

# Product Guide -DNA Library Construction

When we use the NGS technology to sequence the sample, mainly there are several steps such as nucleic acid extraction, library construction, sequencing, data analysis and result verification. Among them, the construction of DNA library is the foundation of the NGS technology. The essence of DNA library construction is the process of converting large-fragment genomic DNA of the sample to be measured into small-fragment DNA, and then adding sequencing adapters at both ends of small-fragment DNA molecules to form effective library molecules that can be sequenced on the machine. The specific construction process of the NGS small fragment DNA library comprises five steps: DNA fragmentation, end repair, A-tailing, ligation and PCR amplification. (The following figure shows the DNA fast library construction process applicable to Illumina platform)

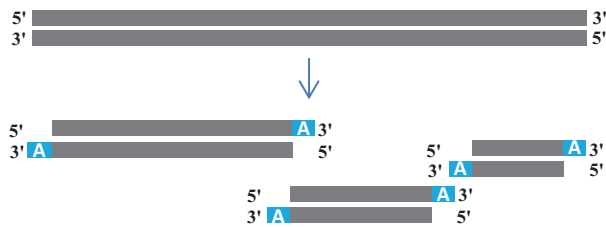
## Fast Library Construction (Applicable to Illumina platform)



(Total time required: 2.5 Hours)		
TIANSeq Fragmentation/End repair/dA-tailing Module (4992350/4992351)	· TIANSeq Fast Ligation Module(4992354/4992355)	· TIANSeq NGS Library Amplification Module (4992373/4992374) · TIANSeq HiFi Amplification Mix (4992371/4992372)

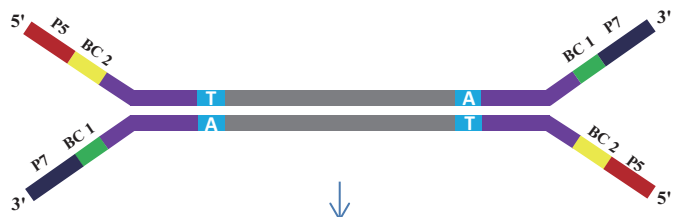
### Step1 TIANSeq Fragmentation/End repair /dA-tailing

- TIANSeq Fragmentation/End repair/dA-tailing Module (4992350/4992351)  
Fragmentation, end repair and A-tailing of gDNA and large fragments of DNA can be completed in one step.
- DNA is fragmented randomly and does not have sequence preference.



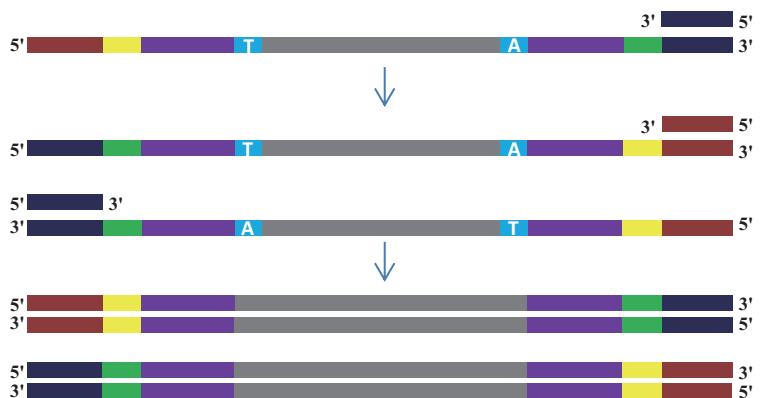
### Step2 Adapter ligation

- TIANSeq Fast Ligation Module (4992354 /4992355) can ligate sequencing adapters to the DNA fragment with dA-tailing at the 3' end.
- This module adopts a one-step reaction process, where fragmented DNA can be directly ligated with adapter without magnetic bead purification.



### Step3 PCR Amplification

- TIANSeq NGS Library Amplification Module (4992373/4992374) contains P5/P7 primer, which is suitable for amplification of library DNA fragments containing P5 and P7 sequences on both sides.
- TIANSeq HiFi Amplification Mix (4992371/4992372) is a new high fidelity PCR amplification premix, which is suitable for PCR amplification of high-throughput sequencing library.



## Product guide of DNA library construction

DNA Library construction	Workflow	Kit		DIY construction scheme		Related materials	
	Fragmentation	TIANSeq DirectFast Library Kit (Illumina) (4992259/4992260)	/	TIANSeq	4992356/4992357		TIANSeq Size Selection DNA Beads (4992358/4992359)
	End repair		TIANSeq	Fragment/Repair/Tailing Module (4992350/4992351)	TIANSeq End repair/dA-Tailing Module (4992352/4992353)	TIANSeq T4 DNA Polymerase TIANSeq Klenow Fragment TIANSeq T4 PNK	
	dA-tailing		TIANSeq Fast DNA Library Kit (Illumina) (4992261/4992262)	TIANSeq Fast Ligation module (4992354/4992355)	TIANSeq Klenow (3'-5' exo-)	TIANSeq Single-Index Adapter (Illumina) (4992641/4992642/4992378)	
	Adapter ligation		TIANSeq NGS Library Amplification Module (4992373/4992374)	TIANSeq T4 DNA Ligase (Rapid)	TIANSeq HiFi Amplification Mix (4992371/4992372)		
	Amplification						

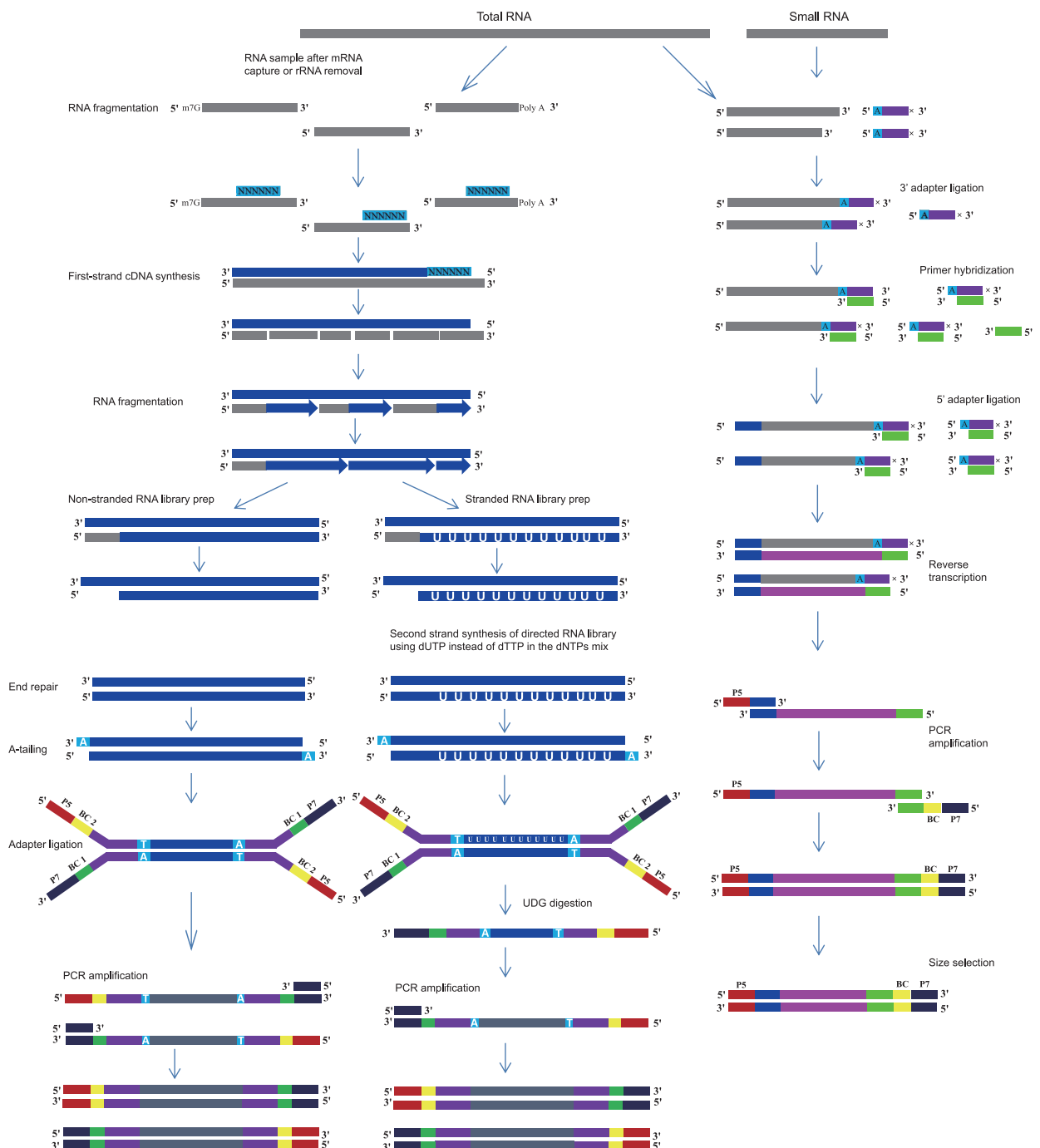
## Product information (Kit and Module)

Features	Product name	Cat. no.	Specification
Fragmentation included	TIANSeq DirectFast Library Kit (Illumina)	4992259	24 rxn
		4992260	96 rxn
Fast library prep (fragmentation-free)	TIANSeq Fast DNA Library Kit (Illumina)	4992261	24 rxn
		4992262	96 rxn

Features	Product name	Cat. no.	Specification
Fragmentation/end repair/dA-tailing	TIANSeq Fragment/Repair/Tailing Module	4992350	24 rxn
		4992351	96 rxn
End repair/dA-tailing	TIANSeq End repair/dA-Tailing Module	4992352	24 rxn
		4992353	96 rxn
Ligation	TIANSeq Fast Ligation module	4992354	24 rxn
		4992355	96 rxn
Amplification	TIANSeq NGS Library Amplification Module	4992373	24 rxn
		4992374	96 rxn
Fragmentation	TIANSeq DNA Fragmentation Module	4992356	24 rxn
		4992357	96 rxn
Quantification	TIANSeq DNA Quantification Kit (Illumina)	4992323	20 µl×500 rxn
DNA size selection & purification	TIANSeq Size Selection DNA Beads	4992358	15 ml
		4992359	60 ml
		4992979	300 ml

## Product Guide -RNA Library Construction

The construction of the next generation sequencing RNA library is mainly divided into mRNA library, lncRNA library and small RNA library according to the types of RNA. The construction process of mRNA and lncRNA library includes RNA enrichment, RNA fragmentation, synthesis of first and second cDNA strands, end repair, A-tailing and PCR amplification. RNA enrichment is a necessary step before the library construction of mRNA and lncRNA for the reason that rRNA accounts for more than 80% of the total RNA, and without rRNA removal, the vast majority of sequencing data of mRNA and lncRNA libraries are rRNA-related data, which is not conducive to a comprehensive and in-depth analysis of mRNA and lncRNA expression differences and sequence changes. The construction process of small RNA library is different from that of mRNA and lncRNA library, which mainly includes 3' adapter ligation, 5' adapter ligation, cDNA synthesis and PCR amplification. The RNA operation flow based on Illumina platform is showed in the following figure:



## Product Selection Guide for RNA Library Construction

mRNA library construction Inc RNA library construction	TIANSeq rRNA Depletion Kit (H/M/R) (4992363/4992364/4992391) Customized rRNA depletion kit by magnetic beads method					
	Step	Kit		DIY construction scheme	Construction materials	
	RNA fragmentation	TIANSeq Fast RNA Library Kit (Illumina) (4992375/4992376)	TIANSeq Stranded RNA-Seq Kit (Illumina) 4993007/4993008	TIANSeq M MLV (RNase H-) TIANSeq RNase Inhibitor	TIANSeq RNA Clean Beads (4992360/4992362/4992867) TIANSeq Size Selection DNA Beads (4992358/4992359)	
	cDNA first strand synthesis					
	cDNA second strand synthesis			TIANSeq RNA Frag/cDNA Synthesis Module (4993005/4993006)		TIANSeq DNA Polymerase I TIANSeq RNase H Note: dNTP containing dUTP is required for directional RNA library construction
	End repair			TIANSeq End Repair/dA-Tailing Module (4992352/4992353)		TIANSeq Klenow (3' 5' exo-)
	A-tailing					TIANSeq T4 DNA Ligase (Rapid)
	Adapter ligation			TIANSeq Fast Ligation Module (4992354/4992355)		TIANSeq HiFi Amplification Mix (dye-)(4992369/4992370/4992371/4992372)
PCR amplification	TIANSeq NGS Library Amplification Module (4992373/4992374)					

Small RNA Library construction	Step	DIY construction scheme
	3' adapter ligation	TIANSeq T4 RNA Ligase 2, Truncated
	5' adapter ligation	TIANSeq T4 RNA Ligase 1
	cDNA synthesis	TIANSeq M MLV (RNase H-) TIANSeq RNase H TIANSeq RNase Inhibitor
	PCR amplification	TIANSeq HiFi Amplification Mix / TIANSeq NGS Library Amplification Module

Features	Product name	Cat.no.	Specification
rRNA depletion	TIANSeq rRNA Depletion Kit (H/M/R)	4992363	6 rxn
		4992364	24 rxn
		4992391	96 rxn
RNA library construction	TIANSeq Fast RNA Library Prep Kit (Illumina)	4992375	24 rxn
		4992376	96 rxn
	TIANSeq Stranded RNA-Seq Kit (Illumina)	4993007	24 rxn
		4993008	96 rxn
Nucleic acid purification	TIANSeq RNA Clean Beads	4992360	5 ml
		4992362	15 ml
		4992867	60 ml
Double-strand cDNA synthesis	TIANSeq RNA Frag/cDNA Synthesis Module	4993005	24 rxn
		4993006	96 rxn