For all you seq...

RNA

RNA Transcription

CaptureSeq — RNA capture sequencing (CaptureSeq) — Design capture probes — Hybridize capture probes — Library enriched with targeted RNAs

RASL-seq — RNA-mediated oligonucleotide annealing, selection, and ligation sequencing (RASL-seq) — Poly A+ RNA — Add targeted primers — Ligate — Selection — Elute fragment — Add bar-coded P7 primer — Indexed cDNA

ClickSeq — Generate RNAseq libraries from stochastically terminated 3′-azido-blocked cDNA fragments (ClickSeq) — RNA — Add semi-random primers and AzNTPs - dNTP mixture — Purify single-stranded cDNA — Click-ligate alkyne-modified adaptor — PCR amplify — cDNA library

3Seq
3-Seq
3′-end-seq
PAS-Seq
SS3-Seq
3′T-fill — 3′-end sequencing for expression quantification (3SEQ) from FFPE samples. Poly(A) site sequencing (PAS-Seq). 3′-end regions of transcripts (3′-end-seq). Strand-specific 3′-end-RNA-seq (SS3-Seq and 3′T-fill) — Partially degraded RNA from FFPE sample — poly A selected fragments — P7 primer, N Random base, V Any base, except T — Anneal primer — Reverse transcribe — 2nd strand cDNA synthesis — Ligate P5 linker — cDNA

3P-Seq — Poly(A)-position profiling by sequencing (3P-Seq) — T1 RNase partial digest — Biotinylated splint primer — Ligate biotinylated primer — Streptavidin purify — Anneal primer and reverse transcribe — RNase H digestion — cDNA

2P-seq — Poly(A)-selected RNA — Poly(A)-primed sequencing (2P-seq) to quantify usage of known poly(A) sites — T1 RNase partial digest — N Random base, V Any base, except T — Reverse transcription — Circularize and PCR — Sequence with primer ending in A_{20} — cDNA

3′-Seq — 3′-Seq to detect 3′ UTR isoforms — Reverse transcribe — Synthesize 2nd strand — Introduce nick at U with RNase HII — Translate nick 5′ to 3′ for 50-75 bases — Blunt end at nick — PCR and purify — cDNA

TIF-Seq
PEAT — Transcript isoform sequencing (TIF-Seq). Similar to paired-end analysis of TSSs (PEAT) strategy — Capped mRNA — Tobacco acid pyrophosphatase (TAP) treatment — Ligate 'Soligocap' oligonucleotide — Reverse transcription — Second strand synthesis — Incorporate biotinylated primers — Circularize and fragment — Purify — cDNA

SMORE-Seq — Simultaneous mapping of RNA ends by sequencing (SMORE-seq) — Capped mRNA — Tobacco acid pyrophosphatase (TAP) treatment — Ligate 5′ adapter — Fragmentation — Ligate 3′ adapter — Reverse transcription — Reverse transcription — PCR and purify — cDNA

TL-seq
TATL-seq — Enzymatic capture of m7G-capped mRNA 5′-ends (TL-seq). Translation-associated TL-seq (TATL-seq) — Capped mRNA — Capped mRNA — Fragmentation and size selection — Phosphatase — Pyrophosphatase — Ligate 5′ adapter — Reverse transcription — Gel purify — Ligate 3′ adapter — Reverse transcription — cDNA

TAIL-seq — Measure genome-wide poly(A) tail lengths (TAIL-seq) — rRNA-depleted RNA — 3′ adaptor ligation — Partial digestion with RNase T1 — Pull down with streptavidin — 5′ end phosphorylation — Gel purify — 5′ adaptor ligation — RT, PCR and purify — cDNA

PAL-seq — Poly(A)-tail length profiling by sequencing (PAL-seq) — Total RNA — Splint oligo — Ligate splint oligo — Partial digest with RNase T1 — Bind to streptavidin beads and wash — Ligate adaptor — Reverse transcribe and release from beads — Anneal sequencing primer and extend with dTTP and biotin-dUTP — Sequence and flow in fluorescent streptavidin. Measure fluorescence intensity — Scan flowcell

FRT-Seq — Flowcell reverse transcription sequencing (FRT-seq) for strand-specific RNA-Seq — Poly A+ RNA — Fragment and dephosphorylate — P7 primer — Gel purify Phosphorylate — P5 primer — Hybridize to flowcell and reverse transcribe

Bubble-Seq — Libraries of restriction fragments that contain replication initiation sites (bubbles) in vivo — Bubble-containing fragment — Restriction digest — Cast fragments in trapping gel — Run gel — Recover bubble-containing plug — DNA extraction — Add sequencing primers — DNA

ChIRP — Chromatin Isolation by RNA Purification (ChIRP) — RNA-binding protein — Crosslink Fragment — Biotinylated tiling oligos Hybridize — Capture on Streptavidin magnetic beads — RNase H — DNA extraction — DNA

CHART — Capture hybridization analysis of RNA targets (CHART) — Crosslink and lyse cells — Hybridize biotinylated probes — Capture, wash and elute — Extract DNA or Western blot analysis of proteins — DNA

RAP — RNA antisense purification (RAP) — Crosslink and lyse cells — Hybridize biotinylated 120 bp antisense probes — Capture, wash and elute — PCR and reverse transcribe — cDNA

GRO-seq
BRIC-Seq
Bru-Seq
BruChase-Seq — Global run-on-sequencing (GRO-seq) 5′-Bromo-uridine immunoprecipitation chase (BRIC-Seq) — Nascent RNA — Br-UTP — Run-on with analog — Isolate and hydrolyze — Bead coated with α-BrdUTP antibody — Elute Cap removal End Repair — Reverse transcription amplification — cDNA

5′-GRO-seq — Detect nascent RNA with a 5′ 7-methyl-guanylated-cap (5′-GRO-seq) — Nascent RNA — Br-UTP — Run-on with analog — DNase treatment and hydrolyze — 3′ dephosphorylate with polynucleotide kinase — Bead coated with α-BrdUTP antibody — 5′ dephosphorylate with calf intestinal-phosphatase — Cap removal — Ligate adaptors with truncated mutant RNA ligase 2 and RNA ligase 1 — Reverse transcription amplification — cDNA

BruDRB-seq — BruDRB-seq — to reveal gene-specific differences in elongation rates — Nascent RNA — DRB — Br-UTP — Transient inhibition of initiated RNAPII — Remove DRB — Synchronized run-on with analog — Isolate and hydrolyze — Bead coated with α Brd-UTP antibody — Elute Cap removal End Repair — Reverse transcription amplification — cDNA

4sUDRB-seq — 4sUDRB-seq — to reveal gene-specific differences in elongation rates in vivo — Nascent RNA — DRB — Transient inhibition of initiated RNAPII — Remove DRB — Synchronized run-on with analog — Biotinylated fragments — Bead coated with streptavidin — Elute — Reverse transcription amplification — cDNA

Repli Seq — Repli Seq—to map temporally ordered replicating DNA — Nascent RNA — BrdU — Run-on with analog — Sort cells and lyse — Bead coated with anti-BrdU antibody — Elute End Repair — Purify — DNA

PRO-seq
NSCR — Precision nuclear run-on and sequencing (PRO-seq) Nascent strand capture and release (NSCR) — Nascent RNA — Single biotin-NTP run-on — Isolate and Hydrolyze — Bead coated with streptavidin — Purify — Reverse transcription — cDNA

NS-seq — Nascent strand sequencing (NS-seq) to discover DNA replication origins and G4 structures — Nascent RNA — DNA replication origin — Lambda 5′ to 3′ exodeoxyribonuclease (λ-exo) — Digestion — Purify — Amplify — DNA

CAGE — Cap-analysis gene expression (CAGE) to map initiation sites of both capped-coding and noncoding RNAs — RNA — Cap — AAUAn — EcoP15 I site — Capped mRNA — N15 random primer with EcoP15 I site — Reverse transcription — Biotinylate — RNase digestion — Capture only complete 5′ ends — Purify cDNA — Add linker and digest with EcoP151 site — cDNA

DeepCAGE — RNA — Cap — AAUAn — Monar — 3′ adapter — 3′ End of nascent transcripts (3′NT) — RNA — Cap — High throughput Cap-analysis gene expression (CAGE) — Capped mRNA — Random primer — Reverse transcription — Transcriptome complex isolated in hypotonic buffers and nonionic detergents — RNase digestion — Insoluble chromatin complex — Mmel digestion — RNA extraction — Purify — Ligate — Reverse transcription — cDNA

NET-Seq — Native elongating transcript sequencing (NET-Seq) — RNA — Cap — Transcriptome complex — Immunoprecipitate complex — RNA extraction — Reverse transcription — cDNA

mNET-seq — Native elongating transcript sequencing technology for mammalian chromatin (mNET-seq) — RNA — Cap — Pol II Transcriptome complex — RNA — mTG — MNase digestion — Soluble chromatin — Immunoprecipitate with Pol II Ab — 5′ phosphorylation with PNK — Purify and size select — Reverse transcription — cDNA

3′-azido-2′,3′-dideoxynucleotide triphosphate (AzNTP)

Copper-catalysed cycloaddition of 3′-azido blocked cDNA to 5′-hexynyl DNA adaptor

Biotin

Photoactivatable Nucleosides

4-thiouridine (4SU)

5-iodouridine (SIU)

4-bromo uridine (SBrU)

6-Thioguanosine (6SG)

1,6-Hexanediol

Br-UTP

5,6-dichlorobenzimidazole 1-D-D-ribofuranoside (DRB)

5′-Bromo-uridine (BrdU)

Locked nucleic acid (LNA)

EDTA

RNA-Protein Interactions

PARE-Seq — Parallel analysis of RNA ends (PARE) — mRNA directed cleavage — Capped mRNA — Fragment RNA — Poly(A) RNA extraction — Ligate adapter — Reverse transcription — Second strand synthesis — Mmel digestion — Purify — Ligate — PCR — cDNA

GMUCT 1.0 — Genome-wide mapping of uncapped transcripts (GMUCT) — Degraded RNA — Poly(A) RNA selection — Ligate 5′ RNA adapter — Add oligo-dT — Reverse transcription — PCR — Fragmentation — 3′ adapter index — 5′ adapter — Ligate adapters — PCR — Indexed cDNA

GMUCT 2.0 — Genome-wide mapping of uncapped transcripts (GMUCT) — Degraded RNA — Poly(A) RNA selection — Ligate 5′ RNA adapter — Random hexamer fused to 3′ primer — Reverse transcription — PCR to add index — Indexed cDNA

RBNS — RNA Bind-n-Seq (RBNS) — High affinity binding site — Random RNA — RNA binding protein with streptavidin binding peptide tag — Incubate with various protein concentrations — Bead coated with streptavidin — Purify — Reverse transcription amplification — cDNA

Ribo-Seq
GTI-Seq
ARTseq™ — Ribo-Seq: Ribosome profiling. Global translation initiation sequencing (GTI-Seq) — RNA — Ribosome — RNase digestion — RNA extraction — rRNA depletion — RNA extraction — Reverse transcription — cDNA

RIP-Seq — RNA immunoprecipitation (RIP) — RNA-protein complex — Immunoprecipitate RNA-protein complex — RNase digestion — RNA extraction — Reverse transcription — cDNA

CLIP
HITS-CLIP
PTB-Seq
PIP-Seq — Crosslinking and immunoprecipitation sequencing (CLIP) or high-throughput sequencing of CLIP cDNA library (HITS-CLIP) — RNA-protein complex — UV 254 nm — RNase T1 digestion — Proteinase K — RNA extraction — Reverse transcription — cDNA

Pol II CLIP — Crosslinking and immunoprecipitation of Pol II (Pol II CLIP) — RNA-protein complex — UV 254 nm — Immunoprecipitate RNA-protein complex — Proteinase K — RNA extraction — Reverse transcription — cDNA

PAR-CLIP — Photoactivatable-Ribonucleoside-Enhanced Crosslinking and Immunoprecipitation (PAR-CLIP) — RNA-protein complex — Incorporate 4-thiouridine (4SU) into transcripts of cultured cells — UV 365 nm — RNase T1 digestion — Proteinase K — RNA extraction — Reverse transcription — cDNA

iCLIP — Individual nucleotide resolution CLIP (iCLIP) — RNA-protein complex — Immunoprecipitate cross-linked RNA-protein complex — Proteinase K — Cross-linked peptides remain after proteinase K digestion — cleavable adapter — cDNA truncates at binding site — Circularize — Linearize and PCR — cDNA

AGO-CLIP — Map miRNA binding to AGO sites in C. elegans — miRNA — RNA-protein complex — Incorporate 4-thiouridine (4SU) into transcripts of cultured cells — UV 365 nm — RNase T1 digestion — T4 RNA ligase — Proteinase K — RNA extraction — Reverse transcription — cDNA

hi-CLIP — RNA hybrid and individual-nucleotide resolution ultraviolet crosslinking and immunoprecipitation (hiCLIP) — Same RNA — Different RNA — RNA-protein complex — UV 254 nm — Partial RNase digestion — Adaptor A — Adaptor B — Immunoprecipitate RBP-RNA complex — Adaptor ligation — Remove 3′ block from adaptor B — Second RNA ligation — Proteinase K RNA extraction — Sequence — Same RNA RNA 1 — Different RNA RNA 1 RNA 2 — Map RNA sequences to the genome

HiTS-RAP — High-throughput sequencing–RNA affinity profiling (HITS-RAP) — Target sequence — Prepare sequencing libraries and sequence first strand Remove second strand DNA — Flowcell — 32-bp Ter sequence — Klenow — Hybridize primer — Synthesize second strand with unmodified nucleotides — Add Escherichia coli replication termination protein (Tus) — Transcribe and halt — Bind labeled protein — Scan flowcell

TRAP-Seq — Targeted purification of polysomal mRNA (TRAP-Seq) — RNA — Cap — Polysomes — Bead — RNA — Reverse transcription — cDNA

DLAF — Directly ligate sequencing adaptors to the first-strand cDNA (DLAF) — AAUAn — mRNA — AAUAn — Random primer — Reverse transcription — AAUAn — RNase digestion — cDNA — Left adaptor — Right adaptor — 3′ Hexanediol — 5′ Phosphate — Ligation in presence of PEG and DMSO — Uracil-specific excision (USER) — PCR and purify — cDNA

miTRAP — miRNA trapping by RNA in vitro affinity purification (miTRAP) — mRNA — RNA-protein complex — Amylose resin — Bind in vitro transcribed bait RNA — Incubate with cell extract — miRNA — Extract RNA-protein complex — miRNA — Isolate miRNA — cDNA

CLASH — Crosslinking, ligation, and sequencing of hybrids (CLASH) — RNA duplex — UV crosslinking — Crosslinked complex — Affinity purification — Ligate ends — Reverse transcription — cDNA

RNA Low-Level Detection

Digital RNA
HiRes-Seq
FREQ-Seq
RNAtag-Seq — Unique molecular barcodes are added after cDNA synthesis for quantitative allele-frequency detection. High-resolution RNA-seq to assess noncoded base substitutions in mRNA (HiRes-Seq) — cDNA — cDNA1 — cDNA2 — Adapters with unique barcodes — Amplify — Some fragments amplify preferentially — Sequence — Align sequences and determine actual ratio based on barcodes — True RNA abundance

Quartz-Seq — Whole-transcript amplification for single-cells (Quartz-Seq) — Add polyA primer with T7 promoter and PCR target — Reverse transcription and Primer digestion — Poly A addition and oligo dT primer with PCR target — Generate second strand — Add blocking primer — Blocking primer with LNA — Enrich with suppression PCR — cDNA

DP-Seq — Designed Primer-based RNA-sequencing strategy (DP-seq) — No secondary structure — Unique sequence — Define set of heptamer primers — PolyA selection — First strand cDNA synthesis — cDNA — Hybridize primers — PCR — DNA

Smart-seq
NanoCAGE
CAGEscan — Switch mechanism at the 5′ end of RNA templates (Smart) — mRNA fragment — First strand synthesis with Moloney murine leukemia virus reverse transcriptase — Adaptor — Second strand synthesis — PCR amplification — Purify — DNA

Smart-seq2 — Switch mechanism at the 5′ end of RNA templates (Smart) — mRNA fragment — First strand synthesis with Moloney murine leukemia virus reverse transcriptase — Locked nucleic acid (LNA) — Template-switching oligo — cDNA synthesis — PCR — Tagmentation — Gap repair, enrichment PCR and PCR purification — Index 2 — Index 1 — Enrichment-ready fragment

UMI Method — Unique molecular identifiers (UMIs) uniquely identify copies derived from each molecule — mRNA fragment — First strand synthesis — Regenerate molecular tag (N10) — Second strand synthesis — PCR amplification — True variant — Random error — Align fragments from every unique molecular tag — DNA

CEL-Seq — Cell expression by linear amplification and sequencing (CEL-Seq) — Cell 1 — Cell 2 — Cell 3 — Add cell barcodes and UMIs during reverse transcription — Second strand RNA synthesis — Pool — Fragment, add adapters and — PCR — Separate cell sequences based on unique indices — DNA

STRT — Single-cell tagged reverse transcription (STRT) — Add oligo-dT primer — cDNA synthesis — Add 3 to 6 cytosines — Template switching primer — Introduce unique index — Pool — Single-primer PCR and purify — Separate cell sequences based on unique indices

TCR Chain Paring — Identify T-cell Receptor (TCR) alpha–beta chain pairing in single-cells — Oil emulsion — TCRα mRNA — TCRβ mRNA — CDR3 — Reverse transcription — TCRα — TCRβ — Amplification — Overlap extension — Blocker primers — Nested PCR amplification — PCR supression of non-fused molecules — CDR3α — CDR3β — DNA

Uridine

Pseudouridine (Ψ)

1-cyclohexyl-(2-morpholino-ethyl)carbodiimide-metho-p-toluene sulfonate (CMCT)

Ψ-CMC adduct

Inosine

Acrylonitrile

N1-cyanoethyl inosine

N⁶-Methyladenosine (m⁶A)

1-methyl-7-nitroisatoic anhydride (1M7)

Acylation

DIBO-biotin "click"

Preparation of acylated RNA for biotin-streptavidin purification. DIBO, dibenzocyclooctyne

Dimethyl sulfate (DMS)

Display methods on mobile device

RNA Modifications

MeRIP-Seq
m6A-seq
m6A-RIP — m6A-specific methylated RNA immunoprecipitation with nextgeneration sequencing (MeRIP-Seq), (m6A-seq). — Methylated RNA — Methylated RNA — RBP — Extract RNA — Fractionate RNA — Immunoprecipitate — Reverse transcription — cDNA

miCLIP — m6A individual-nucleotide-resolution cross-linking and immunoprecipitation (miCLIP) — Methylated RNA — Sheared cellular RNA — Add anti-m6A antibody — UV 365 nm — Immunoprecipitate — Reverse transcription truncated at binding sites — cDNA

Ψ-seq
PSI-seq — Pseudouridine residues — Pseudouridine site identification sequencing (PSI-seq and Ψ-seq) — PolyA selected DNase treated RNA — CMC treatment and OH- treatment — Fragmentation — Adaptor ligation — Reverse transcription — Purification — cDNA

Pseudo-seq — Pseudouridine residues — Pseudo-seq, a method for genome-wide identification — PolyA selected RNA — Fragmentation and size selection — CMC treatment and OH- treatment — Adaptor ligation — Reverse transcription — Size select — Circularize with circligaseTM — PCR and purify — DNA

ICE — Inosine residue — Inosine chemical erasing (ICE) — Inosine residue — Control — Acrylonitrile — N1-cyanoethylinosine — Reverse transcription PCR amplification — Reverse transcription PCR amplification — X — cDNA

RNA Structure

SHAPE-Seq — Selective 2′-hydroxyl acylation analyzed by primer extension sequencing (SHAPE-Seq) — RNA — Barcoded RNA — 1M7 reaction — Reverse transcription — RNA hydrolysis — cDNA

icSHAPE — In vivo click selective 2′-hydroxyl acylation and profiling experiment (icSHAPE) — Cell — In vivo folded RNA — NAI-N_3 — Add azide group — N_3 — DIBO-biotin 'click' — Reverse transcription — Capture on Streptavidin beads — Elute — cDNA

CIRS-seq — Chemical inference of RNA structures (CIRS-seq) — In vivo folded RNA — Proteinase K treatment — RNA — DMS — Reaction — Reverse transcription — RNA hydrolysis — cDNA

SHAPE-MaP — Selective 2′-hydroxyl acylation analyzed by primer extension and mutational profiling (SHAPE-MaP) — SHAPE adducts — 1M7 reaction — Adduct induced mutations — Mn^{2+} — cDNA reaction — Library prep and sequencing — Align sequence and count mutations — Profile

Structure-Seq
DMS-Seq — Use dimethyl sulphate methylation of unprotected adenines and cytosines (Structure-Seq) — +DMS — Control — Structure in vivo — In vivo treatment — Use DMS to methylate the RNA base pairing faces of A and C in loops — Random hexamers — Reverse transcription — Ligate linker — PCR — DNA

SPARE — Specific parallel amplification of 5′ RNA Ends (SPARE) to identify microRNA processing intermediates — Base to loop processing — Loop to base processing — Processing intermediates — RNA-adapter ligation — Processing intermediates — Reverse transcription — Precursor-specific primer — PCR — Sequence

PARS
dsRNA-Seq — Parallel analysis of RNA structure (PARS) also double-stranded RNA (dsRNA-Seq) — PolyA RNA — RNase S1 — RNase V1 — RNase digestion — RNA fragments with 5′ phosphate ends — Random fragmentation — Reverse transcription — cDNA

PARTE — Parallel analysis of RNA structures with temperature elevation (PARTE) — PolyA RNA — 75°C — 25°C — RNAse V1 — RNAse V1 digestion at different temperatures — RNA fragments with 5′ phosphate ends — Random fragmentation — Reverse transcription — cDNA

Frag-seq — Fragmentation sequencing (Frag-seq) — In vitro folded polyA RNA — P1 endonuclease — Endogenous 5′P control — Endogenous 5′OH control — P1 endonuclease digestion — 3′OH — 5′OH — 5′P — T4 kinase — 3′OH — 5′P — reverse transcription — cDNA

CapSeq — 5′ anchored profiling of Pol II transcripts (CapSeq) — Total RNA — Terminator — CIP — TAP — Primer Ligation — Random Primer — Reverse transcription — Purification — cDNA

CIP-TAP — CIP-TAP for identifying capped small RNA (csRNA) species — csRNA — CIP — 3′ primer ligation and gel purify — TAP — 5′ primer ligation — and gel purify — cDNA synthesis — PCR — Purification — cDNA

Key — Yellow highlights indicate the target of the protocol

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TruSeq PCR Free

Double-stranded DNA — Fractionate Size select — End repair Phosphorylate — A-overhang — Add Adaptors — Adaptor ligation — Product ready for cluster generation

TruSeq Nano

Double-stranded DNA — Fractionate Size select — End repair Phosphorylate — A-overhang — Add Adaptors — Adaptor ligation — Denature and amplify — Product ready for cluster generation

TruSeq Custom Amplicon

Double-stranded DNA — Region of interest — Custom Probe 1 — Custom Probe 2 — Add custom probes — Extension and ligation — Add sequencing primers — PCR — Product ready for cluster generation

TruSeq RNA

mRNA — polyA select — Fragment — Random hexamer — First and second strand synthesis

TruSeq Small RNA

Small RNA fragment — Ligate adaptors — Add primer — Reverse transcription — Denature and amplify — Product ready for cluster generation

TruSeq RNA Stranded

RNA fragment — Random primer — Create cDNA — Create second strand cDNA — End repair Phosphorylate A-overhang — Adaptor ligation — Denature and amplify — Product ready for cluster generation

TruSeq RNA Access

Target — Pool stranded RNA-Seq libraries — Biotinylated target probe — Hybridize probes to targets — Capture on streptavidin magnetic beads — Elute — Product ready for cluster generation

TruSeq Targeted RNA Expression

Target — Total RNA — cDNA — Add custom primers — Hybridization — Extension-Ligation — Denature and amplify — Amplification — Product ready for cluster generation

Nextera Library Preparation

Transposase — DNA — Tagmentation — ~300bp — Product ready for cluster generation

Nextera Rapid Capture

Target — Denatured and pooled fragments from Nextera library — Biotinylated target probe — Hybridize probes to targets — Capture on magnetic beads — Elute — Product ready for cluster generation

Nextera Mate Pair

Transposase — Biotinylated junction adapter — DNA — Tagmentation — Circularize — Fragment — Isolate biotinylated fragment — Adaptor ligation — Denature and amplify — Product ready for cluster generation

Sequencing by Synthesis

Adapter hybridizes to flowcell — Reverse strand synthesis — Forward strand — Reverse strand — Remove forward strand — Fold over and hybridize to second primer — Synthesize second strand — Bridge amplification — Thousands of molecules are amplified in parallel — Sequence primer — The reverse strand is cleaved and washed away — With each cycle, four fluorescently tagged nucleotides compete for addition to the growing chain. Only one is incorporated based on the sequence of the template. — The read product is washed away — Index 1 primer — Sequence Index1 — The read product is washed away — Fold over and hybridize to first primer — Deblock P5 primer and add unlabeled bases — Sequence Index2 — Synthesize second strand — Read 2 primer — The forward-strand is cleaved and washed away — The second read is sequenced

Moleculo

~10kb Sheared genomic DNA — End repair — Adapter ligation — Generate clonal pools Amplify — Transposase — Tagmentation — ~600bp — Add indices — Prepared fragments — Pool and purify

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This poster was compiled by the Illumina Scientific Affairs. Additional information, the latest version of the poster, and a comprehensive list of *seq methods, are available at http://www.illumina.com/libraryprepmethods. Please contact Scientific Affairs with any questions, comments, or suggestions.

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