

Materials List for:

A Protocol for Functional Assessment of Whole-Protein Saturation Mutagenesis Libraries Utilizing High-Throughput Sequencing

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URL: <https://www.jove.com/video/54119>

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Materials

Name	Company	Catalog Number	Comments
Typtone	Research Products Intl. Corp.	T60060-1000.0	
Yeast extract	Research Products Intl. Corp.	Y20020-500.0	
Sodium chloride	Fisher Scientific	BP358-212	
Potassium chloride	Sigma-Aldrich	P9333-500G	
Magnesium sulfate	Sigma-Aldrich	M7506-500G	
Agar	Fisher Scientific	BP1423-500	
Tetracycline hydrochloride	Sigma-Aldrich	T7660-5G	
Petri plates	Corning	351029	
MATLAB	Mathworks	http://www.mathworks.com/products/matlab/	
Oligonucleotide primers	Integrated DNA Technologies	https://www.idtdna.com/pages/products/dna-rna/custom-dna-oligos	25 nmol scale, standard desalting
pBR322_AvrII	available upon request		pBR322 plasmid modified to contain AvrII restriction site downstream of the TEM-1 gene
pBR322_OP1 – pBR322_OP5	available upon request		five modified pBR322 plasmids each containing a pair of orthogonal priming sites
Q5 high-fidelity DNA polymerase	New England Biolabs	M0491L	includes 5x PCR buffer and PCR additive (GC enhancer)
15 ml conical tube	Corning	430025	
Multichannel pipettes (Eppendorf ResearchPlus)	Eppendorf		
PCR plate, 96 well	Fisher Scientific	14230232	
96 well plate seal	Excel Scientific	F-96-100	
Veriti 96-well thermal cycler	Applied Biosystems	4375786	
6x gel loading dye	New England Biolabs	B7024S	
Agarose	Research Products Intl. Corp.	20090-500.0	
Ethidium bromide	Bio-Rad	161-0433	
UV transilluminator (FOTO/Analyst ImageTech)	Fotodyne Inc.	http://www.fotodyne.com/content/ImageTech_gel_documentation	
EB buffer	Qiagen	19086	
96-well black-walled, clear bottom assay plates	Corning	3651	
Lambda phage DNA	New England Biolabs	N3011S	
PicoGreen dsDNA reagent	Invitrogen	P7581	dsDNA quantitation reagent, used in protocol step 2.2.4

Victor 3 V microplate reader	PerkinElmer		
DNA purification kit	Zymo Research	D4003	
Microcentrifuge tubes	Corning	3621	
Long-wavelength UV illuminator	Fisher Scientific	FBUVLS-80	
Agarose gel DNA extraction buffer	Zymo Research	D4001-1-100	
AatII	New England Biolabs	R0117S	
AvrII	New England Biolabs	R0174L	
T4 DNA ligase	New England Biolabs	M0202S	
EVB100 electrocompetent E. coli	Avidity	EVB100	
Electroporator (E. coli Pulser)	Bio-Rad	1652102	
Electroporation cuvettes	Bio-Rad	165-2089	
Spectrophotometer (Ultrospec 3100 pro)	Amersham Biosciences	80211237	
50 ml conical tubes	Corning	430828	
Plasmid purification kit	Macherey-Nagel	740588.25	
8 well PCR strip tubes	Axygen	321-10-551	
Qubit dsDNA HS assay kit	Invitrogen	Q32854	dsDNA quantitation reagent
Qubit assay tubes	Invitrogen	Q32856	
Qubit fluorometer	Invitrogen	Q32866	
Ampicillin sodium salt	Akron Biotechnology	50824296	
MiSeq reagent kit v2 (500 cycles)	Illumina	MS-102-2003	
MiSeq desktop sequencer	Illumina	http://www.illumina.com/systems/miseq.html	alternatively, one could sequence on Illumina HiSeq platform
FLASH software	John Hopkins University - open source	http://ccb.jhu.edu/software/FLASH/	software to merge paired-end reads from next-generation sequencing data
AatII_F			GATAATAATGGTTTCTTAGACG TCAGGTGGC
AvrII_R			CTTCACCTAGGTCCTTTTAAAT TAAAAATGAAG
AvrII_F			CTTCATTTTAAATTTAAAAGGA CCTAGGTGAAG
AatII_OP1_R			ACCTGACGTCCGTATTTCAAC TGTCCGGTCTAAGAAACCATT ATTATCATGACATTAAC
AatII_OP2_R			ACCTGACGTCCGCTCACGGA GTGTACTAATTAAGAAACCATT ATTATCATGACATTAAC
AatII_OP3_R			ACCTGACGTGTCGTACGTCTGA ACTTGGGACTTAAGAAACCA TTATTATCATGACATTAAC
AatII_OP4_R			ACCTGACGTCCCGTTCTCGAT ACCAAGTGATAAGAAACCATT ATTATCATGACATTAAC
AatII_OP5_R			ACCTGACGTGTCGTCCGTCGGA GTAACAATCTTAAGAAACCATT TATTATCATGACATTAAC
OP1_F			GACCGGACAGTTGAAATACG
OP1_R			CGACGTACAGGACAATTTCC
OP2_F			ATTAGTACACTCCGTGAGCG
OP2_R			AGTATTAGGCGTCAAGGTCC

OP3_F			AGTCCCAAGTTCAGACGTAC
OP3_R			GAAAAGTCCCAATGAGTGCC
OP4_F			TCACTTGGTATCGAGAACGG
OP4_R			TATCACGGAAGGACTCAACG
OP5_F			AGATTGTTACTCCGACGGAC
OP5_R			TATAACAGGCTGCTGAGACC
Group1_F			ACACTCTTTCCCTACACGAC GCTCTTCCGATCTNNNNNGC ATTTTGCCTACCGGTTTTTGC
Group1_R			GTGACTGGAGTTCAGACGTG TGCTCTTCCGATCTNNNNNTC TTGCCCCGGCGTCAAC
Group2_F			ACACTCTTTCCCTACACGAC GCTCTTCCGATCTNNNNNGA ACGTTTTCCAATGATGAGCAC
Group2_R			GTGACTGGAGTTCAGACGTG TGCTCTTCCGATCTNNNNNGT CCTCCGATCGTTGTCAGAAG
Group3_F			ACACTCTTTCCCTACACGAC GCTCTTCCGATCTNNNNNAG TAAGAGAATTATGCAGTGCTGCC
Group3_R			GTGACTGGAGTTCAGACGTG TGCTCTTCCGATCTNNNNNTC GCCAGTTAATAGTTTGCGC
Group4_F			ACACTCTTTCCCTACACGAC GCTCTTCCGATCTNNNNNCC AAACGACGAGCGTGACAC
Group4_R			GTGACTGGAGTTCAGACGTG TGCTCTTCCGATCTNNNNNGC AATGATACCGCGAGACCC
Group5_F			ACACTCTTTCCCTACACGAC GCTCTTCCGATCTNNNNNCG GCTGGCTGGTTTATTGC
Group5_R			GTGACTGGAGTTCAGACGTG TGCTCTTCCGATCTNNNNNTAT ATGAGTAAACTTGGTCTGACAG
501_F			AATGATACGGCGACCACCGA GATCTACACTATAGCCTACAC TCTTTCCCTACACGAC
502_F			AATGATACGGCGACCACCGA GATCTACACATAGAGGCACA CTCTTTCCCTACACGAC
503_F			AATGATACGGCGACCACCGA GATCTACACCCTATCCTACAC TCTTTCCCTACACGAC
504_F			AATGATACGGCGACCACCGA GATCTACACGGCTCTGAACA CTCTTTCCCTACACGAC
505_F			AATGATACGGCGACCACCGA GATCTACACAGGCGAAGACA CTCTTTCCCTACACGAC
701_R			CAAGCAGAAGACGGCATAC GAGATCGAGTAATGTGACTG GAGTTCAGACGTG
702_R			CAAGCAGAAGACGGCATAC GAGATTCTCCGGAGTGACTG GAGTTCAGACGTG