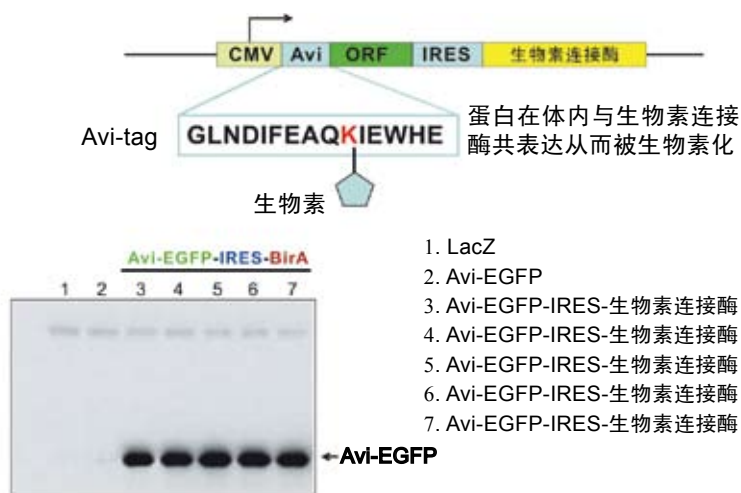


由于生物素连接酶是来源于细菌等低等生物，在高等哺乳动物细胞是没有这类酶，也就无法在哺乳动物细胞内把Avi-Tag ORF融合表达克隆标记上生物素，因此哺乳动物细胞表达的Avi-Tag融合蛋白往往需要体外生物素化，这就大大限制了Avi-Tag的应用。广州复能基因有限公司结合Avi-Tag与IRES技术，建立了在高等哺乳动物细胞内进行酶促催化生物素化的体系，使得哺乳动物细胞表达的Avi-Tag融合蛋白在胞内就被生物素化（<http://www.genecopoeia.com/product/ires/>）（图35）。



Western: 被Sterptavidin-HRP试剂盒检测

图35 293细胞中带Avi-Tag的eGFP被生物素连接酶生物素化。

图片来源: www.genecopoeia.com

原文检索:

<http://www.avidity.com/>

<http://www.fulengen.com>

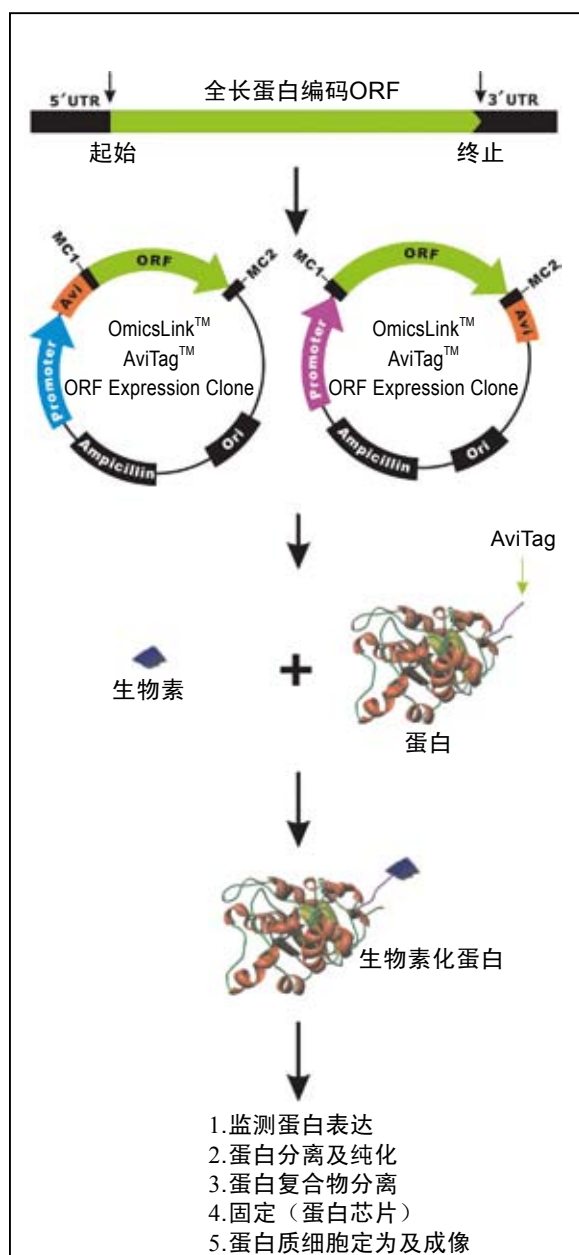


图34 Avi-tag融合表达克隆
图片来源: www.genecopoeia.com

三、多功能复合标签

蛋白标签是生命科学研究、产品开发等必不可少的工具。每种蛋白标签都有其适用的适用范围，通过不同标签之间的有效组合就能大大拓展蛋白标签的应用。例如His-Tag很适合蛋白纯化，但不能提高融合蛋白在*E. coli*中表达的可溶性，而将TrxTM、SUMO可以促进重组蛋白可溶性的标签与His-Tag一起组合，就可达到即提高可溶性重组蛋白表达，有便于纯化的效果，在获得纯化蛋白后，还可以通过SUMO蛋白酶高效地把蛋白标签去除。广州复能基因有限公司构建了一系列的由不同标签组合的载体，可以适合不同的应用（<http://www.genecopoeia.com/tech/omicslink/>）（表10~表13）。

表10 适合于麦胚无细胞表达体系的表达克隆

载体	启动子	宿主细胞	稳定转染	蛋白标签	蛋白酶位点
pReceiver-WG02	T7	cell free	N/A	N-His	Factor Xa
pReceiver-WG03	T7	cell free	N/A	N-HisSUMO	CoolCutter(TM)
pReceiver-WG04	T7	cell free	N/A	N-AviSUMO	CoolCutter(TM)
pReceiver-WG05	T7	cell free	N/A	N-HisAviSUMO	CoolCutter(TM)
pReceiver-WG09	T7	cell free	N/A	HisGST	TEV
pReceiver-WG16	T7	cell free	N/A	N/A	N/A
pReceiver-WG31	T7	cell free	N/A	N-HisSUMOAvi	CoolCutter(TM)
pReceiver-WG33	T7	cell free	N/A	N-TrxHisSUMO	CoolCutter(TM)

表11 适合于原核表达体系的表达克隆

载体	启动子	宿主细胞	稳定转染	蛋白标签	蛋白酶位点
pReceiver-B01	T7	<i>E. Coli</i>	N/A	N-His	N/A
pReceiver-B31	T7	<i>E. Coli</i>	N/A	C-His	N/A
pReceiver-B02	T7	<i>E. Coli</i>	N/A	N/A	N/A
pReceiver-B03	T7	<i>E. Coli</i>	N/A	N-GST	Tev
pReceiver-B04	T7	<i>E. Coli</i>	N/A	N-GST	EK
pReceiver-B05	Tac	<i>E. Coli</i>	N/A	N-GST	Tev
pReceiver-B06	Tac	<i>E. Coli</i>	N/A	N-GST	EK
pReceiver-B07	Tac	<i>E. Coli</i>	N/A	N-MBP	Tev
pReceiver-B08	Tac	<i>E. Coli</i>	N/A	N-MBP	EK
pReceiver-B09	T7	<i>E. Coli</i>	N/A	N-Avi	N/A
pReceiver-B10	Tac	<i>E. Coli</i>	N/A	N-Flag	N/A
pReceiver-B11	Tac	<i>E. Coli</i>	N/A	N-His	N/A
pReceiver-B12	Tac	<i>E. Coli</i>	N/A	HisSUMO	Sumo protease
pReceiver-B13	T7	<i>E. Coli</i>	N/A	HisSUMO	SUMO Protease

表12 适合于哺乳动物细胞表达体系的表达克隆

载体	启动子	宿主细胞	稳定转染	蛋白标签	蛋白酶位点
pReceiver-M01	CMV	Mammalian	Neomycin	N-His	N/A
pReceiver-M02	CMV	Mammalian	Neomycin	N/A	N/A
pReceiver-M29	CMV	Mammalian	Neomycin	N-eGFP	N/A
pReceiver-M03	CMV	Mammalian	Neomycin	C-eGFP	N/A
pReceiver-M15	CMV	Mammalian	Neomycin	N-eYFP	N/A
pReceiver-M16	CMV	Mammalian	Neomycin	C-eYFP	N/A
pReceiver-M32	CMV	Mammalian	Neomycin	N-eCFP	N/A
pReceiver-M33	CMV	Mammalian	Neomycin	C-eCFP	N/A
pReceiver-M04	CMV	Mammalian	Neomycin	N-GST	EK
pReceiver-M05	CMV	Mammalian	Neomycin	N-Avi	N/A

(续表)

pReceiver-M48	CMV	Mammalian	Neomycin	N-Avi+ IRES-Biotin ligase	N/A
pReceiver-M17	CMV	Mammalian	Neomycin	C-Avi	N/A
pReceiver-M06	CMV	Mammalian	Neomycin	N-3xHA	N/A
pReceiver-M07	CMV	Mammalian	Neomycin	C-3xHA	N/A
pReceiver-M08	CMV	Mammalian	Neomycin	C-3xHA-His	N/A
pReceiver-M45	CMV	Mammalian	Neomycin	C-3xHA+ IRES-eGFP	N/A
pReceiver-M09	CMV	Mammalian	Neomycin	C-Myc	N/A
pReceiver-M10	CMV	Mammalian	Neomycin	C-Myc-His	N/A
pReceiver-M47	CMV	Mammalian	Neomycin	C-Myc+ IRES-eGFP	N/A
pReceiver-M11	CMV	Mammalian	Neomycin	N-Flag	N/A
pReceiver-M12	CMV	Mammalian	Neomycin	N-3XFlag	N/A
pReceiver-M13	CMV	Mammalian	Neomycin	C-Flag	N/A
pReceiver-M46	CMV	Mammalian	Neomycin	C-Flag+ IRES-eGFP	N/A
pReceiver-M14	CMV	Mammalian	Neomycin	C-3XFlag	N/A
pReceiver-M24	CMV	Mammalian	Neomycin	N-SNAP	N/A
pReceiver-M26	CMV	Mammalian	Neomycin	C-SNAP	N/A
pReceiver-M49	CMV	Mammalian	Neomycin	N-HaloTag	Tev protease
pReceiver-M50	CMV	Mammalian	Neomycin	C-HaloTag	Tev protease
pReceiver-M55	CMV	Mammalian	Neomycin	N-mCherry	N/A
pReceiver-M56	CMV	Mammalian	Neomycin	C-mCherry	N/A
pReceiver-M60	CMV	Mammalian	Neomycin	IRES-eGFP	N/A

表13 适合于慢病毒表达体系的表达克隆

载体	启动子	宿主细胞	稳定转染	蛋白标签	蛋白酶位点
pReceiver-Lv01	CMV	Stem/primary cell	No	N/A	N/A
pReceiver-Lv31	CMV	Stem/primary cell	No	IRES-eGFP	N/A
pReceiver-Lv36	CMV	Stem/primary cell	No	+ IRES-luciferase	N/A
pReceiver-Lv40	CMV	Stem/primary cell	Neomycin	IRES-Neomycin	N/A
pReceiver-Lv21	CMV	Stem/primary cell	Neomycin	N/A	N/A
pReceiver-Lv02	CMV	Stem/primary cell	No	C-3xHA	N/A
pReceiver-Lv32	CMV	Stem/primary cell	No	C-3xHA + IRES-eGFP	N/A
pReceiver-Lv52	CMV	Stem/primary cell	N/A	C-3xHA+IRES2-eGFP	N/A
pReceiver-Lv06	CMV	Stem/primary cell	Neomycin	C-3xHA	N/A
pReceiver-Lv23	CMV	Stem/primary cell	Neomycin	N-Flag	N/A
pReceiver-Lv03	CMV	Stem/primary cell	No	C-Flag	N/A
pReceiver-Lv33	CMV	Stem/primary cell	No	C-Flag + IRES-eGFP	N/A
pReceiver-Lv53	CMV	Stem/primary cell	N/A	C-Flag+IRES2-eGFP	N/A
pReceiver-Lv07	CMV	Stem/primary cell	Neomycin	C-Flag	N/A

(续表)

pReceiver-Lv19	CMV	Stem/primary cell	Neomycin	N-eGFP	N/A
pReceiver-Lv04	CMV	Stem/primary cell	No	C-eGFP	N/A
pReceiver-Lv08	CMV	Stem/primary cell	Neomycin	C-eGFP	N/A
pReceiver-Lv20	CMV	Stem/primary cell	Neomycin	N-eYFP	N/A
pReceiver-Lv05	CMV	Stem/primary cell	No	C-eYFP	N/A
pReceiver-Lv09	CMV	Stem/primary cell	Neomycin	C-eYFP	N/A
pReceiver-Lv34	CMV	Stem/primary cell	Neomycin	N-eCFP	N/A
pReceiver-Lv61	CMV	Stem/primary cell	No	C-eCFP	N/A
pReceiver-Lv62	CMV	Stem/primary cell	Neomycin	C-eCFP	N/A
pReceiver-Lv35	CMV	Stem/primary cell	No	N-Avi + IRES-Biotin ligase	N/A
pReceiver-Lv26	CMV	Stem/primary cell	Neomycin	N-Avi	N/A
pReceiver-Lv10	CMV	Stem/primary cell	Neomycin	C-Avi	N/A
pReceiver-Lv25	CMV	Stem/primary cell	Neomycin	N-Myc	N/A
pReceiver-Lv17	CMV	Stem/primary cell	Neomycin	C-Myc	N/A
pReceiver-Lv18	CMV	Stem/primary cell	Neomycin	C-Myc-His	N/A
pReceiver-Lv43	CMV	Stem/primary cell	No	C-Myc+ IRES-eGFP	N/A
pReceiver-Lv70	CMV	Stem/primary cell	N/A	C-Myc+IRES2-eGFP	N/A
pReceiver-Lv44	CMV	Stem/primary cell	No	C-Myc+ IRES-eYFP	N/A
pReceiver-Lv47	CMV	Stem/primary cell	Neomycin	C-Myc+IRES-Neomycin	N/A
pReceiver-Lv64	CMV	Stem/primary cell	Neomycin	N-HaloTag	Tev protease
pReceiver-Lv65	CMV	Stem/primary cell	Neomycin	C-HaloTag	Tev protease
pReceiver-Lv69	CMV	Stem/primary cell	N/A	C-Myc+IRES-mCherry	N/A
pReceiver-Lv71	CMV	Stem/primary cell	Puromycin	N-mCherry	N/A
pReceiver-Lv72	CMV	Stem/primary cell	Neomycin	C-mCherry	N/A
pReceiver-Lv41	EF1a	Stem/primary cell	Neomycin	N/A	N/A

(注：感谢筱玥和YORK在本专题制作过程中给予的帮助！)

科研综述 研究前沿 热点话题
 技术方法 专题译述 生命百态
 会议展览 教学视频



www.LifeOmics.com