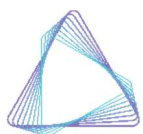


Plasmid-EZ 入门指南



AZENTA
LIFE SCIENCES

azenta.com

目录

1. Plasmid-EZ 简介	03
Plasmid-EZ 产品介绍	
Plasmid-EZ 结果下载	
Plasmid-EZ 结果展示	
2. 质粒基因组注释	08
质粒注释图谱	
如何使用 SnapGene Viewer 查看 GenBank 文件	
Excel 注释查看	
如何打开 FASTA 文件	
3. 数据质量评估	11
读长分布与质量分数查看	
单碱基置信度分数查看	
4. 变异检测	12
基于碱基的变异分析	
多重叠群分析与注释生成	
5. 为什么我的样品组装失败了?	14

Plasmid-EZ 简介

质粒--生命科学研究和生物医药研发的必需品。测序验证是关键质控步骤，在进行下游应用前，跳过测序质控可能会导致后续的异常排查成本增加，包括时间成本和生产成本。

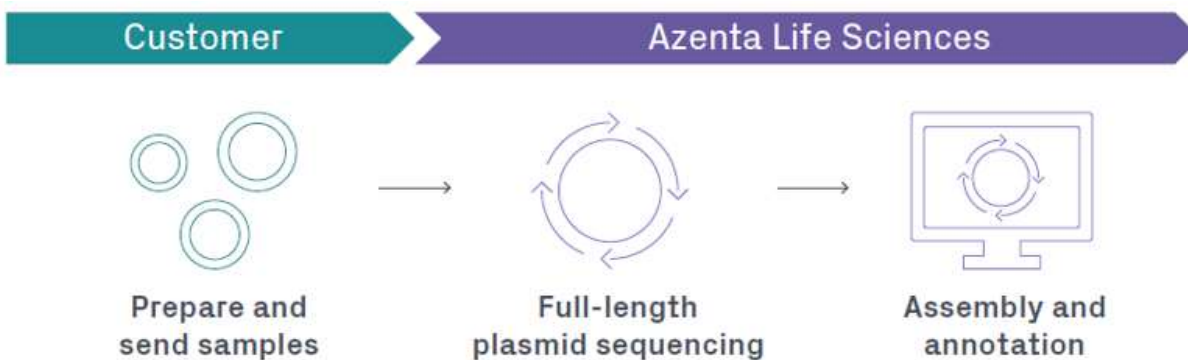


目前质粒测序质控所遇到的难点



Plasmid-EZ 简介

Plasmid-EZ 是一种经济高效，高通量，快速的从质粒到结果可交互式结构验证解决方案。



✓最快1天可出结果，快速高效；

✓不需要提供已知序列信息或测序引物；

✓样本通量可灵活调整；

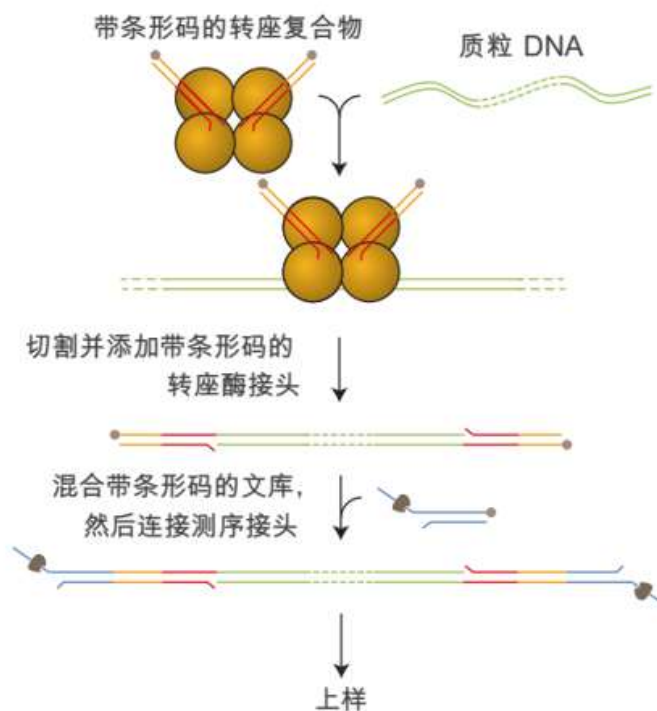
✓测序不受质粒大小限制；

✓测序不受结构复杂、多聚体样本的限制；

✓高准确度。

Plasmid-EZ测序反应流程

1. 带有barcode DNA序列的转座酶与质粒结合
2. 质粒被转座酶打断，同时在打断的位置连接上barcode DNA
3. 混合连有不同barcode DNA的质粒，连接测序接头（每个样品有不同的barcode 序列）
4. 纯化后上机测序



Plasmid-EZ 简介

质粒测序验证过去一直以传统的 Sanger 测序技术为主导，但 Plasmid-EZ 流程简便快速，可以为质粒鉴定提供更全面完整的信息。

Plasmid-EZ与Sanger测序的比较

特点	Sanger测序	Plasmid-EZ
测序区域	一般仅针对目标基因，可通过Primer walking多反应测序拼接获得完整质粒	可直接测完整质粒
引物要求	是	否
PCR要求	是	否
通量	低	高
识别插入元件的方向	无法识别方向	是
解析结构复杂区域	否	是
测出多聚体质粒	否	是
测出混合质粒或杂质DNA	否	是
周期	以4个反应为例，3-4天	最快1天

Plasmid-EZ送样要求

环状质粒

质粒单一，无基因组污染或降解（推荐使用Qubit定量，避免浓度虚高）

片段≤10Kb：浓度 ≥20ng/μl，体积≥20μl；

片段10Kb-20Kb之间：浓度 ≥30ng/μl，体积≥20μl；

片段≥20Kb：浓度 ≥40ng/μl，体积≥20μl；

纯度要求：OD260/280为1.8-2.0，OD260/230≥2.0。

菌液/平板菌落（片段≤20Kb）

菌液：提供体积≥200μl新鲜菌液（OD > 0.8），装于1.5ml离心管中封口保存；

直抽菌液：过夜培养的新鲜大肠杆菌菌液2ml或2ml菌液沉淀；

平板菌落：单菌落，无明显卫星菌落，需将待测的菌落圈选标注并编号。



Plasmid-EZ 简介

Plasmid-EZ 测序结果可以直接在金唯智账户中查看/下载。点击“查看结果”，界面中会显示每个样品的组装状态，您可以点击右上角的“下载”进行结果下载。（结果保存 1 个月，建议及时下载与保存）

订单生成日期	订单号	订单名称	项目名称	业务线	服务类型	保长期	数量	订单状态	操作
☐ 2025/1/8 14:35	80-1613643358			Sanger测序	Plasmid-EZ全质粒测序	常规	24	等待样品	订单详情
☐ 2024/12/17 13:15	80-1613337609			Sanger测序	Plasmid-EZ全质粒测序	常规	1	完成	查看结果

首页 / Sanger测序订单结果

收费数 1 您对这份订单是否满意? [是](#) [否](#)

已提交的样品结果 [下载](#)

订单号 80-1613337609

#	样品名称	组装状态
1	1	success

结果下载后，点击 80-xxxxxxx 文件夹，订单中的每个样本均有独立文件夹。

PGEM

6/6/2025 3:36 PM

File folder

PGEM-1

6/6/2025 3:30 PM

File folder

每个样品文件夹中包含 Result 结果文件夹和交互式报告 (Report.html) 等信息。

Result

6/6/2025 3:36 PM

File folder

src

6/6/2025 3:36 PM

File folder

md5.md5

5/28/2025 1:12 PM

MD5 File

md5check.bat

5/28/2025 1:12 PM

Windows Batch File

md5sum.exe

5/28/2025 1:12 PM

Application

Report.html

5/28/2025 1:12 PM

Firefox HTML Document

```
1234567891011121314151617181920212223242526272829303132333435363738394041424344454647484950515253545556575859606162636465666768697071727374757677787980818283848586878889909192939495969798991001011021031041051061071081091101111121131141151161171181191201211221231241251261271281291301311321331341351361371381391401411421431441451461471481491501511521531541551561571581591601611621631641651661671681691701711721731741751761771781791801811821831841851861871881891901911921931941951961971981992002012022032042052062072082092102112122132142152162172182192202212222232242252262272282292302312322332342352362372382392402412422432442452462472482492502512522532542552562572582592602612622632642652662672682692702712722732742752762772782792802812822832842852862872882892902912922932942952962972982993003013023033043053063073083093103113123133143153163173183193203213223233243253263273283293303313323333343353363373383393403413423433443453463473483493503513523533543553563573583593603613623633643653663673683693703713723733743753763773783793803813823833843853863873883893903913923933943953963973983994004014024034044054064074084094104114124134144154164174184194204214224234244254264274284294304314324334344354364374384394404414424434444454464474484494504514524534544554564574584594604614624634644654664674684694704714724734744754764774784794804814824834844854864874884894904914924934944954964974984995005015025035045055065075085095105115125135145155165175185195205215225235245255265275285295305315325335345355365375385395405415425435445455465475485495505515525535545555565575585595605615625635645655665675685695705715725735745755765775785795805815825835845855865875885895905915925935945955965975985996006016026036046056066076086096106116126136146156166176186196206216226236246256266276286296306316326336346356366376386396406416426436446456466476486496506516526536546556566576586596606616626636646656666676686696706716726736746756766776786796806816826836846856866876886896906916926936946956966976986997007017027037047057067077087097107117127137147157167177187197207217227237247257267277287297307317327337347357367377387397407417427437447457467477487497507517527537547557567577587597607617627637647657667677687697707717727737747757767777787797807817827837847857867877887897907917927937947957967977987998008018028038048058068078088098108118128138148158168178188198208218228238248258268278288298308318328338348358368378388398408418428438448458468478488498508518528538548558568578588598608618628638648658668678688698708718728738748758768778788798808818828838848858868878888898908918928938948958968978988999009019029039049059069079089099109119129139149159169179189199209219229239249259269279289299309319329339349359369379389399409419429439449459469479489499509519529539549559569579589599609619629639649659669679689699709719729739749759769779789799809819829839849859869879889899909919929939949959969979989991000100110021003100410051006100710081009101010111012101310141015101610171018101910201021102210231024102510261027102810291030103110321033103410351036103710381039104010411042104310441045104610471048104910501051105210531054105510561057105810591060106110621063106410651066106710681069107010711072107310741075107610771078107910801081108210831084108510861087108810891090109110921093109410951096109710981099110011011102110311041105110611071108110911101111112111311141115111611171118111911201121112211231124112511261127112811291130113111321133113411351136113711381139114011411142114311441145114611471148114911501151115211531154115511561157115811591160116111621163116411651166116711681169117011711172117311741175117611771178117911801181118211831184118511861187118811891190119111921193119411951196119711981199120012011202120312041205120612071208120912101211121212131214121512161217121812191220122112221223122412251226122712281229123012311232123312341235123612371238123912401241124212431244124512461247124812491250125112521253125412551256125712581259126012611262126312641265126612671268126912701271127212731274127512761277127812791280128112821283128412851286128712881289129012911292129312941295129612971298129913001301130213031304130513061307130813091310131113121313131413151316131713181319132013211322132313241325132613271328132913301331133213331334133513361337133813391340134113421343134413451346134713481349135013511352135313541355135613571358135913601361136213631364136513661367136813691370137113721373137413751376137713781379138013811382138313841385138613871388138913901391139213931394139513961397139813991400140114021403140414051406140714081409141014111412141314141415141614171418141914201421142214231424142514261427142814291430143114321433143414351436143714381439144014411442144314441445144614471448144914501451145214531454145514561457145814591460146114621463146414651466146714681469147014711472147314741475147614771478147914801481148214831484148514861487148814891490149114921493149414951496149714981499150015011502150315041505150615071508150915101511151215131514151515161517151815191520152115221523152415251526152715281529153015311532153315341535153615371538153915401541154215431544154515461547154815491550155115521553155415551556155715581559156015611562156315641565156615671568156915701571157215731574157515761577157815791580158115821583158415851586158715881589159015911592159315941595159615971598159916001601160216031604160516061607160816091610161116121613161416151616161716181619162016211622162316241625162616271628162916301631163216331634163516361637163816391640164116421643164416451646164716481649165016511652165316541655165616571658165916601661166216631664166516661667166816691670167116721673167416751676167716781679168016811682168316841685168616871688168916901691169216931694169516961697169816991700170117021703170417051706170717081709171017111712171317141715171617171718171917201721172217231724172517261727172817291730173117321733173417351736173717381739174017411742174317441745174617471748174917501751175217531754175517561757175817591760176117621763176417651766176717681769177017711772177317741775177617771778177917801781178217831784178517861787178817891790179117921793179417951796179717981799180018011802180318041805180618071808180918101811181218131814181518161817181818191820182118221823182418251826182718281829183018311832183318341835183618371838183918401841184218431844184518461847184818491850185118521853185418551856185718581859186018611862186318641865186618671868186918701871187218731874187518761877187818791880188118821883188418851886188718881889189018911892189318941895189618971898189919001901190219031904190519061907190819091910191119121913191419151916191719181919192019211922192319241925192619271928192919301931193219331934193519361937193819391940194119421943194419451946194719481949195019511952195319541955195619571958195919601961196219631964196519661967196819691970197119721973197419751976197719781979198019811982198319841985198619871988198919901991199219931994199519961997199819992000200120022003200420052006200720082009201020112012201320142015201620172018201920202021202220232024202520262027202820292030203120322033203420352036203720382039204020412042204320442045204620472048204920502051205220532054205520562057205820592060206120622063206420652066206720682069207020712072207320742075207620772078207920802081208220832084208520862087208820892090209120922093209420952096209720982099210021012102210321042105210621072108210921102111211221132114211521162117211821192120212121222123212421252126212721282129213021312132213321342135213621372138213921402141214221432144214521462147214821492150215121522153215421552156215721582159216021612162216321642165216621672168216921702171217221732174217521762177217821792180218121822183218421852186218721882189219021912192219321942195219621972198219922002201220222032204220522062207220822092210221122122213221422152216221722182219222022212222222322242225222622272228222922302231223222332234223522362237223822392240224122422243224422452246224722482249225022512252225322542255225622572258225922602261226222632264226522662267226822692270227122722273227422752276227722782279228022812282228322842285228622872288228922902291229222932294229522962297229822992300230123022303230423052306230723082309231023112312231323142315231623172318231923202321232223232324232523262327232823292330233123322333233423352336233723382339234023412342234323442345234623472348234923502351235223532354235523562357235823592360236123622363236423652366236723682369237023712372237323742375237623772378237923802381238223832384238523862387238823892390239123922393239423952396239723982399240024012402240324042405240624072408240924102411241224132414241524162417241824192420242124222423242424252426242724282429243024312432243324342435243624372438243924402441244224432444244524462447244824492450245124522453245424552456245724582459246024612462246324642465246624672468246924702471247224732474247524762477247824792480248124822483248424852486248724882489249024912492249324942495249624972498249925002501250225032504250525062507250825092510251125122513251425152516251725182519252025212522252325242525252625272528252925302531253225332534253525362537253825392540254125422543254425452546254725482549255025512552255325542555255625572558255925602561256225632564256525662567256825692570257125722573257425752576257725782579258025812582258325842585258625872588258925902591259225932594259525962597259825992600260126022603260426052606260726082609261026112612261326142615261626172618261926202621262226232624262526262627262826292630263126322633263426352636263726382639264026412642264326442645264626472648264926502651265226532654265526562657265826592660266126622663266426652666266726682669267026712672267326742675267626772678267926802681268226832684268526862687268826892690269126922693269426952696269726982699270027012702270327042705270627072708270927102711271227132714271527162717271827192720272127222723272427252726272727282729273027312732273327342735273627372738273927402741274227432744274527462747274827492750275127522753275427552756275727582759276027612762276327642765276627672768276927702771277227732774277527762777277827792780278127822783278427852786278727882789279027912792279327942795279627972798279928002801280228032804280528062807280828092810281128122813281428152816281728182819282028212822282328242825282628272828282928302831283228332834283528362837283828392840284128422843284428452846284728482849285028512852285328542855285628572858285928602861286228632864286528662867286828692870287128722873287428752876287728782879288028812882288328842885288628872888288928902891289228932894289528962897289828992900290129022903290429052906290729082909291029112912291329142915291629172918291929202921292229232924292529262927292829292930293129322933293429352936293729382939294029412942294329442945294629472948294929502951295229532954295529562957295829592960296129622963296429652966296729682969297029712972297329742975297629772978297929802981298229832984298529862987298829892990299129922993299429952996299729982999300030013002300330043005300630073008300930103011301230133014301530163017301830193020302130223023302430253026302730283029303030313032303330343035303630373038303930403041304230433044304530463047304830493050305130523053305430553056305730583059306030613062306330643065306630673068306930703071307230733074307530763077307830793080308130823083308430853086308
```



Plasmid-EZ 简介

Result 文件夹包含 00_QC、01_Assembly 和 02_Annotation 文件夹。

00_QC	6/6/2025 3:36 PM	File folder
01_Assembly	6/6/2025 3:36 PM	File folder
02_Annotation	6/6/2025 3:36 PM	File folder

原始.fastq 格式结果可在 00_QC 文件夹查看。未经处理的测序原始数据包含在 raw.fastq.gz 中，如需查看，可用系统自带解压软件或 7zip 等软件解压获得.fastq 文件，再用文本编辑器（如记事本）等软件打开。

LengthDist.html	5/28/2025 1:08 PM	Firefox HTML Document
Qual.html	5/28/2025 1:08 PM	Firefox HTML Document
raw.fastq.gz	5/28/2025 1:08 PM	GZ File
RawData.stat	5/28/2025 1:08 PM	STAT File

常见结果文件如组装结果（genome.fa 文件）和碱基组成 Excel 文件（Base-composition.xls）均位于 01_Assembly 文件夹中。

All.final.stat.xls	5/28/2025 1:08 PM	Microsoft Excel 97-2003 ...
All.Map.Cov.stat	5/28/2025 1:08 PM	STAT File
barcode1_PGEM.bam	5/28/2025 1:08 PM	BAM File
barcode1_PGEM.bam.bai	5/28/2025 1:08 PM	BAI File
barcode1_PGEM.contig1.depth.html	5/28/2025 1:08 PM	Firefox HTML Document
Base-composition.xls	5/28/2025 1:08 PM	Microsoft Excel 97-2003 ...
genome.fa	5/28/2025 1:08 PM	FA File



质粒基因组注释

首先，打开 Report.html 报告。在样本报告中，您可以先点击“质粒基因组注释”查看已组装序列（又称重叠群，contig）的带注释质粒图谱。将鼠标悬停于图谱上时，会弹出对应区域的信息，该信息也会列于图谱下方的表格中。

一、实验流程

二、生物信息分析流程

三、生物信息分析结果

1 测序数据统计

2 质粒基因组组装

3 质粒基因组注释

质粒测序分析结果报告

1 实验流程

首先，提取待测质粒基因组DNA，构建测序文库。

Nanopore平台建库和文库质检：

取固定量的质粒DNA，使用带barcode的转座酶打断，然后将样本混合后进行纯化，纯化产物再加上统一的接头，构建成适配ONT平台的文库。将构建好的文库稀释后加载到测序芯片上，使用ONT平台进行测序。

Feature	Type	percent identity	percent match length	Description
f1 ori	rep_origin	100.0	100.0	f1 bacteriophage origin of replication; arrow indicates direction of (+) strand synthesis
AmpR promoter	promoter	100.0	100.0	bla
AmpR	CDS	99.76	100.0	β-lactamase; bla; confers resistance to ampicillin
ori	rep_origin	99.83	100.0	high-copy-number ColE1 pMB1 pBR322 pUC origin of replication
MCS	misc_feature	100.0	100.0	pUC18/19 multiple cloning site
lac promoter	promoter	100.0	100.0	promoter for the E. coli lac operon
CAP binding site	protein_bind	100.0	100.0	CAP binding activates transcription in the presence of cAMP. E. coli catabolite activator protein
T7 promoter	promoter	100.0	100.0	promoter for bacteriophage T7 RNA polymerase
SP6 promoter	promoter	100.0	100.0	promoter for bacteriophage SP6 RNA polymerase
lac operator	protein_bind	100.0	100.0	The lac repressor binds to the lac operator to inhibit transcription in E. coli. This inhibition can be relieved by adding lactose or isopropyl-β-D-thiogalactopyranoside (IPTG). lac repressor encoded by lacI
lacZα	CDS	100.0	90.80	LacZα fragment of β-galactosidase; lacZ fragment
bom	misc_feature	100.0	70.92	basis of mobility region from pBR322
lacI	CDS	100.0	8.58	lac repressor; lacI; The lac repressor binds to the lac operator to inhibit transcription in E. coli. This inhibition can be relieved by adding lactose or isopropyl-β-D-thiogalactopyranoside (IPTG).
rop	CDS	100.0	18.75	Rop protein

注释图谱及序列以 GenBank 格式存储于“02_Annotation”文件夹中，该文件可在任意质粒查看软件（如 [SnapGene Viewer](#)）中打开。

	contig1_annot.xls	5/28/2025 1:08 PM	Microsoft Excel 97-2003 ...
	contig1_pLann.csv	5/28/2025 1:08 PM	Microsoft Excel Comma...
	contig1_pLann.gb	5/28/2025 1:08 PM	GenBank DNA
	contig1_pLann.html	5/28/2025 1:08 PM	Firefox HTML Document

质粒基因组注释

注释信息和序列可在“02_Annotation”文件夹内的 contig1_pLann.csv 文件中获取，该文件可使用 Excel 打开。

contig1_annot.xls	5/28/2025 1:08 PM	Microsoft Excel 97-2003 ...
contig1_pLann.csv	5/28/2025 1:08 PM	Microsoft Excel Comma...
contig1_pLann.gbk	5/28/2025 1:08 PM	GenBank DNA
contig1_pLann.html	5/28/2025 1:08 PM	Firefox HTML Document

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R
1	sseqid	start	locat	end	locat	strand	percent ic	full length	length of	percent rr	fragment	database	Feature	Type	Descriptic sequence			
2	f1_ori	1262	1691		1	100	429	429	100	FALSE	snappene f1_ori	rep_origir	f1 bacteri	ACGCGCCCTGTAGCGCGCATTAAAGCGCGCGGGTGTG				
3	AmpR_prc	2049	2154		1	100	105	105	100	FALSE	snappene AmpR pro	promoter	bla	CGCGGAACCCCTATTGTTTATTTTCTAAATACATTCA				
4	AmpR_(2)	2154	3015		1	99.768	861	861	100	FALSE	snappene AmpR	CDS	T ^r -lactama	ATGAGTATTCACATTTCGGTGTGCGCCCTATTCCTTTT				
5	ori	3185	577		1	99.83	589	589	100	FALSE	snappene ori	rep_origir	high-copy	TTGAGATCCTTTTCTGCGCGTAATCTGCTGCTTGCA				
6	MCS_(8)	1021	1078		-1	100	57	57	100	FALSE	snappene MCS	misc_feat	pUC18/19	GAATTCGAGCTCGGTACCCGGGGATCCTCTAGAGTCGA				
7	lac_promc	900	931		1	100	31	31	100	FALSE	snappene lac promo	promoter	promoter	TTTACACTTTATGCTTCGGGCTCGTATGTTG				
8	CAP_bind	864	886		1	100	22	22	100	FALSE	snappene CAP bindi	protein_b	CAP bindi	TAATGTGAGTTAGCTCACTCAT				
9	T7_promo	1080	1099		-1	100	19	19	100	FALSE	snappene T7 promol	promoter	promoter	TAATACGACTCACTATAGG				
10	SP6_prom	996	1015		1	100	19	19	100	FALSE	snappene SP6 promi	promoter	promoter	ATTTAGGTGACACTATAGA				
11	lac_oper	938	955		1	100	17	17	100	FALSE	snappene lac operat	protein_b	The lac re	TTGTGAGCGGATAACAA				
12	lacZ_alpha	1101	1259		1	100	174	158	90.8046	TRUE	snappene lacZ α	CDS	LacZ α frag	ATTCAGTGGCCGTCGTTTACAACGTCGTGACTGGGAA				
13	bom	1718	1818		-1	100	141	100	70.92199	TRUE	snappene bom	misc_feat	basis of m	CTGGCTTAACATATGCGGCATCAGAGCAGATTGTACTGA				
14	lacI	759	852		1	100	1083	93	8.587258	TRUE	snappene lacI	CDS	lac repres	GCGCCCAATACGCAAAACCGCCTCTCCCCGCGCGTTGGC				
15	rop	1919	1955		-1	100	192	36	18.75	TRUE	snappene rop	CDS	Rop prote	CTCGCGCGTTTCGGTGATGACGGTGAAACCTCTGA				

在“01_Assembly”文件夹内提供了 genome.fa 文件，其中包含该重叠群的序列。此文件可使用 SnapGene Viewer、Geneious 等软件打开，也可通过包括 Microsoft Word 在内的任意文本查看器打开。

```

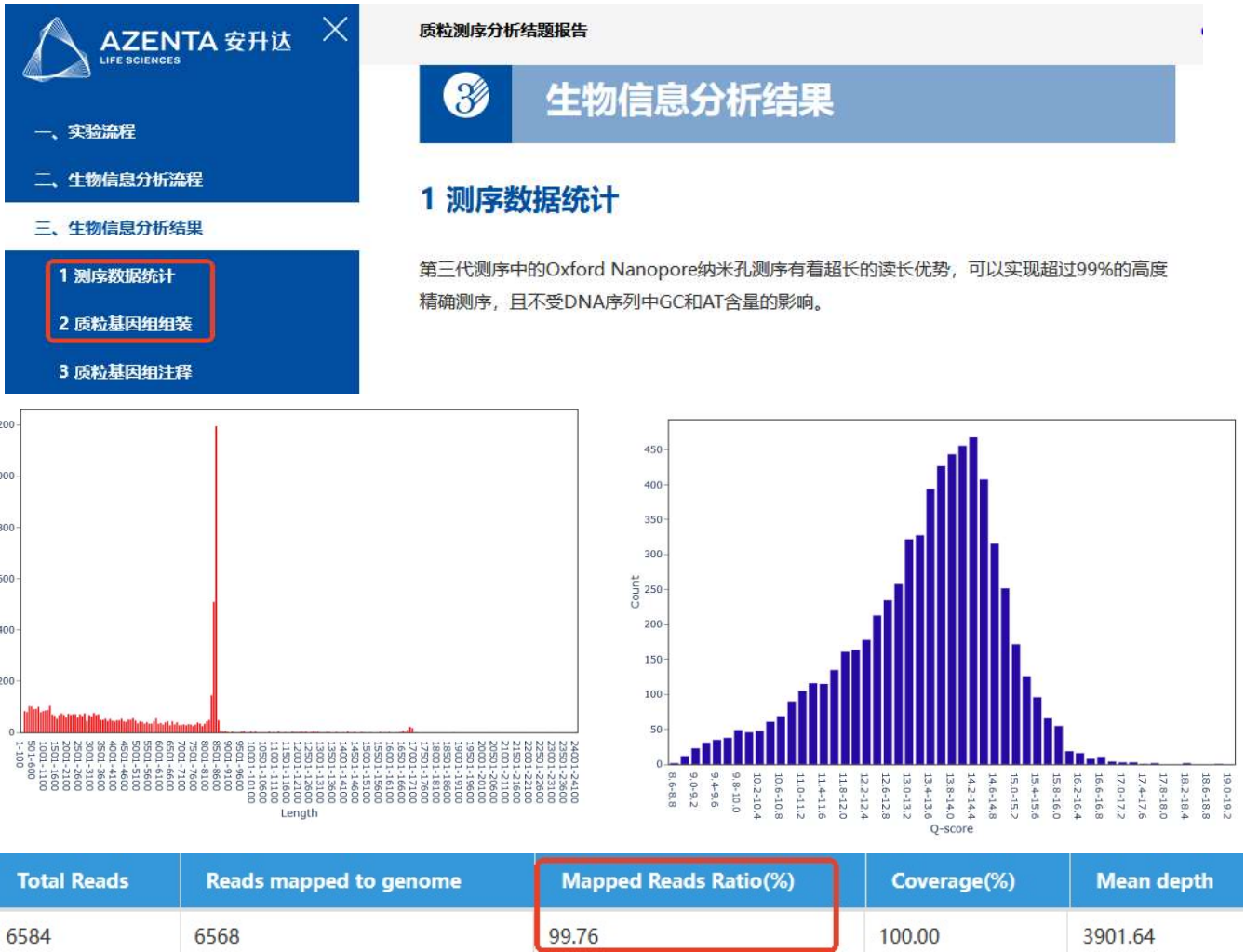
|>PGEM.tig00000003 len=3197 reads=125 class=contig suggestRepeat=yes
suggestBubble=no suggestCircular=yes trim=0-3197
TTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACCACCGCTACCCAGCGGTGGTTTGTGTTGCCGGATCAA
GAGCTACCAACTCTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAAATACTGTTCTTCTAGTGT
AGCCGTAGTTAGGCCACCACTTCAAGAACTCTGTAGCACCGCTACATACCTCGCTCTGCTAATCCTGTTACC
AGTGCGTCTGCCAGTGGCGATAAGTCTGTCTTACCGGGTTGGACTCAAGACGATAGTTACCGGATAAGGCG
CAGCGGTTCGGGCTGAACGGGGGGTTCGTGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGAT
ACCTACAGCGTGAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGCGGACAGGTATCCGGTAAGCGG
CAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCTTGTATCTTTATAGTCTCTGTGCGG
TTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATGAAAAACGCCA
GCAACGCGGCCTTTTACGGTTTCTTGGCCTTTGTGCTGGCCTTTTGCTCACATGTTCTTCTGCGTTATCCCC
TGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGACGCCGAACGACCGAGCGC
AGCGAGTCAGTGAGCGAGGAAGCGGAAGAGCGCCCAATACGCAAAACCGCCTCTCCCGCGCGCTTGGCCGATC
ATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTTA

```



数据质量评估

点击网页报告左侧“测序数据统计”和“质粒基因组组装”，您可以查看到测序质量指标，包括原始测序读长（又称 reads）的长度分布图（红色图表）、reads 质量分布图（蓝色图表）和比对读长百分比（第 3 部分）等信息。



期望观察到的结果:

3

1. 出现与组装长度匹配的清晰质粒峰
2. 大多数 reads $Q > 10$
3. 大多数 reads 能比对至组装数据，通常为 90%以上

变异检测

在 01_Assembly 文件夹中提供了一个碱基组成 Excel 文件（Base-composition.xls），其中包含每个碱基位置处的 reads 数量。

	All.final.stat.xls	5/28/2025 1:08 PM	Microsoft Excel 97-2003 ...
	All.Map.Cov.stat	5/28/2025 1:08 PM	STAT File
	barcode1_PGEM.bam	5/28/2025 1:08 PM	BAM File
	barcode1_PGEM.bam.bai	5/28/2025 1:08 PM	BAI File
	barcode1_PGEM.contig1.depth.html	5/28/2025 1:08 PM	Firefox HTML Document
	Base-composition.xls	5/28/2025 1:08 PM	Microsoft Excel 97-2003 ...
	genome.fa	5/28/2025 1:08 PM	FA File

该文件还为您提供了在该碱基处存在插入或突变的 reads 数量。（由于纳米孔测序原始测序读长存在一定比例的随机突变，此为参考值，具体变异建议以 Sanger 或 NGS 验证结果为准。）

ID	Site	Ref	Depth	A	T	C	G	Indel	Mutation	Other_Variant
contig1	1	A	3178	3178	0	0	0	0	0	- /
contig1	2	C	3282	0	0	3282	0	0	0	- /
contig1	3	G	3451	0	0	0	3451	0	0	- /
contig1	4	G	3790	1	0	0	3789	0	0.0264	- /
contig1	5	G	3801	6	0	0	3795	0	0.1579	+AT(1)]+(/
contig1	6	A	3804	3795	0	0	2	7	0.2366	+C(1)]+G(/

变异检测

组装结果也有可能包含多个序列，如果出现这种情况，01_Assembly 文件夹中的 genome.fa 文件将包含所有组装好的重叠群。

 All.final.stat.xls	5/28/2025 1:08 PM	Microsoft Excel 97-2003 ...
 All.Map.Cov.stat	5/28/2025 1:08 PM	STAT File
 barcode1_PGEM.bam	5/28/2025 1:08 PM	BAM File
 barcode1_PGEM.bam.bai	5/28/2025 1:08 PM	BAI File
 barcode1_PGEM.contig1.depth.html	5/28/2025 1:08 PM	Firefox HTML Document
 barcode1_PGEM.contig2.depth.html	5/28/2025 1:08 PM	Firefox HTML Document
 Base-composition.xls	5/28/2025 1:08 PM	Microsoft Excel 97-2003 ...
 genome.fa	5/28/2025 1:08 PM	FA File

在 SnapGene Viewer 中打开此文件，您将看到所有重叠群的列表，以及来自组装程序的一些详细信息。



例如，若组装得到了两个重叠群：一个是完整的环状质粒，另一个并非完整环状序列。那么，第二个重叠群很可能是组装过程中产生的组装伪像，而非真正的变异。

相反，如果看到多个环状重叠群，且用于生成重叠群的 reads 数量较多（e.g., > 30），则这可能代表样本中存在多个质粒变体。

如果想查看任何其他重叠群的注释，只需将序列复制粘贴到 [Plannote](#) 中。这将生成带有 GenBank 文件的注释以及包含注释部分的 CSV 文件。

如果所有重叠群都只有少量 reads，则可能表明样本的组装质量较低或样品本身数据量较少。

为什么我的样品组装失败了？

若样本组装失败，Result 文件夹中将仅包含 00_QC 文件夹。文件夹内包含原始数据、样本读长、数据质量等相关信息。

 00_QC	6/6/2025 3:03 PM	File folder
 LengthDist.html	6/6/2025 3:03 PM	Firefox HTML Document
 Qual.html	6/6/2025 3:03 PM	Firefox HTML Document
 raw.fastq.gz	6/6/2025 3:03 PM	GZ File
 RawData.stat	6/6/2025 3:03 PM	STAT File

组装失败的最常见原因是样本浓度未达到所需的送样要求，或存在样品浓度虚高问题。浓度过低可能导致建库过程中片段化加剧及 / 或样本测序读长（reads）数量不足。我们强烈建议您在送样前使用 Qubit 或等效仪器检测样本浓度，以降低失败风险。

从测序读长长度分布图可一定程度上解释出现不同组装结果的原因：左图测序读长峰形单一，表明样本为单克隆质粒，通常可成功组装；中图出现多个峰，说明样本中可能存在片段缺失、重组或多聚体质粒等情况，可能出现多条组装结果；右图测序读长以短片段为主，不存在主峰，说明样本中可能存在严重降解或浓度虚高等情况，高概率会导致组装失败。

