9. Materials

9.1 Chemicals

Acetic Acid (glacial)	Fisher
Acetone	Fisher
Acetonitrile	Glen Research
Agarose	Gibco BRL
8-Aminoguanosine	Sigma
Ammonium Hydroxide	Fisher
Ammonium Persulfate	Kodak
Ampicillin	Sigma
ATP	Sigma
Bromophenol Blue	Sigma
Butanol	Fisher
Chloroform	Sigma Aldrich
CTP	Sigma
7-Deaza-GTP	Ambion
7-Deaza-Guanosine	Ambion
dGTP	Sigma
3'-dGTP	TriLink Biotech
DNA-Ladder (100 bp)	Gibco BRL
DNA-Phosphoramidites	Applied Biosystems
dNTPs	Pharmacia
DTT	Invitrogen
EDTA	Sigma
Ethanol	Aaper Alcohol
Ethidium Bromide	Fisher
Formic Acid	Fisher
Glycerol	Fisher
GMP	Sigma
GDP	Sigma
GTP	Sigma
GTP-γ-S	Sigma
Guanosine	Sigma
Guanosine-5'-O-(1-Thiotriphosphate)	Trilink Biotech
Hydrochloric Acid	Fisher
ITP	Sigma
IPTG	Fisher
Isoamylalcohol	Fisher
Lithium Fluoride	Sigma
Magnesium Chloride	Sigma

Manganese Chloride	Sigma
1-Methyl-Guanosine	Sigma
N^2-Methyl-2'-Deoxy-GTP	TriLink Biotech
2'-O-Methyl-GTP	TriLink Biotech
3'-O-Methyl-GTP	TriLink Biotech
Phenol	Fisher
Potassium Phosphate	Sigma
2-Propanol	Fisher
Radiochemicals	NEN
Sigmacote	Sigma
Sodium Chloride	Fisher
Sodium Hydroxide	Fisher
Spermidine	Sigma
TEMED	Bio-Rad
6-Thio-GTP	Jena Bioscience
Thiopropyl Sepharose 6B	Pharmacia
Tris-HCl	Fisher
Triton X-100	Fisher
tRNA	Boehringer Mannheim
TTP	Sigma
UTP	Sigma
XTP	Jena Bioscience

9.2 Media

LB-Medium

10 g/l bacto-tryptone
5 g/l yeast extract
10 g/l NaCl, pH 7.5

LB-Agar Plates

12 g/l agar (w/v) in LB-medium
autoclave 20 min, 120°C
cool down to ~50°C
add ampicillin to 100 mg/ml
pour into sterile petri plates

9.3 Buffers

5x DNA Sample Buffer

50 mM EDTA, pH 8.0
50% (w/v) glycerol
Orange G

1x GTP-Selection Buffer
200 mM KCl
10 mM KH_2PO_4, pH 6.2
5 mM $MgCl_2$
50µM EDTA

10x PCR Buffer (Boehringer Mannheim)
50 mM KCl
10 mM Tris-HCl, pH 9.0
0.1% Triton X-100
1.5 mM $MgCl_2$

5x Reverse Transcription Buffer (Invitrogen)
250 mMTris-HCl, pH 8.3
375 mM KCl
15 mM $MgCl_2$

10x TBE-Buffer
900 mM Tris-borate
20 mM EDTA

10x TE
100 mM Tris-HCl, pH 8.0
10 mM EDTA

10x Transcription Buffer
400 mM Tris-HCl, pH 7.8
50 mM DTT
30 mM $MgCl_2$
20 mM spermidine
0.01% Triton X-100

9.4 Laboratory Equipment

Camera	Sharp
Cary 1E UV-Visible Spectrophotometer	Varian
Centrifuge J6-M	Beckman
DNA-Synthesizer Expedite	Millipore
Electroeluter	Schleicher & Schuell
Freezer -20°C	Puffer Hubbard
Freezer -80°C	Revco
Fridge 4°C	Marvel
Geiger Counter	Ludlum Instruments

Gel Dryer	Bio-Rad
Gel Electrophoresis Systems	Bio-Rad / BRL / Owl
Heating Blocks	Fisher, Labline Instruments
Incubator	VWR Scientific
Lyophilizer	Virtis
Magnetic Stirrer	Ika
Microcentrifuge	Fisher
Microwave	Sharp
Power Supply	Bio-Rad
pH-Meter	Corning
PhosphorImager Storm 840	Molecular Dynamics
Phosphor Screens	Molecular Dynamics
Photometer	Agilent
Pipetmen P2, P20, P200, P1000	Gilson / ThermoLabsystems
Precision Scale	Denver Instrument Company
Scale	Sartorius
Scintillation Counter LS 6500	Beckman
Speed Vac	Savant
Thermocycler PCR	MJ Research / Perkin Elmer
Table-Top Centrifuge	Eppendorf
UV-Transilluminator	UVP
Vortex	Fisher
Water Bath	Brinkmann
Water Destillation System	Barnstead

9.5 Other Materials and Equipment

Amicon Membranes	Millipore
Elutrap BT1 & BT2 Membranes	Schleicher & Schuell
Chromatography Paper 3MM	Whatman
Gloves	Fisher
96-Well Plates	Costar / Falcon
Micro Bio-Spin Chromatography Columns 30	Bio-Rad
Microcon YM-30 Spinfilters	Millipore
Parafilm	Sigma
Plate Sealing Film	3M
PCR Tubes	MJ Research
Petri Dishes	Falcon
Pipette Tips	Fisher, Marsh, USA Scientific, Art Molecular BioProducts
Reaction Tubes, 0.5 ml	Sarstedt
Reaction Tubes, 1.5 ml	Sarstedt
Reaction Tubes, 2.0 ml	Fisher

Scintillation Vials MINIS 2000, 6 ml	VWR
Sterile filters 0.2 µm	Gelman Sciences
Syringes	Becton Dickinson

9.6 Enzymes

DNase I	Promega
Superscript Reverse Transcriptase	Invitrogen
T7 RNA-Polymerase	NEB
Taq DNA Polymerase	Gibco BRL

9.7 Kits

TOPO TA Cloning Kit	Invitrogen
QIAprep Spin Miniprep Kit	Qiagen
QIAquick PCR Purification Kit	Qiagen

9.8 Oligonucleotides and Randomized Pool Sequences

All oligonucleotides were synthesized on a Perseptive Biosystems Expedite Synthesizer 8900.
Sequences of oligonucleotides used in these studies are given in 5' to 3' orientation.

Pool 40: universal 5’ primer for doped re-selection:
5’-ATG TAA TAC GAC TCA CTA TAG GAG CCG ACT AAT GAT TAA T-3’

oM7D30: 3’ primer for doped re-selection with sequence M7D30:
5’-GCC ATG TAT GGC GCG ATT GC-3’

o7G27: 3’ primer for doped re-selection with sequence M7G27:
5’-GAA CAG TTC GCA TGC TCG GG-3’

o7G32: 3’ primer for doped re-selection with sequence M7G32:
5’-GAC ATT GCG GCC TAA TCG CG-3’

o8G6: 3’ primer for doped re-selection with sequence M8G6:
5’-GCC TAA GCA TCC GTG ACA GG-3’

o8G11: 3’ primer for doped re-selection with sequence M8G11:
5’-GGG ATT CCG CAA TGG CTA CG-3’

o8G24: 3’ primer for doped re-selection with sequence M8G24:
5’-GCC ATT CTT GGC ACT GGT GG-3’

o8G30: 3’ primer for doped re-selection with sequence Class II:
5’-GCG ATG TTA GCG GAC TCT CG-3’

oWT: 3’ primer for doped re-selection with sequence Class III:
5’-GAA TGC CCG ATG TAG GAC GG-3’

Randomized Pool Sequences for Doped Re-Selections

Pools for re-selection were composed of constant primer sequences at the 5’ and 3’ end and a randomized central region containing the following nucleotide mixtures leading to a 21% chance of mutagenesis at each position:
5: A mixed with 21% of an equimolar mixture of all phosphoramidites
6: C mixed with 21% of an equimolar mixture of all phosphoramidites
7: G mixed with 21% of an equimolar mixture of all phosphoramidites
8: T mixed with 21% of an equimolar mixture of all phosphoramidites

M7D30
5’-GCCATGTATGGCGCGATTGC756557886588676788788787686886858755658786568ATTAATCATTAGTCGGCTCCTATAGTGAGTCGTATTAACAT-3’

M7G27
5’-GAACAGTTCGCATGCTCGGG685888655875587687788856876675676576655655767656865588567657887888568766756576568ATTAATCATTAGTCGGCTCCTATAGTGAGTCGTATTAACAT-3’

M7G32
5’-GACATTGCGGCCTAATCGCG778765756687678786787686886786887566 5ATTAATCATTAGTCGGCTCCTATAGTGAGTCGTATTAACAT-3’

M8G6
5’-GCCTAAGCATCCGTGACAGG775576887678858587876868868768866585685787657675665888565688676876ATTAATCATTAGTCGGCTCCTATAGTGAGTCGTATTAACAT-3’

M8G11
5’-GGGATTCCGCAATGGCTACG585876656567557868778887687667557657865565576858655867555767 56ATTAATCATTAGTCGGCTCCTATAGTGAGTCGTATTAACAT-3’

M8G24
5’-GCGATTCTTGGCACTGGTGG67676857885677565768856788857878 76

868868568766755765786567585866558857556775788ATTAATCATTAGTCG
GCTCCTATAGTGAGTCGTATTAACAT-3′

Class II
5′-GCGATGTTAGCGGACTCTCG685788757666876785856787686886677 6
86788657ATTAATCATTAGTCGGCTCCTATAGTGAGTCGTATTAACAT-3′

Class III
5′-GAATGCCCGATGTAGGACGG57785666567578778888857655568767 55
765788658777858668888577878787ATTAATCATTAGTCGGCTCCTATAGTGAGTC
GTATTAACAT-3′