

STRUCTURAL GENOMIC QUESTIONNAIRE

Center Name: SECSG – University of Georgia

Date: April 3, 2003

Step	Method/Vendor	Contact Person (e-mail)/Location
Cloning (<i>P. furiosus</i>)		
Primer Vendor	MWG www.mwgbiotech.com	fjenney@bmb.uga.edu Univ of Georgia, Athens, GA
Expression strains used	BL21(DE3)/pRIL, C41,C43 (Stratagene, Avidis)	fjenney@bmb.uga.edu Univ of Georgia, Athens, GA
Cloning Method(s)	Standard restriction/ligation cloning, modified pET vectors (Novagen)	fjenney@bmb.uga.edu Univ of Georgia, Athens, GA
Fusion Tags	AHHHHHHGS- (N-terminal His tag)	fjenney@bmb.uga.edu Univ of Georgia, Athens, GA
Cell Opening Method	Sonication and/or freeze/thaw + lysozyme	fjenney@bmb.uga.edu Univ of Georgia, Athens, GA
Expression/Purification (<i>P. furiosus</i>)		
Expression analysis (gels)	10-20% Criterion SDS-PAGE (Bio-Rad)	fjenney@bmb.uga.edu Univ of Georgia, Athens, GA
Purification equipment	Gravity flow affinity columns (Bio-Rad), AKTA Prime (Amersham Pharmacia)	fjenney@bmb.uga.edu Univ of Georgia, Athens, GA
Affinity Resin(s)	Ni-NTA (Qiagen or Novagen)	fjenney@bmb.uga.edu Univ of Georgia, Athens, GA
Ion Exchange Columns	Not routinely used	fjenney@bmb.uga.edu Univ of Georgia, Athens, GA
Sizing Columns	Superdex sizing gel filtration columns, G30,75, and 200 (Amersham Pharmacia)	fjenney@bmb.uga.edu Univ of Georgia, Athens, GA
Equipment (<i>P. furiosus</i>)		
Robot for cloning, Mini-purification	Mostly done by hand (multi-channel pipetters), Beckman FX liquid handler	fjenney@bmb.uga.edu Univ of Georgia, Athens, GA
Fermentors	2.8L Shake flasks (450ml cultures), BioFlow3000 (New Brunswick)	fjenney@bmb.uga.edu Univ of Georgia, Athens, GA
Crystallization robots	Cartesian HonyBee (Feb 2003), ORYX-6 (Mar 2002)	ashah@sgx3.bmb.uga.edu Univ of Georgia
Date purchased		
Quality Control (<i>P. furiosus</i>)		
Mass Spec	Perkin Elmer API 1plus mass spectrometer (electrospray)	fjenney@bmb.uga.edu Univ of Georgia, Athens, GA
Other	Metal analysis by Inductively Coupled Plasma emission mass spectrometry (ICP-MS)	fjenney@bmb.uga.edu Univ of Georgia, Athens, GA

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Step	Method/Vendor	Contact Person (e-mail)/Location
Cloning (<i>Human</i>)		
Primer Vendor	Integrated DNA Technologies (www.idtdna.com)	tdailey@uga.edu Univ of Georgia, Athens, GA
Expression strains used	JM109 (New England Biolabs) or Top10 (Invitrogen)	tdailey@uga.edu Univ of Georgia, Athens, GA
Cloning Method(s)	Custom TOPO vector and standard restriction/ligation cloning, pTrcHis vectors (Invitrogen)	tdailey@uga.edu Univ of Georgia, Athens, GA
Fusion Tags	MGGSHHHHHHGMASAL- (N-term) and MBP w/TEV cleavage site (N-term)	tdailey@uga.edu Univ of Georgia, Athens, GA
Cell Opening Method	Sonication	tdailey@uga.edu Univ of Georgia, Athens, GA
Expression/Purification (<i>Human</i>)		
Expression analysis (gels)	4-20% Ready Gel SDS-PAGE (Bio-Rad)	mmayer@secsg.org Univ of Georgia, Athens, GA
Purification equipment	Gravity flow affinity columns (Bio-Rad), BioLogicLP (Bio-Rad)	mmayer@secsg.org Univ of Georgia, Athens, GA
Affinity Resin(s)	TALON (Clontech)	mmayer@secsg.org Univ of Georgia, Athens, GA
Ion Exchange Columns	Not routinely used	mmayer@secsg.org Univ of Georgia, Athens, GA
Sizing Columns	Hi-Prep Sephacryl S-100 (Amersham Pharmacia)	mmayer@secsg.org Univ of Georgia, Athens, GA
Equipment (<i>Human</i>)		
Robot for cloning, Mini-purification	BioRobot 9600 (Qiagen)	tdailey@uga.edu Univ of Georgia, Athens, GA
Fermentors	2.8L Shake flasks (1 L cultures)	tdailey@uga.edu Univ of Georgia, Athens, GA
Crystallization robots	Cartesian HonyBee (Feb 2003)	ashah@sgx3.bmb.uga.edu Univ of Georgia
Date purchased	ORYX-6 (Mar 2002)6	
Quality Control (<i>Human</i>)		
Mass Spec	Not routinely used	
Other	4-20% Ready Gel SDS-PAGE (Bio-Rad)	mmayer@secsg.org

STRUCTURAL GENOMIC QUESTIONNAIRE

Center Name: SECSG – UAB

Date: April 3, 2003

Step	Method/Vendor	Contact Person (e-mail)/Location
Cloning (<i>C. elegans</i>)		
Primer Vendor	Invitrogen	mingluo@uab.edu , UAB
Expression strains used	BL21(DE3), BL21(DE3)pLys, BL21(DE3)/AI, Novagen	mingluo@uab.edu
Cloning Method(s)	GATEWAY™, Invitrogen	mingluo@uab.edu
Fusion Tags	AHHHHHHGS- (N-terminal His tag)	mingluo@uab.edu
Cell Opening Method	Sonication and/or freeze/thaw + lysozyme	mingluo@uab.edu
Expression/Purification (<i>C. elegans</i>)		
Expression analysis (gels)	10-20% Criterion SDS-PAGE (Bio-Rad) Agilent 2000	mingluo@uab.edu
Purification equipment	Gravity flow affinity columns (Bio-Rad), AKTA Prime (Amersham Pharmacia)	mingluo@uab.edu
Affinity Resin(s)	Ni-NTA (Qiagen)	mingluo@uab.edu
Ion Exchange Columns	HiTrao, Resource (Pharmacia)	mingluo@uab.edu
Sizing Columns		
	Superdex sizing gel filtration columns, G30,75, and 200 (Amersham Pharmacia)	mingluo@uab.edu , UAB
Equipment (<i>C. elegans</i>)		
Robot for cloning, Mini-purification	Beckman FX liquid handler abd Biomek 2000	mingluo@uab.edu
Fermentors	96-well block plates 2.8L Shake flasks	mingluo@uab.edu
Crystallization robots Date purchased	Cartesian HoneyBee (Feb 2003), Nanoscreen (In-house), Beckman FX (March, 2002)	mingluo@uab.edu
Quality Control (<i>C. elegans</i>)		
Mass Spec	mass spectrometer, UAB Facility	mingluo@uab.edu , UAB
Other	Agilent 2000 Electrophoresis	mingluo@uab.edu , UAB