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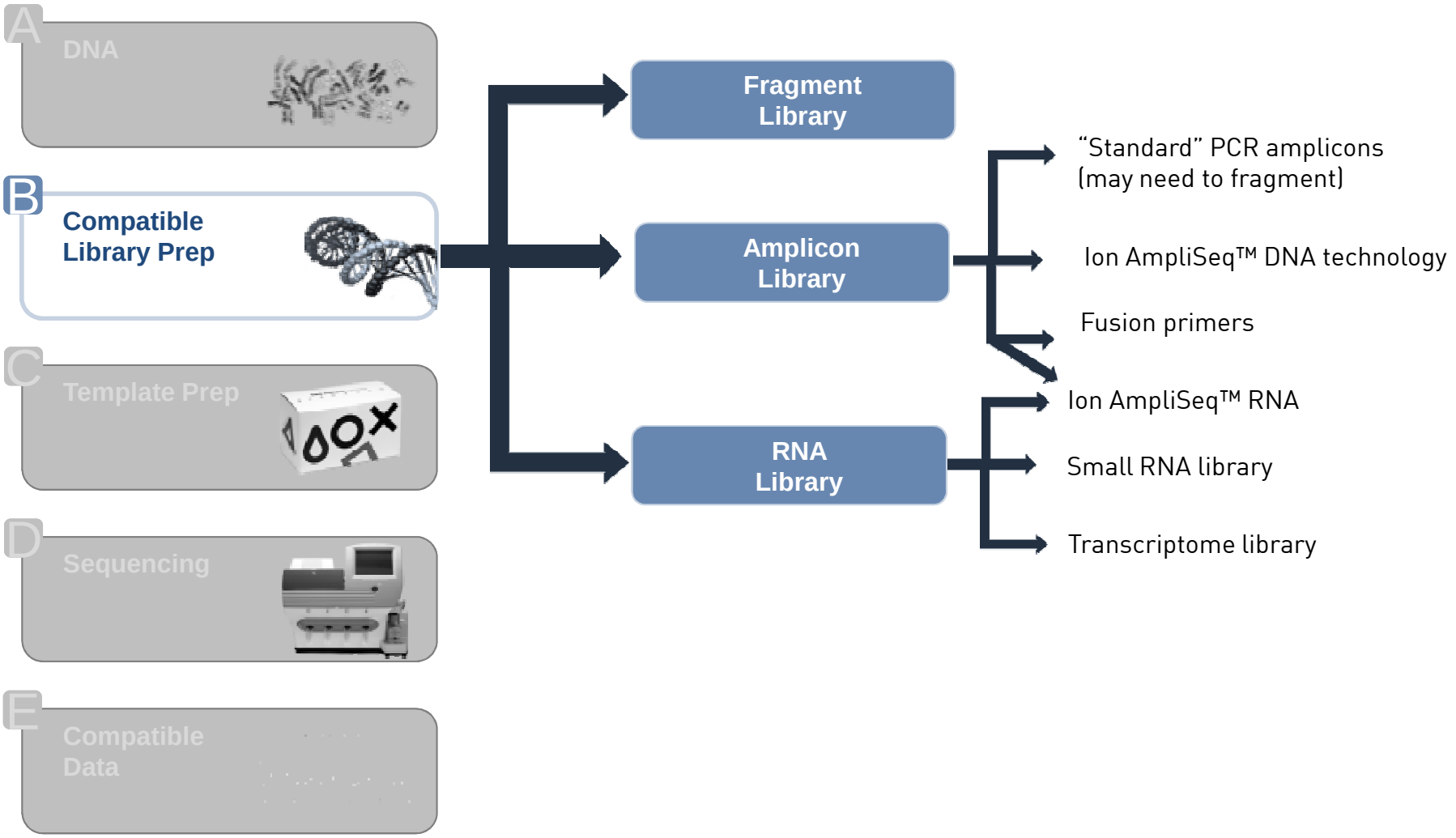
Library Protocols Discussion

The world leader in serving science

Ion Workflow – Library Prep



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Ion Workflow – Library Prep

gDNA / Long Amplicon Libraries	Fragmentation (Size dependent)	End Repair (for physical shearing)	Adapter Ligation, Nick repair	Size selection	Amplification* (if needed)	Qualify & Quantify	~2-3 hrs
Short Amplicon Libraries	PCR	Clean Up	Quantification				~3 hrs
Ion AmpliSeq™ (**DNA**) Libraries	Multiplex PCR	Digest Primers, Phosphorylate	Adapter Ligation	Purify	Amplification* (if needed)	Qualify & Quantify	~3.5 hrs
Ion AmpliSeq™ **RNA** Libraries	Reverse Transcript-ion	Multiplex PCR	Digest Primers	Adapter Ligation	Purify	Amplification	Qualify & Quantify	~5 hrs
Ion RNA Libraries	Prepare WT or miRNA	Hybridization / Adapter Ligation	Reverse transcription	Size selection	Amplification	Qualify & Quantify	~5-6 hrs

extra option: Ion Library Equalizer™ Kit

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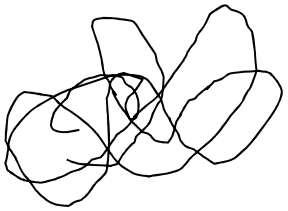
Library Prep: Fragment Workflow for gDNA

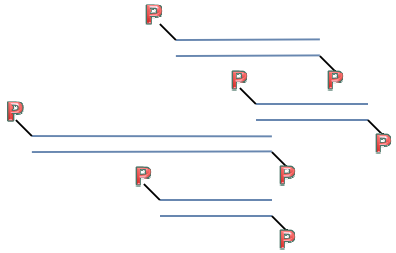


50-100 ng
or 1 ug DNA

Shear
DNA

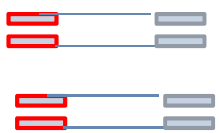
Fragmented DNA

Blunt End
Repair
(for mechanical
shearing only)



End Polished Fragments

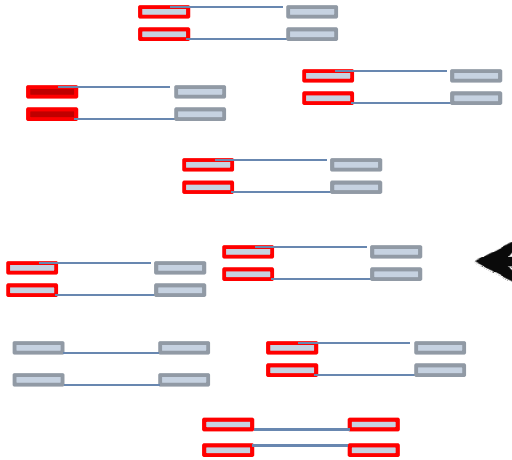
Adapter Ligation,
nick translation
& Size Selection



Adapter Ligated
Library

PCR Amplify
(if needed)

Optional:
Normalization
with Ion Library
Equalizer™ Kit



Amplified, Adapter Ligated Library

Template
Preparation

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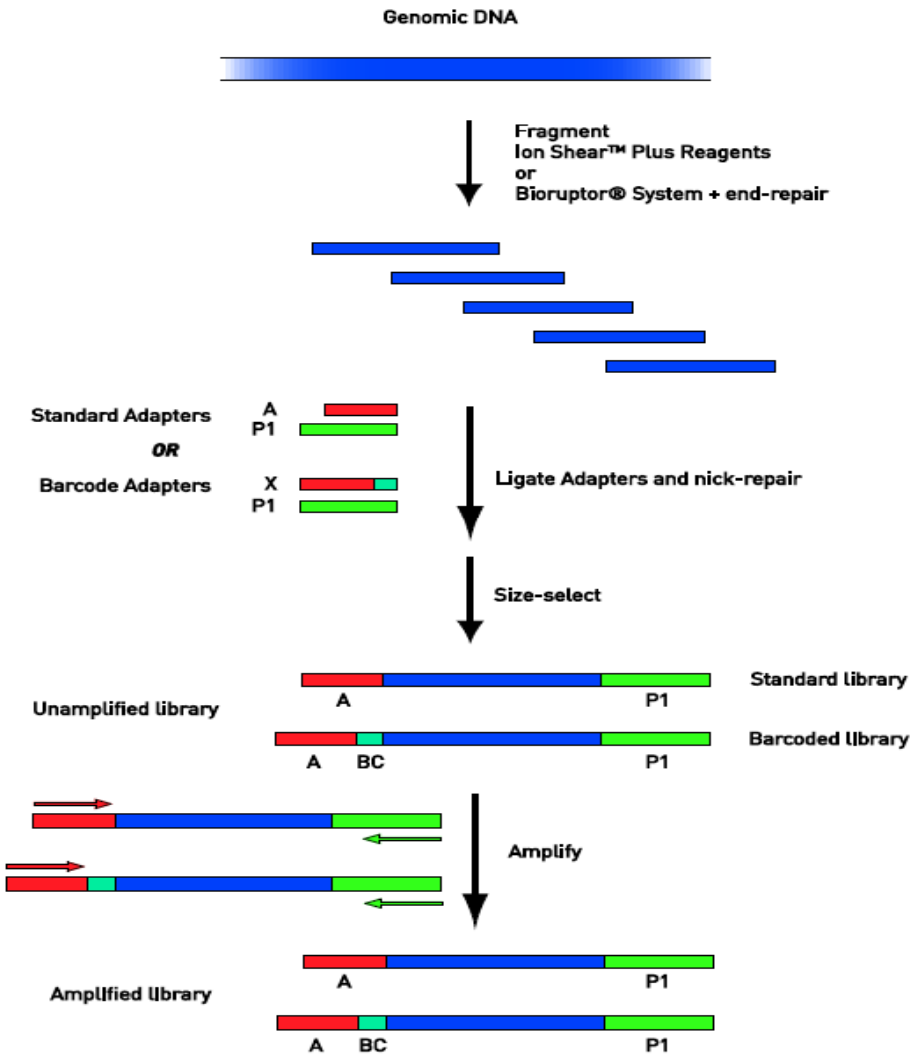
Ion Fragment Library Workflow



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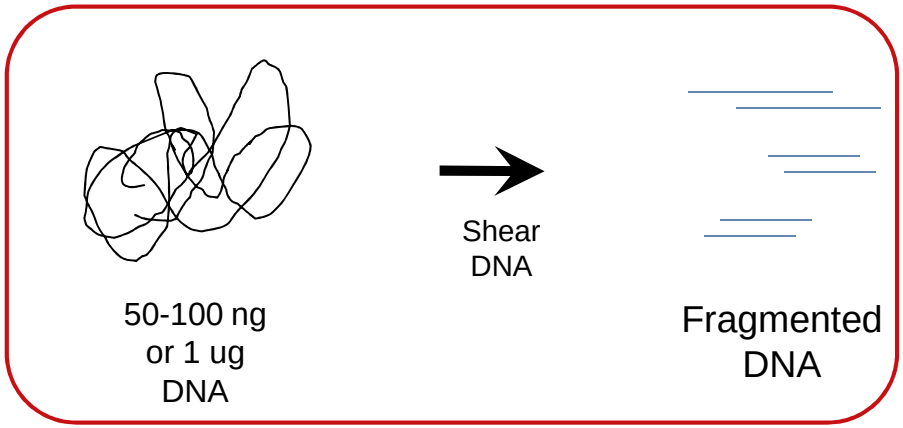
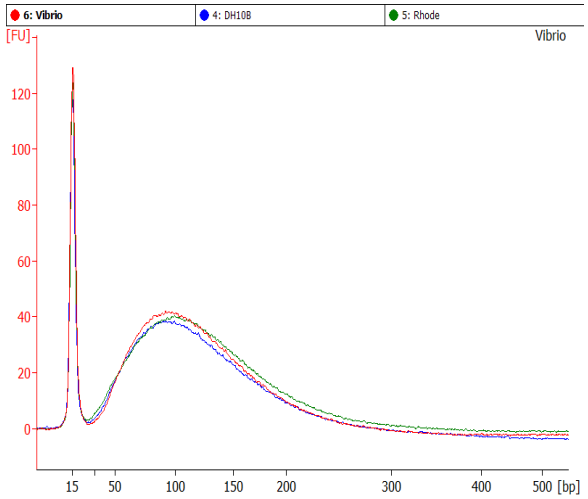
Ion Fragment Library
Fragmentation Options



38 % GC → 65% GC



Two methods for Fragmentation

Enzymatic fragmentation:

- Fragmented DNA is ready for adapter ligation (end-repair not needed).

Ultrasonic fragmentation:

- Fragmented DNA is end-repaired and purified to prepare for ligation to Ion adapters.

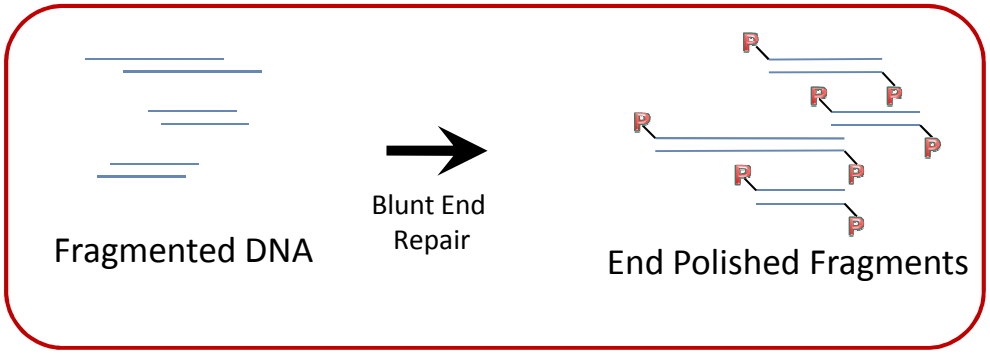
- Different sequencing read lengths require different sizes of library inserts

- Shorter library insert size require more (enzymatic) incubation times

- Check fragment size after shearing, using Bioanalyzer® (optimal sensitivity) or E-Gel® system

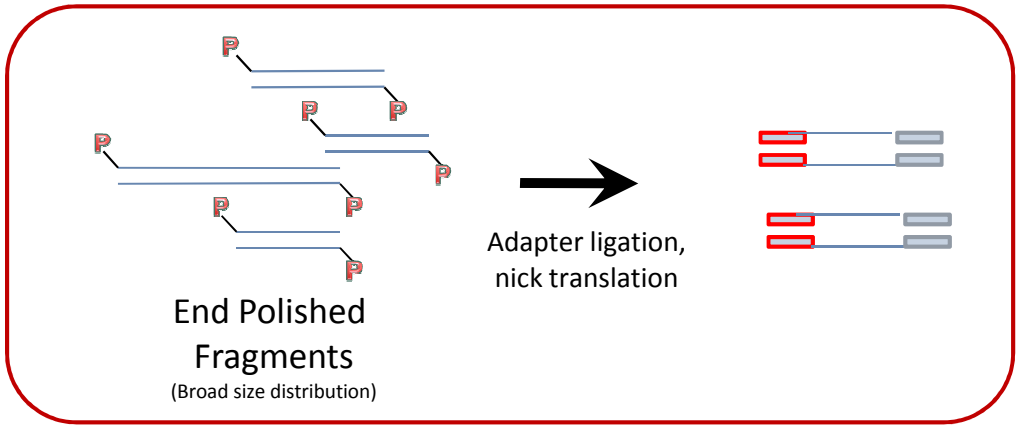
Ion Fragment Library

End Repair
(physical shearing only)



Enzymatic shearing creates blunt ends, so end repair of DNA sheared enzymatically is *not* needed.

Adapter Ligation & Nick Translation



Don't freeze-thaw the 10x ligase buffer

Prepare single-use aliquots of 10x ligase buffer

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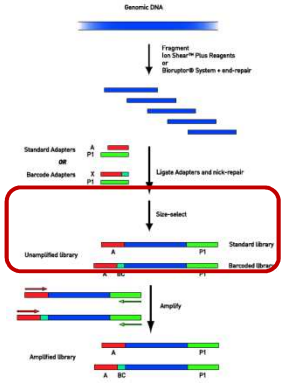
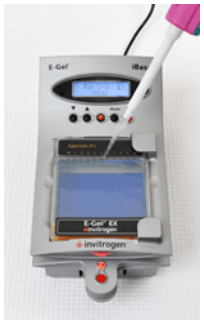
Ion Fragment Library: Size Selection Options





Size-selection options		20–90 min
Method	E-Gel® SizeSelect™ 2% Agarose	Pippin Prep™ instrument
Features	Quicker; Broader size distribution	Automated; Tighter size distribution results in more consistent library size
Time	400-base-read libraries: ~35 min 300-base-read libraries: ~30 min 100–200-base-read libraries: ~20 min	90 min

Alternative method: Manual gel size selection method is available:

Ion Torrent Community → Products → PGM → User Guides and Bulletins → Documents

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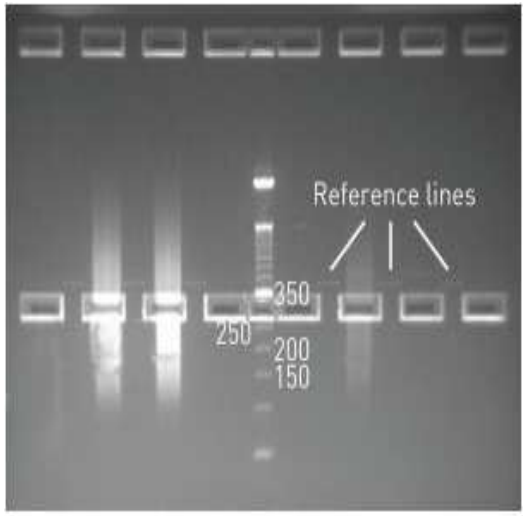
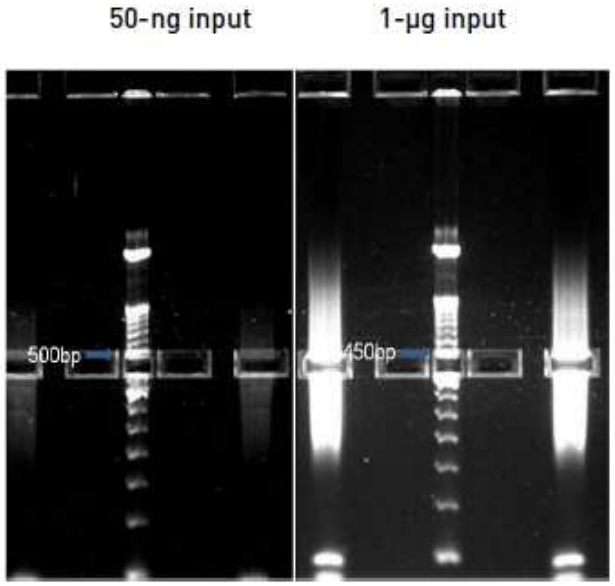
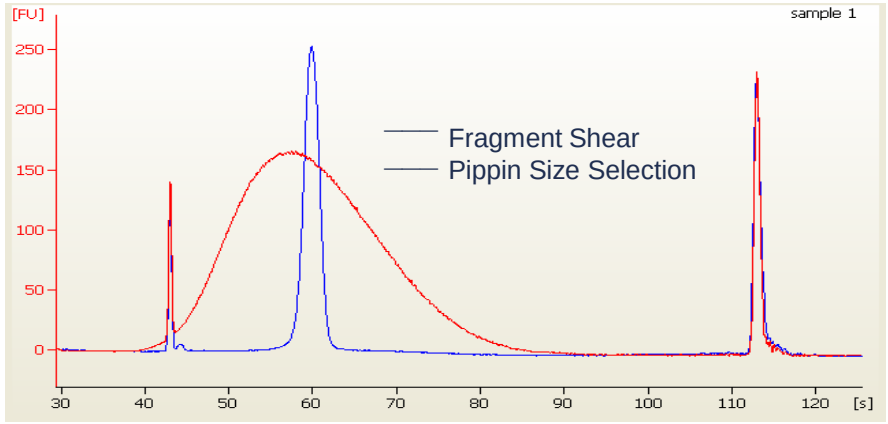
Ion Fragment Library
Size Selection: E-Gel® SizeSelect™ Gels



200-base-read library gel



400-base-read library gel



Size selection is necessary for maximization of sequencing yield

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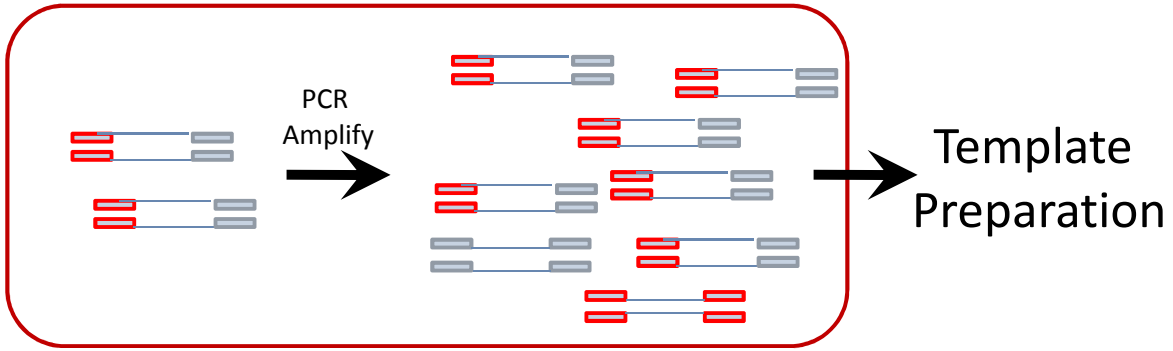
Ion Fragment Library Amplification (if needed)

Advantage

- Can generate sufficient library amount from limited initial input
- Helps increase efficiency of library: higher representation of productive fragments (with both A & P1 adaptors)

Disadvantage

- May introduce bias (control by limiting cycle #)
- Much higher cycle numbers can result in concatemers, etc.



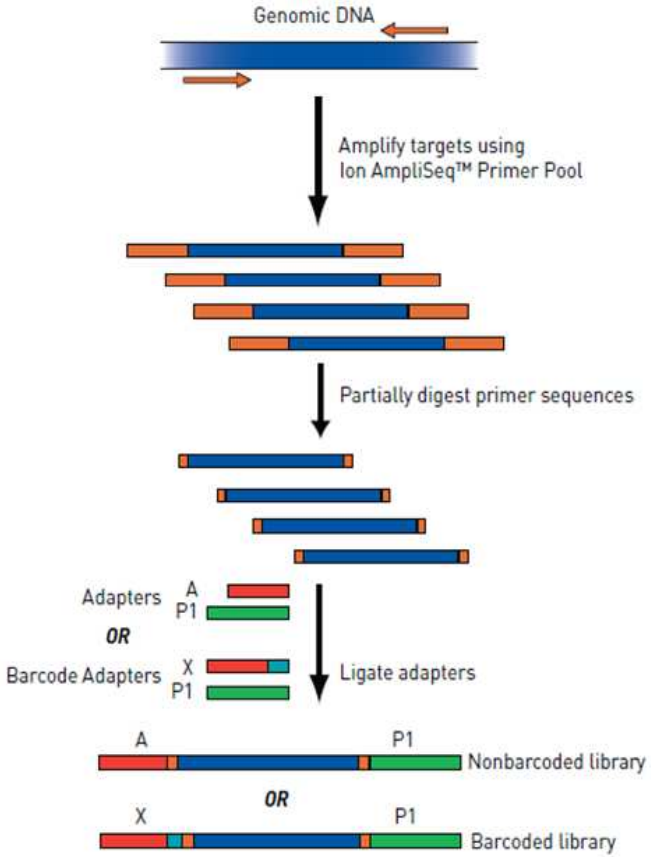
How many cycles should I use to amplify my library?

Number of cycles by library input		
DNA	50-100 ng	1 ug
Cycles	8	5

Amplification conditions can vary by application, see appropriate User Guides or User Bulletins.

Ion AmpliSeq™ DNA and Amplicon Library Prep

extra option: Ion Library Equalizer™ Kit



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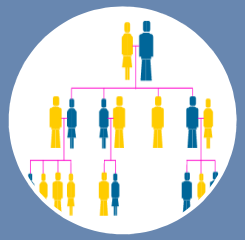
Ion AmpliSeq™ DNA Target Selection Solutions

Ion AmpliSeq™ Cancer Hotspot Panel v2



Ion AmpliSeq™ Inherited Disease Panel

Ion AmpliSeq™ Comprehensive Cancer Panel

Ready-to-Use Panels

Ion AmpliSeq™ Designer

www.ampliseq.com

NEW! Ion AmpliSeq™ Community Panels:

Designed with leading researchers

Ion AmpliSeq™ Library Kit 2.0

Core reagent kit for library construction

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Ion AmpliSeq™ Cancer Hotspot Panel v2

The **Ion AmpliSeq™ Cancer Hotspot Panel v2** is now expanded to allow translational and clinical researchers to fast-track oncology research by surveying "hotspot" regions of 50 oncogenes and tumor suppressor genes, with wide coverage of the *KRAS*, *BRAF*, and *EGFR* genes.

This panel, with improved primer design, contains 207 primer pairs in a single tube and requires only 10 ng of DNA, enabling researchers to sequence challenging samples like FFPE tissue. The convenient predesigned panel allows researchers to focus on data generation and analysis, not on the labor-intensive primer design and target selection steps. While hybridization-based target selection methods require 7 to 72 hours to complete both target selection and library preparation, Ion AmpliSeq™ technology makes it possible to complete the entire process in just 3.5 hours using simple PCR reactions. Additionally, Ion AmpliSeq™ library construction steps are automated using standard 96-well plate–based protocols—further simplifying the workflow and allowing large projects to be rapidly completed without additional sample transfer steps.

Ion AmpliSeq™ Cancer Hotspot Panel v2		
Targets	Hotspot regions, including ~2,800 COSMIC mutations of 50 oncogenes and tumor suppressor genes, with wide coverage of the *KRAS*, *BRAF*, and *EGFR* genes	
Amplicon length	111–187 bp, average 154 bp	
Primer pool size	207 primer pairs in 1 tube	
Input DNA required	Only 10 ng per DNA sample	
Time-to-results	10 hours (DNA to annotated variants)	
Sample multiplexing (observed performance)	Ion 314™ Chip: 2 samples, ~1,400x average coverage Ion 316™ Chip: 8 samples, ~1,400x average coverage Ion 318™ Chip: 16 samples, ~1,400x average coverage	
	Specification	**Observed performance (Ion 314™ Chip)**
Coverage uniformity*	>95%	>98%
On-target reads†	>90%	>96%
Average depth of coverage	NA	>2,000x
SNP detection sensitivity	NA	98% detection rate for 5% variant frequency at positions with average sequencing coverage from 1,000x to 4,000x

*Coverage uniformity = percentage of bases covered at ≥20% of the mean coverage
†On-target reads = percentage of reads that mapped to target regions out of total mapped reads per run

Simplicity

Only 10 ng of input DNA required, enabling sequencing of FFPE samples

Scalability

Sequence one sample or multiplex barcoded samples using any of the three Ion Chips, producing up to 1 Gb of total sequence

Supports automation for 96-well plate–based protocols, enabling large projects to be rapidly processed

Speed

Single-day workflow with 3.5-hour library construction

Use the Ion AmpliSeq™ Cancer Panel with Ion 314™, Ion 316™, or Ion 318™ Chips

Dataset for this panel is available at lifetechnologies.com/ioncommunity

life technologies™

The Ion AmpliSeq™ Cancer Panel targets 50 genes

ABL1	*EZH2*	*JAK3*	*PTEN*
AKT1	*FBXW7*	*IDH2*	*PTPN11*
ALK	*FGFR1*	*KDR*	*RB1*
APC	*FGFR2*	*KIT*	*RET*
ATM	*FGFR3*	*KRAS*	*SMAD4*
BRAF	*FLT3*	*MET*	*SMARCB1*
CDH1	*GNA11*	*MLH1*	*SMO*
CDKN2A	*GNAS*	*MPL*	*SRC*
CSF1R	*GNAQ*	*NOTCH1*	*STK11*
CTNNB1	*HNF1A*	*NPM1*	*TP53*
EGFR	*HRAS*	*NRAS*	*VHL*
ERBB2	*IDH1*	*PDGFRA*	
ERBB4	*JAK2*	*PIK3CA*	

TaqMan® Mutation Detection Assays are available for the genes listed above.

*Gene list available www.lifetechnologies.com/ampliseqready

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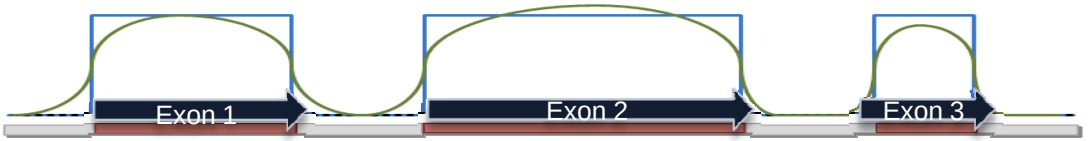
Ion AmpliSeq™ Comprehensive Cancer Panel (CCP)



IDH1	KRAS	MLH1	NFKB2	PIK3C2B	RARA	SOCS1	TOP1
IDH2	LAMP1	MLL	NIN	PIK3CA	RB1	SOX11	TP53
IGF1R	LCK	MLL2	NKX2-1	PIK3CB	RECQL4	SOX2	TPR
IGF2	LIFR	MLL3	NLRP1	PIK3CD	REL	SRC	TRIM24
IGF2R	LPHN3	MLLT10	NOTCH1	PIK3CG	RET	SSX1	TRIM33
IKBKB	POT1	MMP2	NOTCH2	PIK3R1	RHOH	STK11	TRIP11
IKBKE	LPP	MN1	NOTCH4	PIK3R2	RNASEL	STK36	TRRAP
IKZF1							
IL2	ABL1	AURKA	BMPR1A	CDK4	CTNNB1	EPHB4	FANCD2	FZR1
IL21R	ABL2	AURKB	BRAF	CDK6	CYLD	EPHB6	FANCF	G6PD
IL6ST	ACVR2A	AURKC	BRD3	CDK8	CYP2C19	ERBB2	FANCG	GATA1
IL7R	ADAMTS20	AXL	BRIP1	CDKN2A	CYP2D6	ERBB3	FAS	GATA2
ING4	AFF1	BAI3	BTK	CDKN2B	DAXX	ERBB4	FBXW7	GATA3
IRF4	AFF3	BAP1	BUB1B	CDKN2C	DCC	ERCC1	FGFR1	GDNF
IRS2	AKAP9	BCL10	CARD11	CEBPA	DDB2	ERCC2	FGFR2	GNA11
ITGA10	AKT1	BCL11A	CASC5	CHEK1	DDIT3	ERCC3	FGFR3	GNAQ
ITGA9	AKT2	BCL11B	CBL	CHEK2	DDR2	ERCC4	FGFR4	GNAS
ITGB2	AKT3	BCL2	CCND1	CIC	DEK	ERCC5	FH	GPR124
ITGB3	ALK	BCL2L1	CCND2	CKS1B	DICER1	ERG	FLCN	GRM8
JAK1	APC	BCL2L2	CCNE1	CMPK1	DNMT3A	ESR1	FLI1	GUCY1A2
JAK2	AR	BCL3	CD79A	COL1A1	DPYD	ETS1	FLT1	HCAR1
JAK3	ARID1A	BCL6	CD79B	CRBN	DST	ETV1	FLT3	HIF1A
JUN	ARID2	BCL9	CDC73	CREB1	EGFR	ETV4	FLT4	HLF
KAT6A	ARNT	BCR	CDH1	CREBBP	EML4	EXT1	FN1	HNF1A
KAT6B	ASXL1	BIRC2	CDH11	CRKL	EP300	EXT2	FOXL2	HOOK3
KDM5C	ATF1	BIRC3	CDH2	CRTC1	EP400	EZH2	FOXO1	HRAS
KDM6A	ATM	BIRC5	CDH20	CSF1R	EPHA3	FAM123B	FOXO3	HSP90AA1
KDR	ATR	BLM	CDH5	CSMD3	EPHA7	FANCA	FOXP1	HSP90AB1
KEAP1	ATRX	BLNK	CDK12	CTNNA1	EPHB1	FANCC	FOXP4	ICK
KIT							
KLF6							

- Targets coding exons in 409 human oncogenes and tumor suppressor genes
- ~16,000 amplicons
- Detection of known COSMIC somatic mutations

*Gene list available www.lifetechnologies.com/ampliseqready

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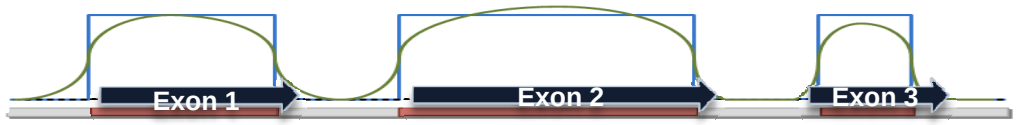
Ion AmpliSeq™ Inherited Disease Panel (IDP)



Category	Genes	Diseases
Developmental	75	193
Heart disease	62	159
Metabolic	28	46
Neuromuscular	87	245
Other	73	210
Total	325	853

- >700 disease states based on OMIM database
- 325 genes
- ~10,500 amplicons

* Disease groups and gene list available at ampliseq.com

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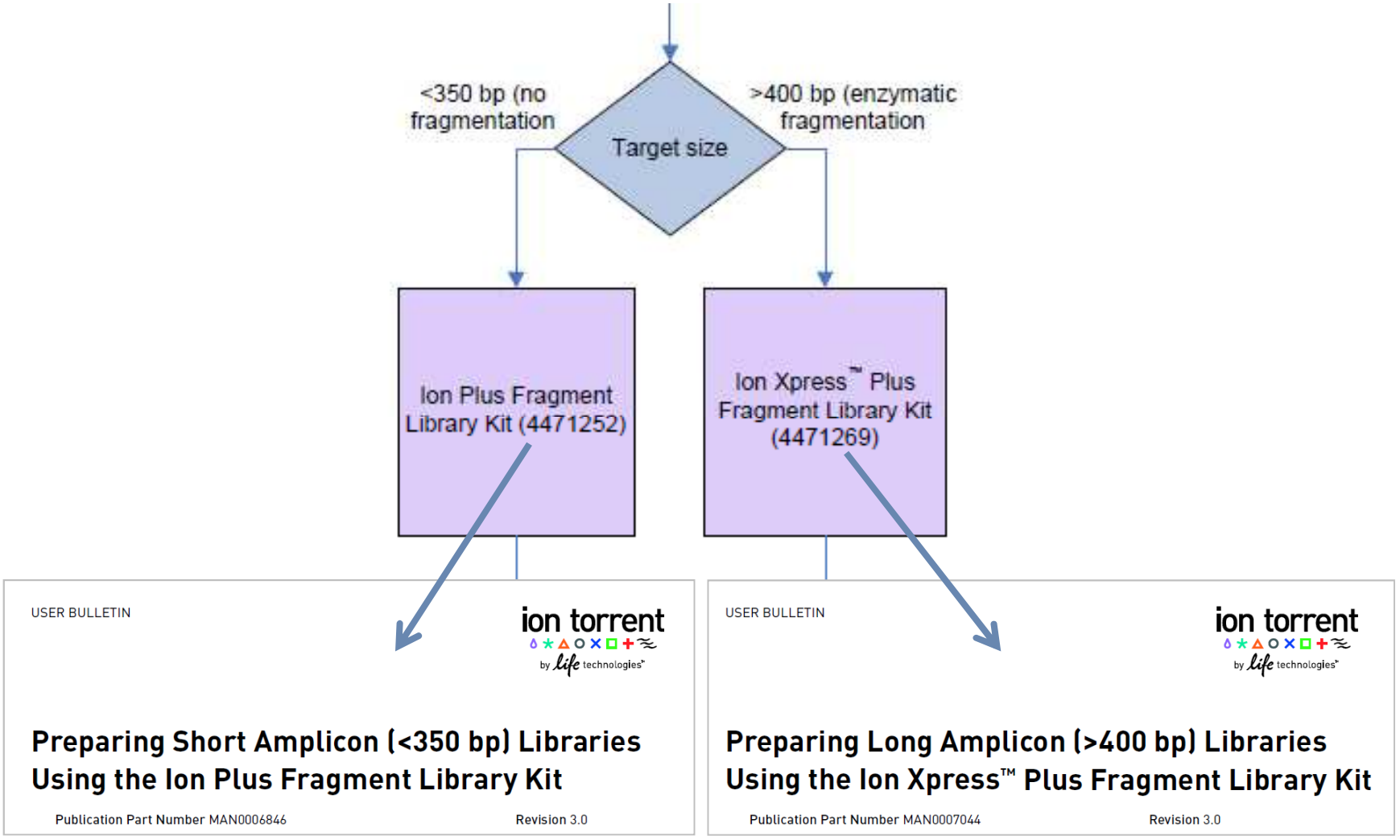
DNA Amplicon Libraries: “Standard” DNA Amplicons



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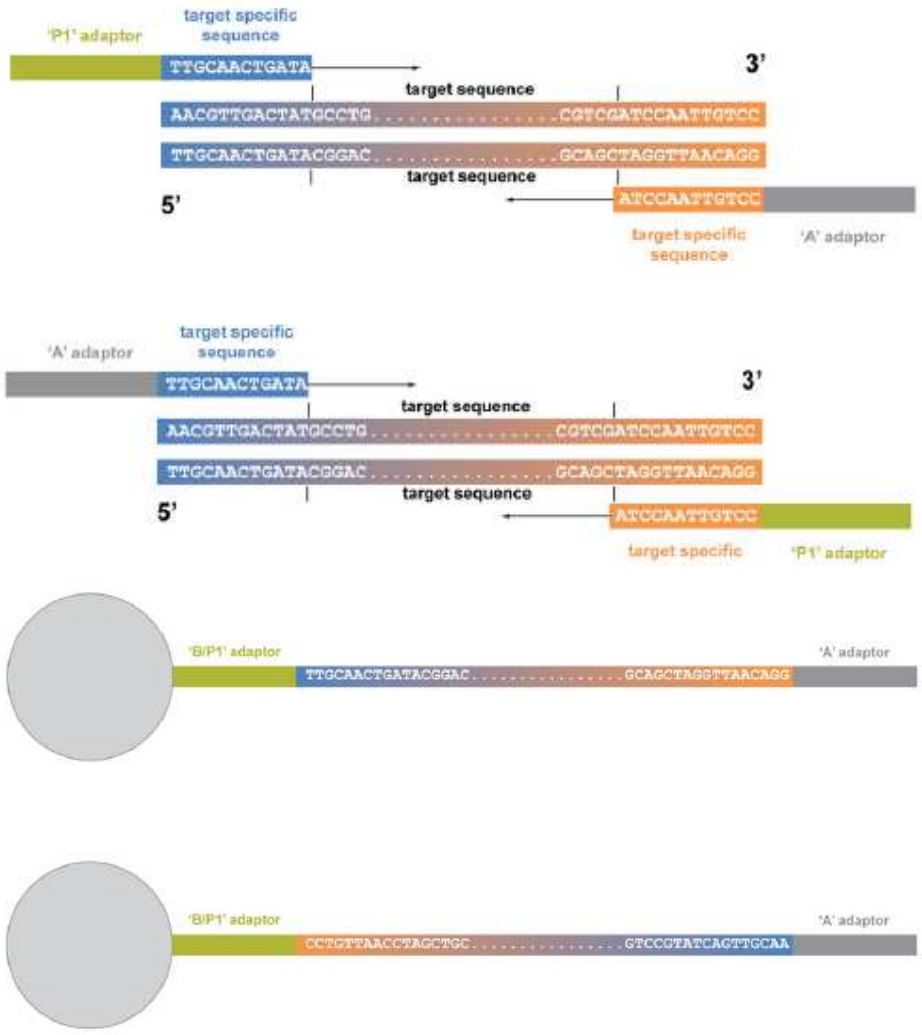
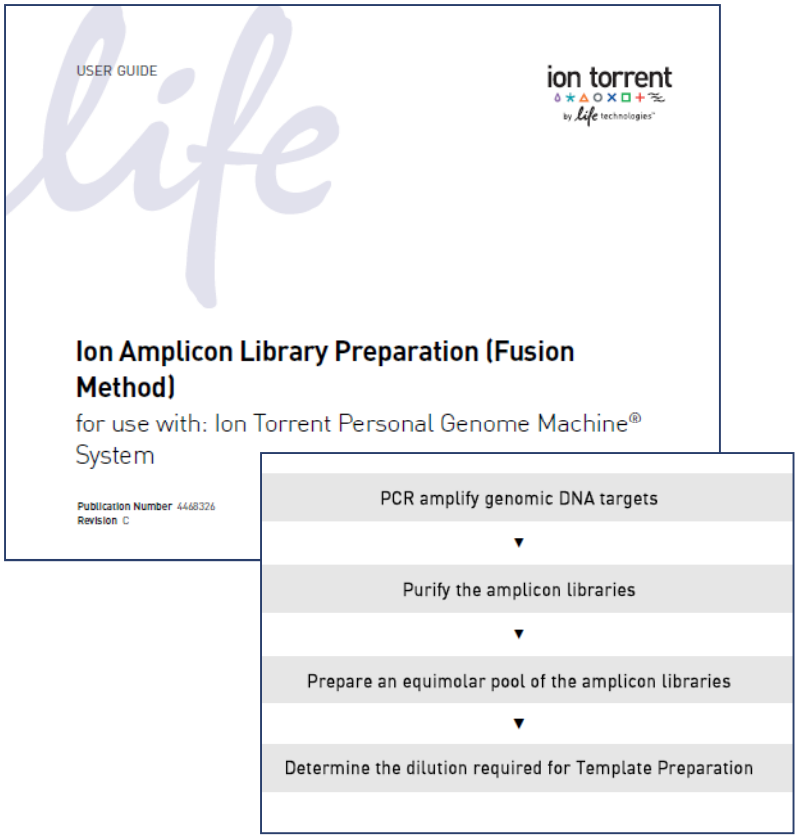
DNA Amplicon Library: Fusion Method





NOTE: No Ion Library Kit is needed for Fusion Method.

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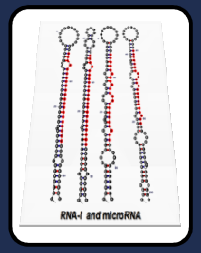
Ion AmpliSeq™ RNA Panels

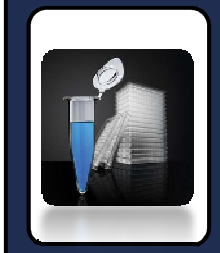


RNA Cancer Panel

- 50 genes
- Corresponds to genes in Ion AmpliSeq™ Cancer Hotspot v2
- 5 ng FFPE or 500 pg unfixed RNA

RNA Apoptosis Panel

- 267 genes
- TaqMan® Assay validated
- 5 ng FFPE or 500 pg unfixed RNA



RNA Custom Panels

- Over 20,000 human genes with thorough coverage of the coding genome
- Single amplicon per gene
- 1 pool design
- 5 ng FFPE or 500 pg unfixed RNA

ambion by life technologies

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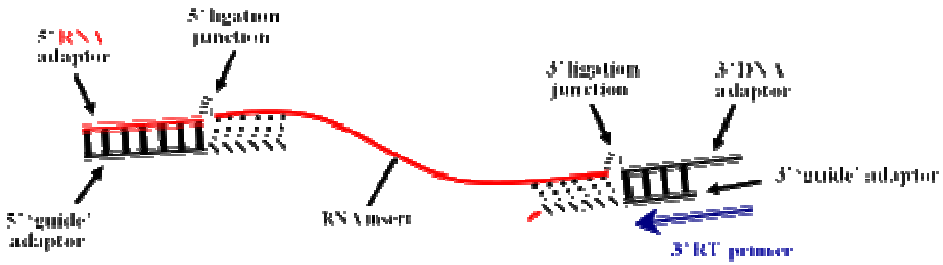
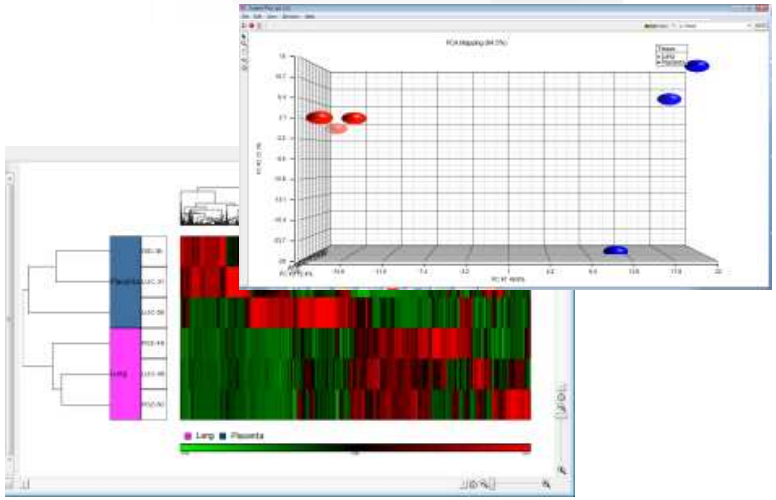
Ion Total RNA-Seq Kit v2

- Create whole transcriptome (WT) or small RNA libraries
- Preserves strand information
- Adaptor and RT primer sequences for PGM™ sequencing, for inputs of 100 ng total RNA or 1 ng miRNA
- Optimized Workflow – 5-6 hrs for small RNA and for WT libraries

Hands-on Lab Course
Also available!





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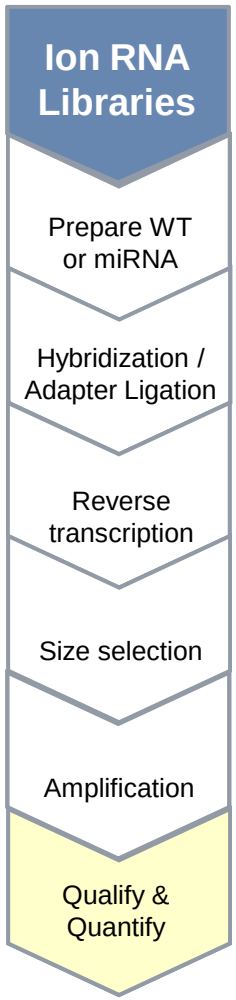
Ion RNA Library Prep



~5-6 hrs

Preparation of whole transcriptome RNA

poly (A) RNA or rRNA-depleted total RNA

↓

Fragment the RNA

↓

Clean up the RNA

Preparation of small RNA

Obtain total RNA, then determine the quality

↓

Purify/enrich small RNA, if necessary

↓

Quantify small RNA sample and determine input amount

↓

Amplified library construction

Hybridize and ligate RNA adapters

↓

Perform reverse transcription

↓

Purify cDNA

↓

Amplify cDNA

↓

Purify amplified DNA

↓

Assess yield and size distribution of amplified DNA

↓

Preparation of templated Ion Sphere™ Particles and run on Ion Sequencer

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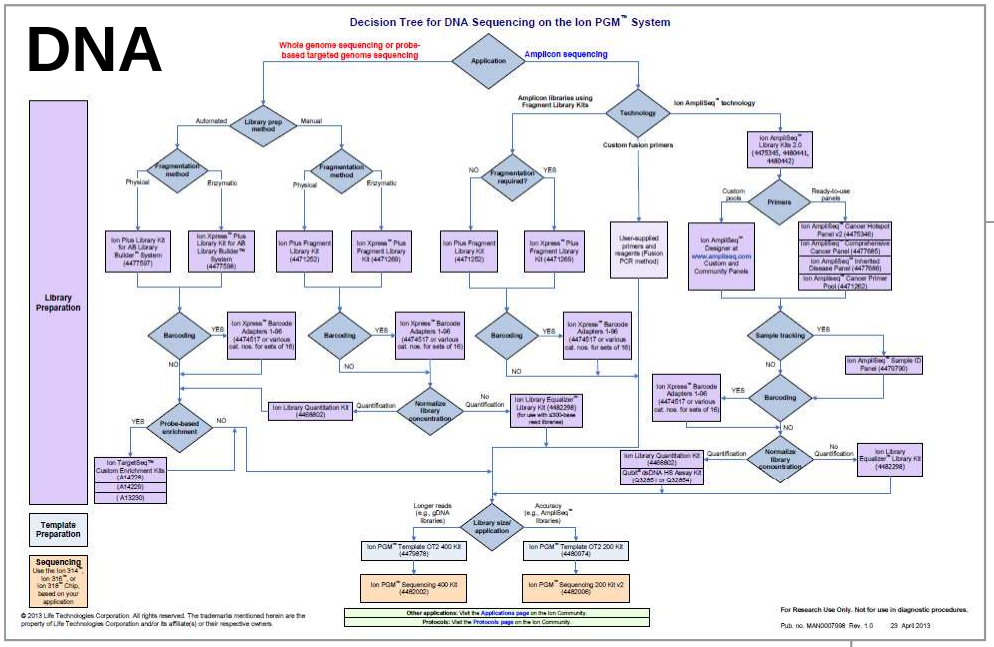
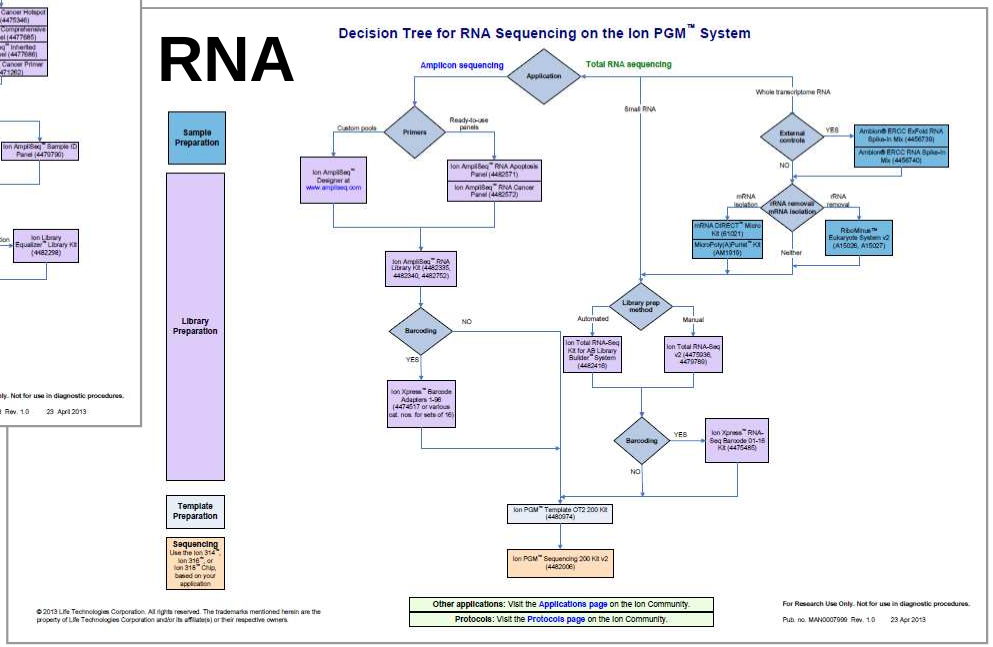
Ion Kit Guides: Decision Trees

Check for updates!

DNA



RNA



Select library preparation method & match kit

Ion Torrent Community → Protocols → PGM Protocols → Documents

http://ioncommunity.lifetechnologies.com/docs/DOC-1804

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Template Dilution Factor

How much library is needed for template preparation?

PGM™ System - Template Dilution Factor

- Specific range of library input is critical for optimal results
- This ratio needs to be optimized per library
  - Range listed below is a good starting point, but ideal point may fall outside this range
  - Begin with 1x TDF, and then scale up or down depending on results of templating reaction (Qubit® fluorometer values of pre-enrichment samples and/or sequencing data)

Note**: Consult ***specific library prep protocol for optimal range of DNA input per library type

Determine the dilution factor that results in a concentration of ~26 pM. This concentration is suitable for downstream template preparation.

Use the following formula:

Dilution factor = (Library concentration in pM)/26 pM

Example:

The library concentration is 10,000 pM.

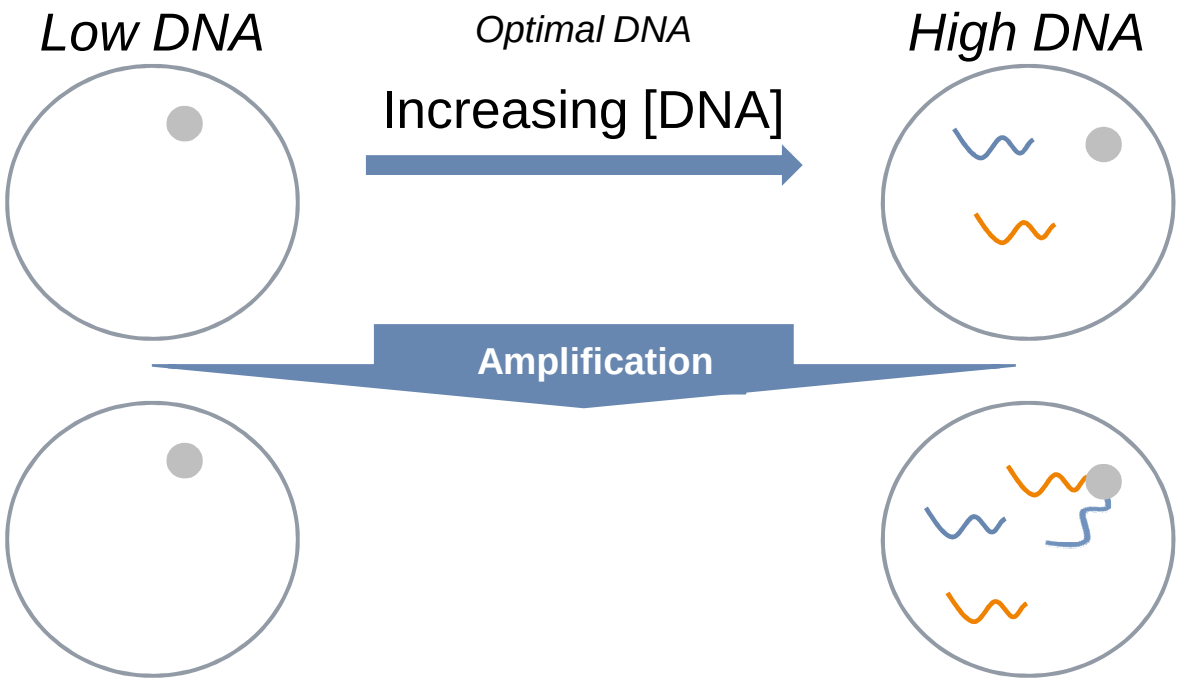
Dilution factor = 10,000 pM/26 pM = 385

Thus, 1 µL of library mixed with 385 µL of Low TE (1:385 dilution) yields approximately 26 pM. Use this library dilution for template preparation.

- Accurate qualification & quantitation of library DNA is critical (qPCR, Bioanalyzer® analysis)

PGM™ System - Template Dilution Factor

Optimizing the library input concentration



No read

Too little library input can result in insufficient positive, or "live", spheres for sequencing

"Mixed" read

Too much library input to template prep can result in too many "mixed" reads

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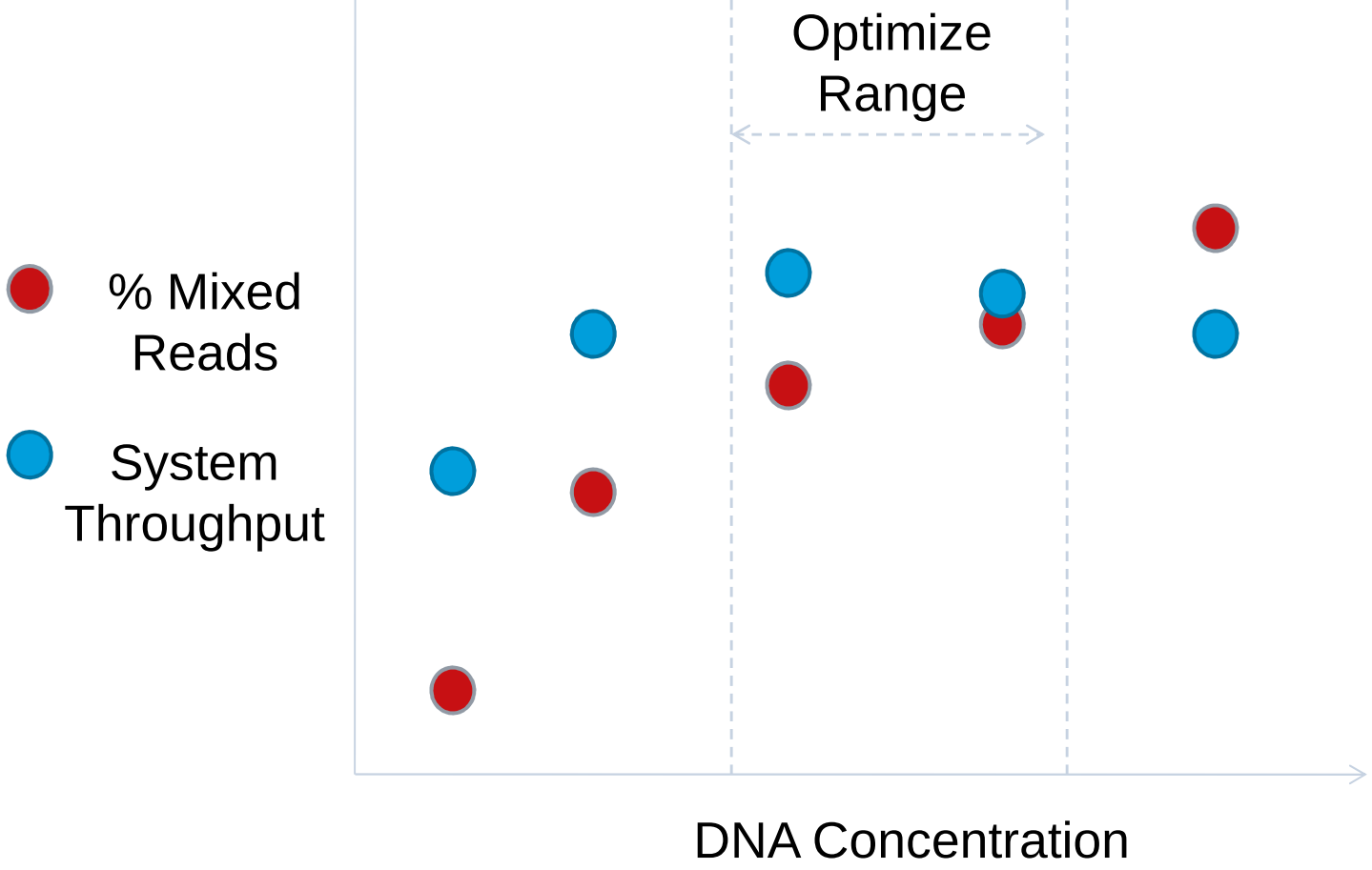
PGM™ System - Template Dilution Factor



Optimal DNA input will maximize system throughput

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PGM™ System - Template Dilution Factor

What is percent of ISPs that are templated?

Amplification onto ISPs

Enrichment of templated ISPs

Use Qubit® fluorometer

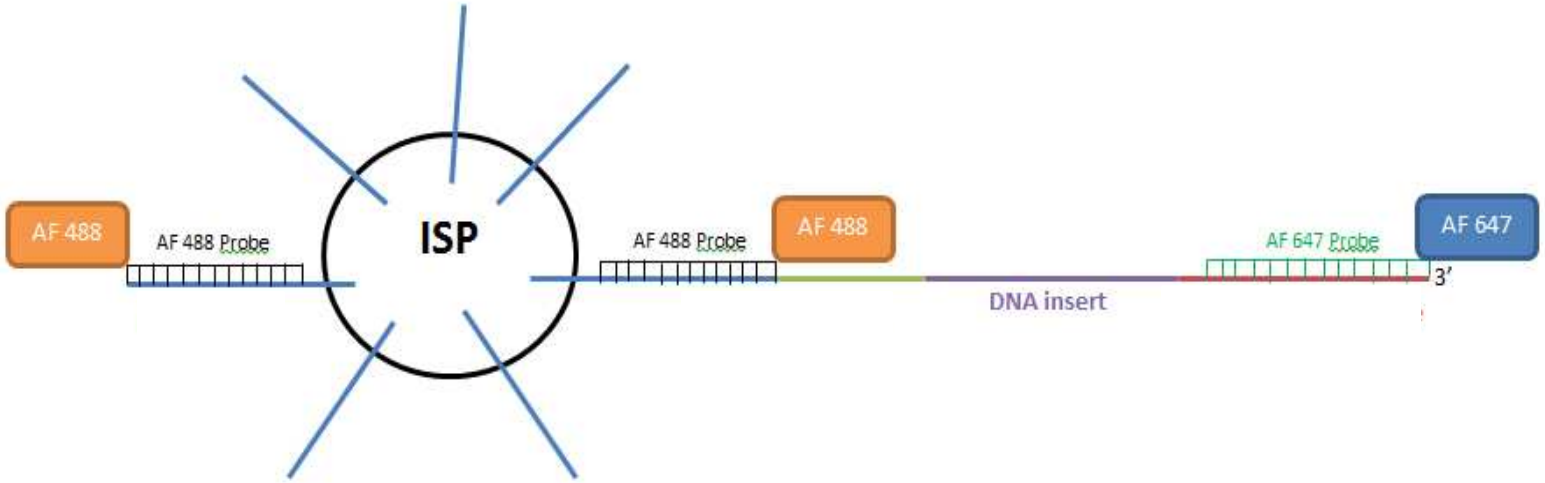
ISP Quality Assessment

Qubit® 2.0 Fluorometer

- Protocol and Excel® program calculator available on the Ion Community
- Calculate template-positive ISPs by measuring fluorescent ratio:

Value = 10% to 30% templated ISPs

(proper TDF /correct amount of input library DNA)



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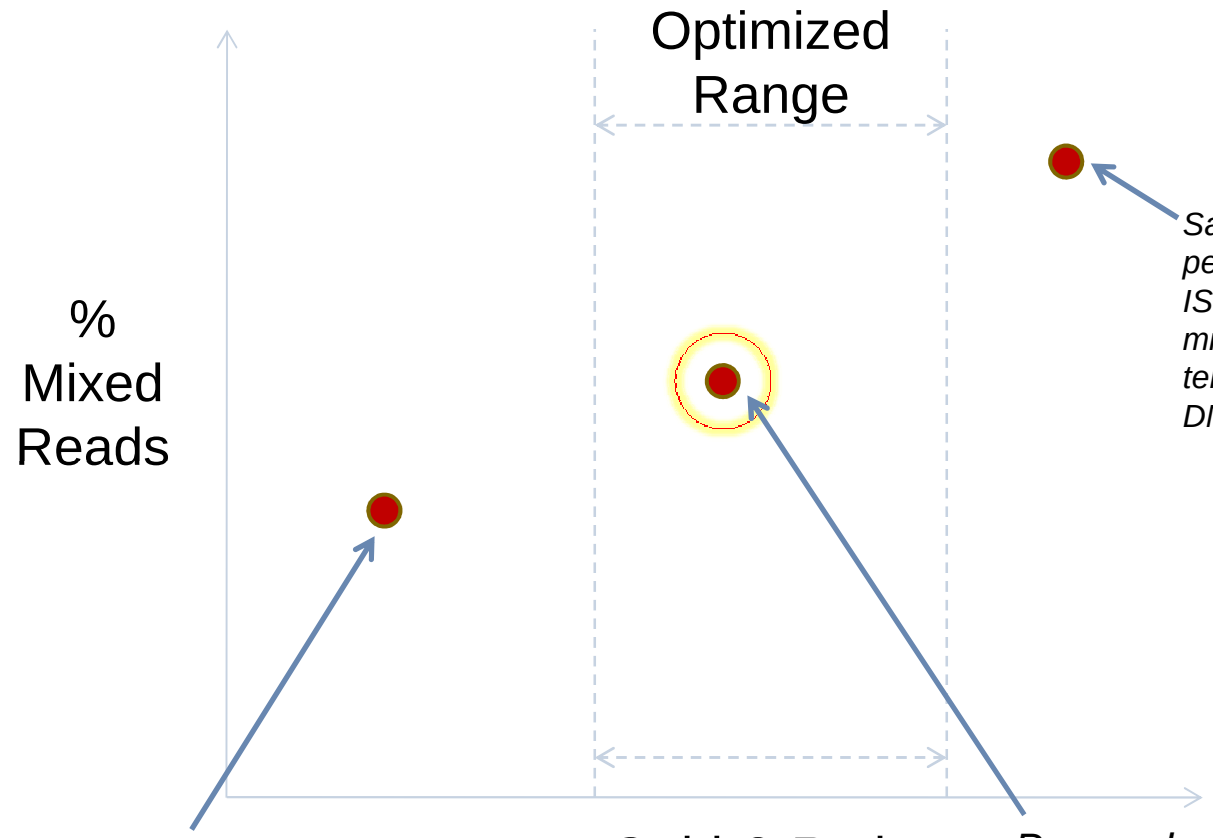
PGM™ System - Template Dilution Factor



Sample has higher than optimal percentage of template-positive ISPs. Proceed with knowledge that mixed reads will be higher -or- redo template prep reaction with less input DNA.

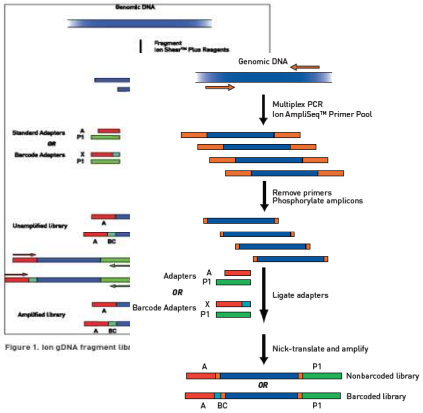
Sample has lower than optimal percentage of ISPs. Proceed with knowledge that ISP recovery will likely be lower -or- redo template prep reaction with more input DNA.

*Proceed with sample if it is **within** optimal range of Qubit® fluorometer values.*
Select this TDF!!!

NEW! The Ion Library Equalizer™ Kit

"Simple library normalization without quantitation or dilutions"

Library | **Template** | **Sequence** | **Analysis**



Ion AmpliSeq™ Libraries
or
Ion gDNA Fragment Libraries

Ion Library Equalizer™ Kit

Ion OneTouch™ 2 System

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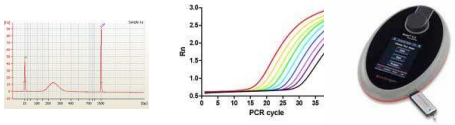
Library Quantification & Normalization

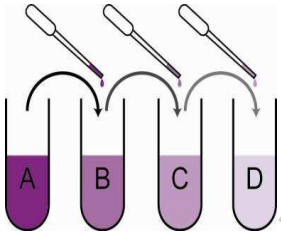
Current workflow

DNA quantification & calculation

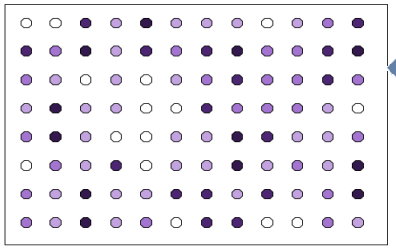
Library dilution



Bioanalyzer® or qPCR or Qubit®

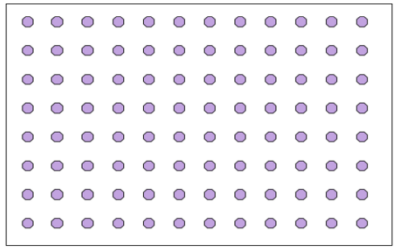


Libraries with varying DNA concentration



Ion Library Equalizer™ Kit

Libraries with normalized DNA concentration



NEW! *alternate workflow*

Bead-Based Kit

1) Amplify 2) Capture & 3) Elute

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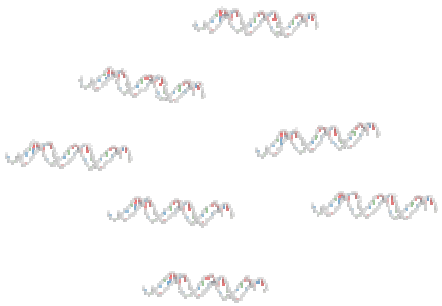
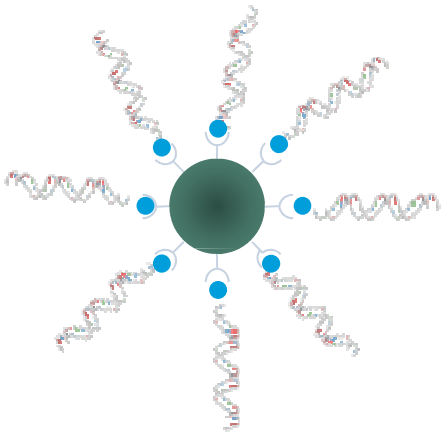
Ion Library Equalizer™ Kit: Simple Workflow

1) Amplify



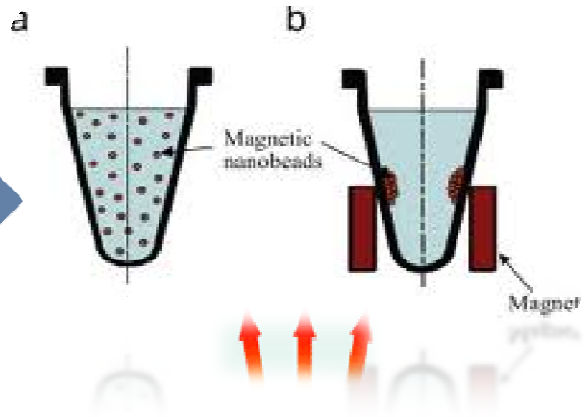
Amplify DNA Libraries with Equalizer™ Primers
(replaces existing amplification step)

2) Capture



Capture libraries on Equalizer™ Beads

3) Elute



Heat to elute libraries and separate beads

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