinant proteins 189
recombinant viruses 156
recombinase-mediated cassette exchange (RMCE) 130
recombinase-mediated inversion process 128
recombinases 2, 3, 94
recombination
- mechanism 127, 177
- product 79
- sequences 74
- system 2
replicating minicircles 140–149
- from cells to organs 155, 156
- clonal behavior 141–143
- Bi-MC systems 143, 144
- combination of excision and RMCE strategies 151–153
- emerging extensions, and refinements 149–156
- episomal status, proof and persistence 147–149
- establishment and maintenance parameters 141
- gene therapy, novel vector vehicles 115–123
- independent expression units, chromatin domains 116–123
- chromosome-based expression strategies 123
- S/MAR actions, multifold and context dependent 119–121
- S/MARs, stress-induced duplex destabilization 121–123
- S/MARs, unifying principle 118, 119
- MC size reduction, “in vivo evolution” 144–146
- MC withdrawal at will 153, 154
- minimalization approaches 137–156
- nonviral vectors, avoiding genomic disturbances 116
- pronuclear injection, and somatic cell nuclear transfer 155
- replicating nonviral episomes 123–137
- ARS and S/MARs, common (SIDD-) properties 125, 126
- EBV paradigm, establishment and maintenance 132–136
- shortcomings and solution 132–137
- S/MAR plasmids, verification of concept 126–132
- vector size limitations 136, 137
- yeast ARS principle 125
- transcriptional termination and polyadenylation, intricate interplay 146, 147
- transient and stable expression systems, limitations 115
replication minimal models 126
repressor titration 9
restriction endonuclease 3
restriction enzymes 151
restriction test 103
retroviruses 37
ribosomal binding site (RBS) 28, 29
RNA-based plasmid maintenance system 29
RNA binding motif 123
RNA–DNA hybrid 27, 28
RNAII-like sequence (RLS) 29
RNA polymerase 8
RNA–RNA interaction 23, 26, 28

s
Saccharomyces cerevisiae 19
SAF-A/hnRNP-U protein 156
scaffold attachment factor A (SAFA) 116
scaffold/matrix attachment regions (S/MARs) 97, 115, 116, 118, 119, 145, 152, 190
– access to replication machinery 107–110
– actions, multifold and context dependent 119, 121
– based episomes 133
– 800 bp element, context-dependent and antisilencing actions 120
– chromosome-based expression strategies 123
– common (SIDD-) properties 125, 126
– elements 109, 120, 146, 156, 180
– minicircles 99, 156
– vectors, episomal replication potential 104, 105
– minimized parental plasmid 145
– plasmids 141, 142, 154, 155
– cell and nuclear permeation 128, 129
– nuclear association sites 129, 130
– in prolonged gene expression in vitro 100
– RMCE-based elaboration following establishment 130–132
– S/MAR transcription 126–128
– transfection principles 128, 129
– verification of concept 126–132
– stress-induced duplex destabilization 121–123
– vectors 107, 134
– in bovine and murine zygotes 101, 102
– scaffold–reassociation assays 122
– sequence-specific recombinases 177
Ser-dependent variants 125
SIDD profiles 146
simian virus 40 (SV40) 126
site-specific recombinases (SSRs) 2, 123
small silencing RNA (siRNA) 203
– electrotransfer 206
S/MARs. See scaffold/matrix attachment regions (S/MARs)
sodium hydroxide solution 75
soft lithography 84
somatic cell nuclear transfer (SCNT) 155, 189
Southern blot analysis 101, 103, 147, 148
sperm mediated gene transfer (SMGT) 155

stress-induced duplex destabilization (SIDD) 107, 122
supercoiled domain model 119
SV40-eGFP-S/MAR minicircle vector 101
system-specific sequences 74

t
tag sequence 77, 78
T cells 13, 18, 66
telomerase reverse transcriptase (TERT) 196
teratoma-initiating cells (TICs) 153
tetracycline operator (Tet-operator) 29
Tet-repressor 28–30
therapeutic genes 2, 23, 37, 205
therapeutic plasmids 24
– antibiotic resistance-free selection approaches 26
– CoE1-type plasmids, replication control 26–28
– design principles 24, 25
– efficient gene transfer 32, 33
– improving sequence composition 32
– MINIback concept 28–30
– improved production processes by MINIback plasmids 30, 31
– novel design, and problem of selection 25, 26
thyA gene 52
thymidine 32
tissue-targeted gene delivery 206
toll-like receptor 9 (TLR9) 32, 93, 208
– signaling 135
total aerobic microbial count (TAMC) 87
total combined yeast/mold count (TYMC) 87
TOTO-1 fluorescence 66
transcription 118
– augmentation 118
– cassettes 127
– supercoiled domain model 119
– termination/polyadenylation 146
– unit 127
transfections
– mdx with microdystrophin-encoding pFAR4 50
– transient 71
– in vitro and in vivo study 49–51
transformations
– efficiency 13
– of native plasmid pEGFP-N1 61
transgenes
– DNA copy number 105
– expression 104, 169
– FISH visualization 149
Index

trichostatin A (TSA) 121

tumor xenografts 185

u
UbC-S/MAR plasmid 101
ubiquitous chromatin opening element 136
unmethylated plasmids 198
unpairing elements (UEs) 107

v
vector establishment, in correct nuclear compartment 107
vector systems, types 71
viral gene transfer 183

w
West Nile virus
  – infection in horses 38
  – vaccine 23

x
Xer-cise™ technology 11

y
Yeast ARS principle, verification for mammalian cells 125
Yersinia pestis 16

z
zero-CpG plasmids 4