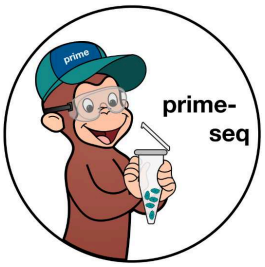
Apr 04, 2022 Version 2

prime-seq V.2

Scientific Reports

DOI
dx.doi.org/10.17504/protocols.io.81wgb1pw3vpk/v2



Aleksandar Janjic[1], Lucas Esteban Wange[1], Johannes JWB Bagnoli[1], Johanna Geuder[1], Phong Nguyen[1], Daniel Richter[1], Christoph Ziegenhain[2], Wolfgang Enard[1], Beate Vieth[1], Wolfgang Enard[1]

[1]Ludwig-Maximilians-Universität München; [2]Karolinska Institute Stockholm

Epi Aging Lab

Aleksandar Janjic
Ludwig-Maximilians-Universität München

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.81wgb1pw3vpk/v2

External link: https://genomebiology.biomedcentral.com/articles/10.1186/s13059-022-02660-8

Protocol Citation: Aleksandar Janjic, Lucas Esteban Wange, Johannes JWB Bagnoli, Johanna Geuder, Phong Nguyen, Daniel Richter, Christoph Ziegenhain, Wolfgang Enard, Beate Vieth, Wolfgang Enard 2022. prime-seq. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.81wgb1pw3vpk/v2**Version created by **Aleksandar Janjic**

Manuscript citation:
Janjic, A., Wange, L.E., Bagnoli, J.W. et al. Prime-seq, efficient and powerful bulk RNA sequencing. Genome Biol 23, 88 (2022). https://doi.org/10.1186/s13059-022-02660-8

Protocol status: Working
We use this protocol and it's working

Created: April 03, 2022

Last Modified: April 04, 2022

Protocol Integer ID: 60237

Keywords: bulk RNA sequencing, RNA-seq, transcriptomics, SCRB-seq, barcoding bulk rna, standard bulk rna, bulk rna, direct rna isolation step, rna, efficient library generation by early barcoding, cell rna, efficient library generation, seq cost, seq protocol, early barcoding, seq

Disclaimer

DISCLAIMER – FOR INFORMATIONAL PURPOSES ONLY; USE AT YOUR OWN RISK

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to **protocols.io** is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgment, advice, diagnosis, or treatment. Any action you take or refrain from taking using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with **protocols.io**, can be held responsible for your use of the information contained in or linked to this protocol or any of our Sites/Apps and Services.

Abstract

Cost-efficient library generation by early barcoding has been central in propelling single-cell RNA sequencing. Here, we optimize and validate prime-seq, an early barcoding bulk RNA-seq method. We show that it performs equivalently to TruSeq, a standard bulk RNA-seq method, but is fourfold more cost-efficient due to almost 50-fold cheaper library costs. We also validate a direct RNA isolation step, show that intronic reads are derived from RNA, and compare cost-efficiencies of available protocols. We conclude that prime-seq is currently one of the best options to set up an early barcoding bulk RNA-seq protocol from which many labs would profit.

Guidelines

- All reagents and plastic-ware can be found in the 'Materials' section.
- Use only RNase free supplies and clean all surfaces and tools with RNase Away prior to working
- Make sure all steps involving cell lysate and RNA before reverse transcription are carried out swiftly and on ice.
- All primer sequences are listed below:

	A	B	C	D	E	F
	Oligo	**Vendor**	**Purification**	**Working Conc.**	**Sequence**	**Notes**
	Barcoded Oligo-dT (E3V7NEXT)	Sigma	Cartridge	10 µM	ACACTCTTTCCCTACACGACGCTCTTCCGATCT[12 bp BC]NNNNNNNNNNNNNNNVTTTTTTTTTTTTTTTTTTTTTTTTTTTTTVN	
	Template Switching Oligo (TSO) (E5V7NEXT)	Sigma	RNase-Free HPLC	100 µM	Biotin-ACACTCTTTCCCTACACGACGCrGrGrG	
	Preamp Primer (SINGV6)	Sigma	Standard Desalting	10 µM	Biotin-ACACTCTTTCCCTACACGACGC	
	3' enrichment primer (P5NEXTPT5)	Sigma	Standard Desalting	5 µM	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCT	
	i7 Index Primer (Nextera)	IDT	Trugrade	5 µM	CAAGCAGAAGACGGCATACGAGAT[i7]GTCTCGTGGGCTCGG	
	i5 Index Primer (TruSeq)	IDT	Trugrade	5µM	AATGATACGGCGACCACCGAGATCTACAC[i5]ACACTCTTTCCCTACACGACGCTCTTCCGATCT	
	prime-seq Adapter AntiSense	IDT	Standard Desalting	1.5 µM	/5Phos/CTGTCTCTTATACACATCT	Duplexed DNA
	prime-seq Adapter Sense	IDT	Standard Desalting	1.5 µM	GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGT	Duplexed DNA

Specific barcoded oligodT (E3V7NEXT) sequences:

E3V7_Set1.txt

E3V7_Set2.txt

Materials

MATERIALS

- DNase I Reaction Buffer - 6.0 ml **New England Biolabs** **Catalog #**B0303S
- DNase I (RNase-free) - 1,000 units **New England Biolabs** **Catalog #**M0303S
- Deoxynucleotide Solution Mix - 40 umol of each **New England Biolabs** **Catalog #**N0447L
- Exonuclease I (E.coli) - 3,000 units **New England Biolabs** **Catalog #**M0293S
- Quant-it™ PicoGreen® dsDNA Assay Kit **Life Technologies** **Catalog #**P7589
- ß -mercaptoethanol **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**M3148
- QuantiFluor(R) RNA System **Promega** **Catalog #**E3310
- Proteinase K solution, 20 mg ml – 1 **Ambion** **Catalog #**AM2546
- 5 M Sodium chloride (NaCl) **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**S5150-1L
- Agilent High Sensitivity DNA Kit **Agilent Technologies** **Catalog #**5067-4626
- Buffer RLT Plus **Qiagen** **Catalog #**1053393
- Maxima H Minus Reverse Transcriptase (200 U/uL) **Thermo Fisher Scientific** **Catalog #**EP0752
- NEBNext Ultra II FS DNA Library Prep with Sample Purification Beads - 24 rxns **New England Biolabs** **Catalog #**E6177S
- EDTA **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**E7889
- Ethanol absolute **Carl Roth** **Catalog #**9065.4
- Igepal **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**I8896
- KAPA HiFi 2x RM **Kapa Biosystems** **Catalog #**KR0370
- Poly(ethylene glycol) **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**89510
- UltraPure DNase/RNase Free Distilled Water **Catalog #**10977-049
- Trizma hydrochloride solution **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**T2694
- Aluminium seals for cold storage **Catalog #**391-1275
- Filter tips 96 low retention 10 uL **Catalog #**771265
- PCR Seals **Thermo Scientific** **Catalog #**AB0558
- twin.tec 96-well DNA LoBind Plates **Eppendorf** **Catalog #**0030129504
- Sera-Mag Speed Beads **GE Healthcare** **Catalog #**65152105050250
- Sodium Azide **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**S2002-100G

Troubleshooting

Safety warnings

Please follow all Manufacturer safety warnings and recommendations.

Before start

Wipe bench surfaces with RNAse Away and keep working environment clean.

Preparation

12m

1 Clean all surfaces and pipettes with RNase Away 5m

2 Thaw frozen buffers and primers on ice 10m

3 Prepare 80% EtOH (approximately 45 mL for 96 samples) 2m

4 **When running the protocol for the first time prepare Cleanup Beads (see end of the protocol)!** 45m

5 prime-seq can be used on lysate or extracted RNA. It is essential, however, that the samples either have the same input or that they are normalized after the RNA is extracted, otherwise sequencing depth per sample will be impacted. Based on your starting material, please follow one of the following cases:

STEP CASE

Lysate (similar input), Direct Lysis 106 steps

Follow this case if you are testing samples that have **similar input** (i.e. the expected RNA amount is the same between samples). The steps here will guide you in digesting residual proteins in your samples, extracting the RNA, digesting DNA, preparing RNA-seq libraries, and finally sequencing.

Example: investigating the genotype effect on transcription in 5,000 neurons

First Time Setup

6 When running the direct lysis protocol for the first time, prepare Bead Binding Buffer (see end of the protocol)!

Sample Collection

7 Prepare **Lysis Buffer** according to the number of samples. 2m

	Reagent	Well	Plate

	RLT Plus Buffer	99 µL	10.89 mL
	β-mercaptoethanol	1 µL	110 µL
	Total	**100 µL**	**11 mL**

Note

If sample volume exceeds 25 % of total lysate, use 2x TCL buffer (Qiagen, #1070498) + 1 % *β*-mercaptoethanol

8 Add 100 µL of **Lysis Buffer** to each well of a semi-skirted 96-well PCR plate 1m

9 Add cells or tissue to wells

Note

Cells

Minimum: 100 cells, Optimum: 10,000 cells

Make sure that the same number of cells are used for each sample. Large differences between cells will impact distribution of sequencing reads and can potentially affect normalization.

Note

Tissue

If samples are difficult to lyse they should be homogenized using a tissue homogenizer.

Tissue should be a relatively small and not exceed more than 1000 ng of RNA. Tissue samples should be normalized by weight and be the same type of tissue.

Large differences between tissue samples will impact distribution of sequencing reads and can potentially affect normalization.

If you are unsure if the samples will contain the same amount of RNA, it is best to switch to the "*Lysate (variable)*" case in Step 13.

10 Transfer 50 µL of **lysate** to a new plate, return one plate immediately to -80 C freezer to save as a backup 1m

Note

Conversely, one can prepare two plates during sorting with 50 µL of lysis buffer.

Proteinase K Digest 30m

11 Add 1 µL **Proteinase K** (20 mg/mL) and 1 µL **EDTA** (25 mM) to each well 1m

12 Incubate for 00:15:00 at 50 °C and then heat inactivate the Proteinase K for 00:10:00 at 75 °C 25m

Bead Clean Up 20m

13 Mix each bulk sample (50 µL per well) with 100 µL of **Cleanup Beads (22% PEG)** 1m

14 Incubate for 00:05:00 at 20 °C (Room Temp) 5m

> Note
>
> While binding, prepare **DNase I Mix** (Step 28)

15 Place on magnet stand until clear (~3 min) and then discard supernatant 3m

16 Wash with 100 µL of **80% EtOH** while the plate is on the magnet. Discard the supernatant 2m

> Note
>
> After adding EtOH, incubate for 30 s so that all beads are bound to magnet.

17 Repeat wash step once more 2m

18 Air dry beads for 00:03:00 3m

> Note
>
> Depending on temperature and humidity, the beads may dry faster. Therefore it is important to regularly check the beads and avoid over-drying.

DNAse I Digest 1m

19 Add 5 µL H2O and **resuspend beads by vortexing vigorously** 2m

> Note
>
> If you encounter bead clumping at this step, try to resuspend the beads by vigorously pipetting the samples. We have generated high quality prime-seq libraries despite heavy clumping.

20 Prepare **DNase I Mix** 3m

	Reagent	Well	Plate
	DNase I	1 µL	110 µL
	DNase I Buffer (10x)	2 µL	220 µL
	Bead Binding Buffer (2x)	10 µL	1.1 mL
	H2O	2 µL	220 µL
	Total	**15 µL**	**1.65 mL**

21 Add 15 µL of **DNase I Mix** and mix by pipetting 2m

22 Incubate DNase I Mix and beads for 00:10:00 at 20 °C (Room Temp) 10m

23 Heat inactivate the DNase I by adding 1 µL of **EDTA (100 mM)** and incubating for 00:05:00 at 65 °C 6m

24 Place plate on magnet stand until clear (~3 min) and discard the supernatant. 3m

25 Wash with 100 µL of **80% EtOH** while the plate is on the magnet. Discard the supernatant 2m

26 Repeat wash step once more 2m

27 Air dry beads for 00:05:00 5m

Note

Depending on temperature and humidity, the beads may dry faster. Therefore it is important to regularly check the beads and avoid over-drying.

Note

While drying, prepare **Reverse Transcription Mix**.

Reverse Transcription 5m

28 Prepare **Reverse Transcription Mix** 5m

	Reagent	Well	Plate
	Maxima H Minus RT	0.15 µL	16.5 µL
	Maxima RT Buffer (5x)	2 µL	220 µL
	dNTPs (25 mM)	0.4 µL	44 µL
	TSO (E5V7NEXT) (100 uM)	0.1 µL	11 µL
	UltraPure Water	2.35 µL	258.5 µL
	Total	**5 µL**	**550 µL**

29 Add 4 µL **H2O** to the beads 1m

> Note
>
> The 4 µL of water can be combined with the Reverse Transcription Mix by increasing the water in Row 6 from 2.35 µL to 6.35 µL.
>
> If working with many samples, or if using a stepper pipette or robot, we find that it is better to add some water separately to prevent the beads from drying too much.

30 Add 5 µL **Reverse Transcription Mix** 1m

31 Add 1 µL of **Barcoded oligodT (E3V7NEXT) (10 µM)** per well 2m

32 Incubate for 01:30:00 at 42 °C 1h 30m

cDNA Pooling & Purification 5m

33 Place the plate on a magnet 3m

34 Pool the supernatant of all wells into a 2 mL tube 10m

35 Add 10 µL of **Cleanup Beads (22% PEG)** ***per sample*** for a 1:1 ratio (e.g. 240 µL for 24 samples) 5m

> Note
>
> The EDTA in the **Cleanup Beads (22% PEG)** will inactivate the RT.

36 Incubate for 00:05:00 at Room temperature to allow binding of the cDNA onto beads 5m

37 Place the tube on the magnet stand until clear (~3 min) and discard supernatant 3m

38 Wash with 1 mL of **80% EtOH** while the tube is on the magnet, discard the supernatant 1m

> Note
>
> Volume of EtOH should be adjusted depending on the number of samples. More samples will require more EtOH to cover the beads completely.

39 Repeat wash step once more 1m

40 Air dry beads for 00:05:00 5m

> Note
>
> Depending on temperature and humidity, the beads may dry faster. Therefore it is important to regularly check the beads and avoid over-drying.

41 Elute the beads in 17 µL of **UltraPure Water** 1m

42 Incubate for 00:05:00 at RT and transfer to a new PCR tube or plate 5m

Exonuclease I Treatment 35m

43 Add 2 µL of **ExoI Buffer (10x)** and 1 µL of **Exonuclease I**. Incubate as follows: 35m

	Step	Temperature	Time
	Incubation	37 C	20 min

	Heat Inactivation	80 C	10 min
	Storage	4 C	∞

44 Mix each sample (20 µL per well) with 16 µL of **Cleanup Beads (22% PEG)** for a 1:0.8 ratio 1m

45 Incubate for 00:05:00 at Room temperature to allow binding of the cDNA onto beads 5m

46 Place the tube on the magnet stand until clear (~3 min) and discard supernatant 3m

47 Wash with 50 µL of **80% EtOH** while the tube is on the magnet, discard the supernatant 1m

48 Repeat wash step once more 1m

49 Air dry beads for 00:05:00 5m

> Note
>
> Depending on temperature and humidity, the beads may dry faster. Therefore it is important to regularly check the beads and avoid over-drying.

50 Elute the beads in 20 µL of **UltraPure Water** 1m

51 Incubate for 00:05:00 at RT and transfer to a new PCR tube or plate 5m

Full length cDNA Amplification 1m

52 Prepare **Pre Amplification Mix** 1m

	Reagent	1x
	KAPA HiFi 2x RM	25 µL
	Pre-amp Primer (SINGV6) (10 uM)	3 µL
	UltraPure Water	2 µL
	Total	**30 µL**

53 Add 30 µL **Pre Amplification Mix** to sample 1m

54 Incubate the Pre Amplification PCR as follows: 1h 30m

	Step	Temperature	Time	Cycles
	Initial Denaturation	98 C	3 min	1 cycle
	Denaturation	98 C	15 sec	10 cycles*
	Annealing	65 C	30 sec	
	Elongation	72 C	4 min	
	Final Elongation	72 C	10 min	1 cycle
	Storage	4 C	∞	

Note

Adjust the number of cycles based on input (sample number, cell number, or concentration).

As a rule of thumb we assume big cells like embryonic stem cells to contain 10 pg of total RNA and small cells like T-cells ~ 1-2 pg

As a general guide we recommend:

	Total RNA Input	Cycles
	10 ng	16
	50 ng	14
	100 ng	12
	500 ng	10
	1000 ng	9

cDNA Bead Purification

1m

55 Mix sample with 40 µL **Clean Up Beads (22% PEG)** for a ratio of 1:0.8 — 1m

56 Incubate for 00:05:00 at 20 °C (Room Temp) — 5m

57 Place the tube on the magnet stand until clear (~3 min) and discard supernatant — 3m

58 Wash with 100 µL of **80% EtOH** while the tube is on the magnet, discard the supernatant — 1m

59 Repeat wash step once more — 1m

60 Air dry beads for 00:05:00 — 5m

Note

Depending on temperature and humidity, the beads may dry faster. Therefore it is important to regularly check the beads and avoid over-drying.

61 Elute cDNA in 10 µL **UltraPure Water** 1m

62 Incubate for 00:05:00 at RT and transfer to a new PCR tube or plate 5m

Note

Stopping Point. Samples can be safely stored at -20 °C and protocol can be continued at a later date.

cDNA Quantification and Quality Check 45m

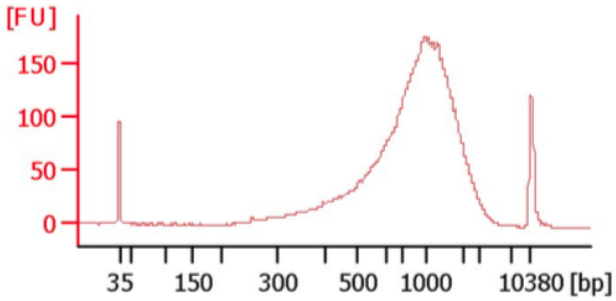
63 Quantify the cDNA using the **Quant-iT PicoGreen dsDNA assay kit** or equivalent Qubit following the manufacturer's protocol. Use 1 µl of clean cDNA for quantification. 10m

64 Quality check the cDNA using the Agilent 2100 Bioanalyzer with **High Sensitivity DNA Analysis Kits**. 45m

Note

Passing the cDNA quality check does not guarantee that the data will be of high quality, however, if the cDNA fails the quality check it will usually not yield good libraries and will therefore generate lower quality data.

Expected result



Library Preparation

65

Note

Before starting, read the library preparation section carefully as there are a few steps that are very time sensitive.

66 Prepare **Fragmentation Mix** 1m

	Reagent	1x
	Ultra II FS Reaction Buffer	1.4 µL
	Ultra II FS Enzyme Mix	0.4 µL
	cDNA (4-8 ng/µL)	2.5 µL
	TE	1.7 µL
	Total	**6 µL**

Note

Ensure that the Ultra II FS Reaction Buffer is completely thawed. If a precipitate is seen in the buffer, pipette up and down several times to break it up, and quickly vortex to mix. Place on ice until use.

Note

Vortex the Ultra II FS Enzyme Mix for 5-8 seconds prior to use for optimal performance.

67 Vortex the **Fragmentation Mix** for 00:00:05 and immediately proceed to step 67 10s

68 Incubate the Fragmentation reaction as follows: 40m

	Step	Temperature	Time
	Pre-Cool	4 C	∞
	Fragmentation	37 C	5 min
	A Tailing and Phosphorylation	65 C	30 min
	Storage	4 C	∞

Note

Set heated lid to 75° C. Make sure the lid is at the correct temperature before you start the reaction.
Skip the first incubation step once you have added your samples.

Adapter Ligation 20m

69 Prepare **Adapter Ligation Mix** 1m

	Reagent	1x
	NEBNext Ultra II Ligation Master Mix	6 µL
	NEBNext Ligation Enhancer	0.2 µL
	prime-seq Adapter (1.5 µM)	0.5 µL
	Total	**6.7 µL**

70 Add 6.7 µL **Adapter Ligation Mix** to each replicate 1m

71 Incubate for 00:15:00 at 20 °C 15m

Note

Turn off heated lid

72 Add 37.3 µL Buffer EB to Samples 1m

73 Mix Sample with 26 µL **SPRI select beads** 1m

Note

We use SPRI Select Beads here instead of our home made 22% Clean Up beads for their guaranteed QCed size selection properties.

Note

The volume of SPRI select beads used during library size selection can be adjusted based on desired library size. Optimization for your samples may be required.

74 Incubate for 00:05:00 at 20 °C (Room Temp) 5m

75 Place the plate on the magnet stand until clear and **transfer** 76 µL **supernatant to clean well**. 3m

Note

Be careful not to discard! This is your sample!

76 Mix supernatant with 10 µL **SPRI select beads** 1m

Note

The volume of SPRI select beads used during library size selection can be adjusted based on desired library size. Optimization for your samples may be required.

77 Incubate for 00:05:00 at 20 °C (Room Temp) 5m

78 Place the plate on the magnet stand until clear and discard supernatant 3m

79 Wash with 150 µL of **80% EtOH** while the plate is on the magnet, discard the supernatant 1m

80 Repeat wash step once more 1m

81 Air dry beads for 00:05:00 5m

Note

Depending on temperature and humidity, the beads may dry faster. Therefore it is important to regularly check the beads and avoid over-drying.

82 Elute samples in 11 µL **0.1X TE** (dilute 1X TE Buffer 1:10 in water) for 00:05:00 5m

Library PCR 45m

83 Transfer 10.5 µL of samples to clean wells

84 Add 1 µL of **Index Primer (Nextera i7, 5 uM)** to each well

> Note
>
> This is the unique index that will be used for demultiplexing libraries.

85 Add 1 µL of **Index Primer (TruSeq i5, 5 uM)** to each well

> Note
>
> Alternatively the universal primer P5NEXTPT5 can be used in case the second index will not be sequenced.

86 Prepare **Library PCR Mix** by adding 12.5 µL

> Note
>
> Although scaled down, there will not be sufficient Q5 Master Mix (M0544L) in the kit. This item will have to be ordered separately.

87 Incubate the **Library PCR** reaction as follows:

	Step	Temperature	Time	Cycles
	Initial Denaturation	98 C	30 sec	1 cycle

	Denaturation	98 C	10 sec	10 cycles *
	Annealing/Elongation	65 C	1 min 15 sec	
	Final Elongation	65 C	5 min	1 cycle
	Storage	4 C	∞	

Note

Adjust the number of cycles based on cDNA input.

As a general guide we recommend:

	cDNA Input	Cycles
	20 ng	10
	10 ng	11
	5 ng	12

Double Size Selection

25m

88 Add 25 µL Buffer EB to Index PCR

89 Mix Index PCR with 26 µL **SPRI select beads**

Note

We use SPRI Select Beads here instead of our home made 22% Clean Up beads for their guaranteed QCed size selection properties.

Note

The volume of SPRI select beads used during library size selection can be adjusted based on desired library size. Optimization for your samples may be required.

90 Incubate for 00:05:00 at 20 °C (Room Temp)

91 Place the plate on the magnet stand until clear and **transfer** 76 µL **supernatant to clean well**.

Note

Be careful not to discard! This is your library.

92 Mix supernatant with 10 µL **SPRI select beads**

Note

The volume of SPRI select beads used during library size selection can be adjusted based on desired library size. Optimization for your samples may be required.

93 Incubate for 00:05:00 at 20 °C (Room Temp)

94 Place the plate on the magnet stand until clear and discard supernatant.

95 Wash with 150 µL of **80% EtOH** while the plate is on the magnet, discard the supernatant

96 Repeat wash step once more

97 Air dry beads for 00:05:00

Note

Depending on temperature and humidity, the beads may dry faster. Therefore it is important to regularly check the beads and avoid over-drying.

98 Elute in 15 µL **UltraPure Water**.

99 Incubate for 00:05:00 and then place on magnet until clear. Transfer eluted library to new well.

Note

Stopping point. The libraries can be safely stored at -20 °C until they will be QCed and sequenced.

QC and quantification

45m

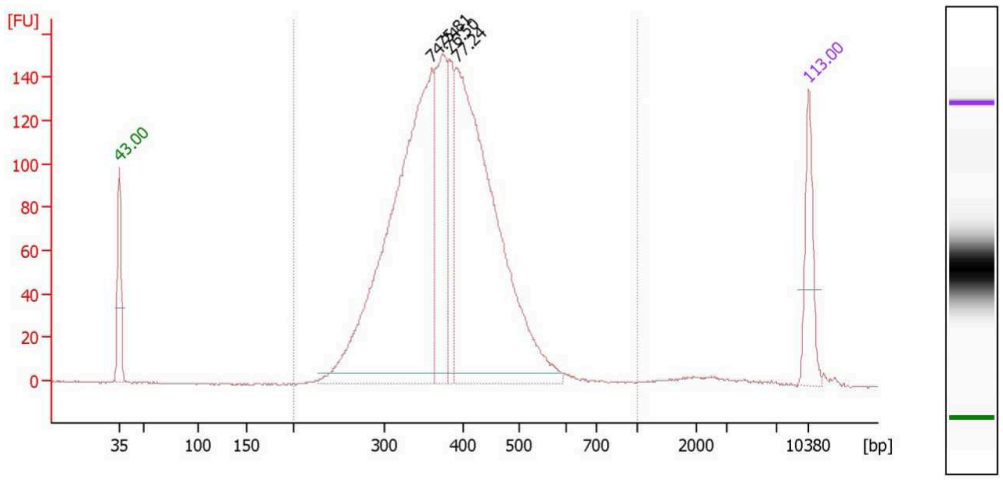
100 Quantify and quality control the library using the Agilent 2100 Bioanalyzer with **High Sensitivity DNA Analysis Kits**.

Note

Bulk libraries often yield high concentrations, which should be diluted to get accurate molarity measurements on the Bioanalyzer. Ideally, do not load more than 2 ng onto the chip.

Expected result

Libraries will typically exceed 1-5 ng/µl concentration



Sequencing

1m

101 Samples should be submitted according to your Sequencing Facility specifications. prime-seq is compatible with Illumina Sequencing.

At least 8 cycles are required for the Index Read (i7) and 28 cycles for the Read 1 (BC+UMI). Dual index sequencing can be done when using patterned flowcells. Read 2 (DNA) should be adjusted based on the quality of the genome being mapped to, but for human and mouse 50 cycles is sufficient.

Some potential sequencing options:

	A	B	C	D	E	F
	Sequencer	**Read 1**	**Read 2**	**Index Read (i7)**	**Index Read (i5)**	**Kit**
	NovaSeq	28	94	8	8	NovaSeq SP v1.5 100 cycle

	A	B	C	D	E	F
	NextSeq 500/550	28	56	8	0	NextSeq 500/550 HiOut v3 75 cycle
	NextSeq 1000/2000	28	94	8	8	NextSeq 1000/2000 P2 100 cycle
	NextSeq 2000	28	52	8	0	NextSeq 2000 P3 50 cycles
	HiSeq	28	114	8	0	HiSeq 3000/4000 150 cycles

Prepare Cleanup Beads (22% PEG)

10m

102 Prepare **PEG Solution (22%)** by adding all ingredients to a 50 mL falcon tube

10m

	Reagent	Amount
	PEG 8000	11 g
	NaCl (5M)	10 mL
	Tris-HCl (1M, pH 8.0)	500 µL
	EDTA (0.5M)	100 µL
	IGEPAL (10% solution)	50 µL
	Sodium Azide (10% solution)	250 µL
	UltraPure Water	up to 49 mL
	Total	**49 mL**

Note

Do not add the total amount of water until after PEG is completely solubilized

103 Incubate at 40 °C and vortex regularly until PEG is completely dissolved 10m

104 Resuspend **Sera-Mag Speed Beads** carefully and pipette 1000 µL of bead suspension into a 1.5 mL tube 1m

105 Place on magnet stand and remove storage buffer 1m

106 Add 1000 µL of **TE Buffer** (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) and resuspend beads 30s

107 Place on magnet stand and remove supernatant 30s

108 Repeat wash step one more time 1m

109 Add 900 µL **TE Buffer** (10 mM Tris-HCl, pH 8.0, 1 mM EDTA) and resuspend beads 30s

110 Add the washed **Sera-Mag Speed Beads** to the **PEG Solution (22%)** and mix well 1m 30s

Note

The final **Cleanup Beads (22% PEG)** can be aliquoted and stored at 4 °C for up to six months

Prepare Bead Binding Buffer 10m

111 Prepare **Bead Binding Buffer (2x)** 10m

	Reagent	
	PEG 8000	1.1 g

	NaCl (5 M)	1 mL
	Tris-HCl (1 M, pH 8.0)	50 µL
	Igepal (10% solution)	5 µL
	Sodium Azide (10% solution)	25 µL
	H2O	to 5 mL
	Total	**5 ml**

Note

The **Bead Binding Buffer (2x)** can be stored at Room temperature for up to six months.