Ligation Sequencing Kit family

1. Ligation-based sequencing kits

   Oxford Nanopore have multiple ligation-based sequencing kits:

   - Ligation Sequencing Kit V14 (SQK-LSK114)
   - Ligation Sequencing Kit XL V14 (SQK-LSK114-XL)
   - Ligation Sequencing Kit (SQK-LSK112)
   - Ligation Sequencing Kit XL (SQK-LSK112-XL)
   - Ligation Sequencing Kit (SQK-LSK110)
   - Ligation Sequencing Kit (SQK-LSK109)
   - Ligation Sequencing Kit XL (SQK-LSK109-XL)
   - Cas9 Sequencing Kit (SQK-CS9109)
   - Native Barcoding Kit 24 V14 (SQK-NBD114.24)
   - Native Barcoding Kit 96 V14 (SQK-NBD114.96)

   The Ligation Sequencing Kits are compatible with gDNA, amplicons, or cDNA starting material. The Cas9 Sequencing Kit requires gDNA as input.

   The Ligation Sequencing Kits (SQK-LSK109 and SQK-LSK109-XL) and the Cas9 Sequencing Kit are supplied with a Flow Cell Priming Kit (EXP-FLP002) or Flow Cell Priming Kit XL (EXP-FLP002-XL) for the XL kits. The upgraded Ligation Sequencing Kits (SQK-LSK110, SQK-LSK112 and SQK-LSK112-XL) both contain flow cell priming reagents within the kits and do not require additional Flow Cell Priming Kits (EXP-FLP002).

   The most recently release Ligation Sequencing Kit V14 (SQK-LSK114) and Ligation Sequencing Kit XL V14 (SQK-LSK114-XL) contain flow cell priming reagents which have been reformulated to be compatible with the improved Kit 14 adapter and R10.4.1 nanopore.

2. Ligation Sequencing Kit

   Kit overview:

   SQK-LSK114

   This is an Early Access product. For more information about out Early Access programmes, please see this article on product release phases.

   The Ligation Sequencing Kit V14 (SQK-LSK114) is our newest ligation-based sequencing kit optimised to achieve sequencing accuracies of over 99% (Q20+), with high output on our latest nanopore: R10.4.1. Further improvements include duplex sequencing which allows users to sequence both the template DNA strand and the complement strand. For more information, see the Kit 14 sequencing and duplex basecalling document.

   This Kit 14 upgrade includes previous updates such as higher capture rate of DNA to enable lower flow cell loading amounts, and fuel fix technology. Please note, due to the higher capture of the adapter, it is important to follow the flow cell loading recommendations in the protocols.

   SQK-LSK112

   The Ligation Sequencing Kit (SQK-LSK112) was our previous upgrade from the Ligation Sequencing Kit (SQK-LSK110) and is optimised for high accuracy. The upgrade included a new sequencing adapter (Adapter Mix H, AMX H) which improved accuracies to over 99% (Q20+), required lower flow cell loading amounts and contained the fuel fix technology to run long experiments without the need for fuel addition during a run.

   Please note, this kit (SQK-LSK112) is a legacy product and will soon be discontinued. We recommend all customers to upgrade to the latest chemistry for their relevant kit which is available on the Store. If customers require further support for any ongoing critical experiments using a Legacy product, please contact Technical Support via email: support@nanoporetech.com.

   SQK-LSK110

   The Ligation Sequencing Kit (SQK-LSK110) is an upgrade from the Ligation Sequencing Kit (SQK-LSK109) and is optimised for high output. The upgrade contains new fuel fix adapter (Adapter Mix F, or AMX-F) to use significantly less fuel during a sequencing run to improve sequencing output. We have also upgraded the loading beads to Loading Beads II (LBII) and Loading Solution (LS) for users who do not use loading beads for their experiments.

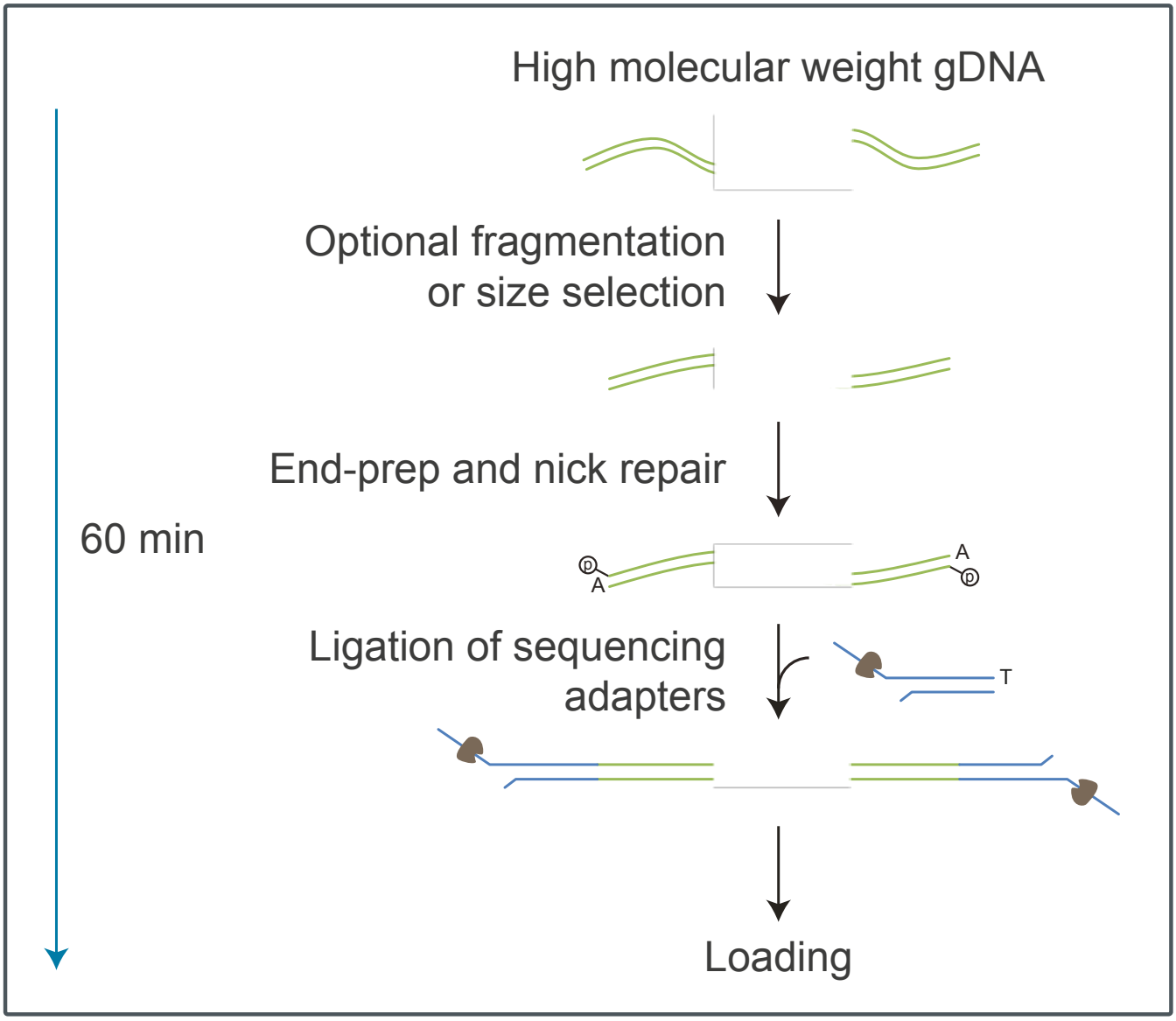
   SQK-LSK109

   This is a ligation-based sequencing kit to offer a flexible method of preparing sequencing libraries from dsDNA (e.g. gDNA, cDNA or amplicons) and is optimised to generate maximum output. Since release, this kit has undergone multiple upgrades and we recommend customers to upgrade to our latest chemistry to achieve the best results.

   For all Ligation Sequencing Kits (SQK-LSK109, SQK-LSK110, SQK-LSK112 and SQK-LSK114), the library preparation is simple and highly versatile, accommodating any double-stranded DNA sample input of 100–200 fmol of short fragment libraries (<10 kb) or 1 μg of long fragment libraries (>10 kb). Fragment length can be controlled by optional fragmentation or size selection.
   This kit is also compatible with upstream processes such as targeted enrichment by sequence capture and whole-genome amplification.

   Workflow:

   The library preparation involves two enzymatic steps. The first enzymatic step repairs any damage in the DNA molecules, such as nicks and generates uniform ends with 5’ phosphates and 3’ adenine overhangs. The second enzymatic step ligates the sequencing adapters which have complementary thymine tails with the dA-tailed template.



Sample input recommendations:

After DNA extraction, we recommend obtaining accurate measurements of how much DNA you have and assessing fragment length. It is important to ensure that you are putting the correct amount of material into the library preparation.
The recommended methods for quantifying your DNA samples are:

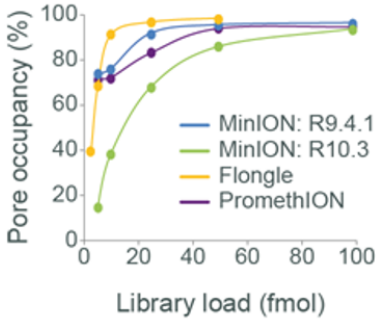
- Mass: Qubit Fluorometer and Qubit dsDNA BR Assay Kit
- Size:
  - Samples that are <10 kb: Agilent 2100 Bioanalyzer
  - Samples that are >10 kb: Agilent Femto Pulse
  - Oxford Nanopore Flongle
- Purity: Nanodrop 2000 Spectrophotometer

For information regarding how to quantify the mass of DNA samples for library preparation input, please refer to the Sample input section below.
Depending on fragment lengths, we recommend different starting inputs:

- For short fragment samples (<10 kb), we recommended a molar input of:
  - 100-200 fmol for R9.4.1 (FLO-MIN106 and FLO-PRO002)
  - 150-300 fmol for R10.3 (FLO-MIN111)
  - 100-200 fmol for R10.4 (FLO-MIN112 and FLO-PRO112)
  - 100-200 fmol for R10.4.1 (FLO-MIN114 and FLO-PRO114M)
  - 50-100 fmol for Flongle flow cells (FLO-FLG001)
- For long fragment samples (>10 kb) we recommend:
  - 1 μg is required for R9.4.1 (FLO-MIN106 and FLO-PRO002)
  - 2 μg is required for R10.3 (FLO-MIN111)
  - 1 μg is required for R10.4 (FLO-MIN112 and FLO-PRO112)
  - 1 μg is required for R10.4.1 (FLO-MIN114 and FLO-PRO114M)

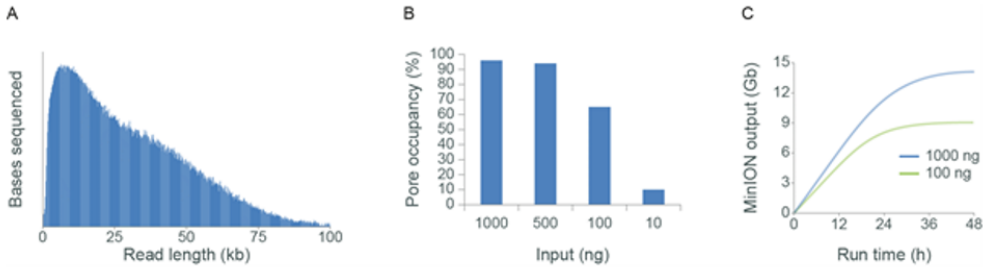
- 500 ng is required for Flongle flow cells (FLO-FLG001) Please note, we found that molar quantification is difficult and unreliable for samples consisting of long fragments.

We have found these starting inputs are almost always enough for optimal pore occupancy. The graph below illustrates pore occupancy of long fragment libraries using different mass input.



The relationship between input into the library preparation and flow cell output. Sequencing libraries were prepared using various starting inputs of a gDNA sample consisting of long molecules, and then run on the various flow cell types. The resulting pore occupancy is shown, and it was found that an input of ~1 μg is sufficient for optimal pore occupancy on R.9.4.1 flow cells and an input of ~2 μg was sufficient for optimal pore occupancy on R10.3 flow cells.

If you start with less than 1 μg, it is possible that output will decrease as pore occupancy may deteriorate and if pores are not always sequencing, output could be compromised. For more information on pore occupancy, please see the Sample input and recommendations section. However, even when starting with as little as 100 ng of high molecular weight DNA, we have observed outputs of ~10 Gb from R9.4.1 MinION/GridION flow cells and ~30 Gb from PromethION flow cells. As you decrease input below 100 ng, pore occupancy significantly deteriorates, and we recommend considering amplification (by PCR or multiple displacement amplification) to generate more template.



Flow cell output may be decreased when starting with less than 1 μg of HMW gDNA. In the experiment above, gDNA extracted from human cells (GM24385) was titrated as input into the Ligation Sequencing Kit. The resulting libraries were sequenced on MinION (R9.4.1 flow cells). Panel A: The typical read length distribution of a library. Panel B: Starting with 1 μg of high molecular weight DNA results in efficient pore occupancy. As input mass decreases, the number of threadable molecules decreases and this leads to reduced pore occupancy. Panel C: The reduction in pore occupancy at a lower input mass means that the pores spend less time sequencing, which results in a reduced flow cell output.

Output can be increased at low inputs by shearing high molecular weight templates (for example, using a Covaris g-TUBE or Megaruptor®). For more details, see 'Optional fragmentation of gDNA' in the Sample input and recommendations section.
Any double-stranded DNA input may be used for the Ligation Sequencing Kit. However, amplicons and cDNA can be prepared using other kits:

- Amplicons: use the Four-primer PCR protocol with the PCR Sequencing Kit (SQK-PSK004) or PCR Barcoding Kit (SQK-PBK004) for multiplexing
- cDNA: use the cDNA-PCR Sequencing Kit (SQK-PCS111) or the PCR-cDNA Barcoding Kit (SQK-PCB111.24) for multiplexing, or the Direct cDNA Sequencing Kit (SQK-DCS109)

Ligation Sequencing Kit components:

Currently, multiple Ligation Sequencing Kits are available. However, due to the chemistry upgrade, the kit reagents differ, as outlined in the table below.

SQK-LSK109 kit components	SQK-LSK110 kit components	SQK-LSK112 kit components	SQK-LSK114 kit components
DNA Control Strand (DCS)	DNA Control Strand (DCS)	DNA Control Strand (DCS)	DNA Control Sample (DCS)
Long Fragment Buffer (LFB)	Long Fragment Buffer (LFB)	Long Fragment Buffer (LFB)	Long Fragment Buffer (LFB)
Short Fragment Buffer (SFB)	Short Fragment Buffer (SFB)	Short Fragment Buffer (SFB)	Short Fragment Buffer (SFB)
Ligation Buffer (LNB)	Ligation Buffer (LNB)	Ligation Buffer (LNB)	Ligation Buffer (LNB)
Elution Buffer (EB)	Elution Buffer (EB)	Elution Buffer (EB)	Elution Buffer (EB)
Sequencing Buffer (SQB)	Sequencing Buffer II (SBII)	Sequencing Buffer II (SBII)	Sequencing Buffer (SB)
Adapter Mix (AMX)	Adapter Mix F (AMX F)	Adapter Mix H (AMX H)	Ligation Adapter (LA)
Loading Beads (LB)	Loading Beads II (LBII)	Loading Beads II (LBII)	Library Beads (LIB)
Sequencing Tether (SQT)	Loading Solution (LS)	Loading Solution (LS)	Library Solution (LIS)

SQK-LSK109 kit components	SQK-LSK110 kit components	SQK-LSK112 kit components	SQK-LSK114 kit components
	Flush Buffer (FB)	Flush Buffer (FB)	Flow Cell Flush (FCF)
	Flush Tether (FLT)	Flush Tether (FLT)	Flow Cell Tether (FCT)

- **DNA Control Sample (DCS)**
  - The DNA Control Sample (DCS) is a 3.6 kb amplicon of the Lambda phage genome. The control sample is included in the library and added to DNA samples during DNA repair and end-prep for troubleshooting purposes.
  - EPI2ME workflows automatically detect the control sample reads to assess if sample preparation and basecalling has been successful. This is especially useful when this information cannot be quickly determined from experimental data.
  - Note: The name of this reagent varies slightly between kit versions. However, the DCS acronym remains the same.
- **Sequencing Buffer (SQB)**
  - The Sequencing Buffer, included in the SQK-LSK109 kit, provides fuel and the optimal chemical conditions for powering DNA translocation through the nanopore.
- **Sequencing Buffer II (SBII)**
  - This is an upgraded component in the SQK-LSK110 kit to improve shipping and storage stability of the buffer.
- **Sequencing Buffer (SB)**
  - This component was upgraded in SQK-LSK114 to improve sequencing by reducing variability in current levels during experiment runs.
- **Short and Long Fragment Buffers**
  - Short Fragment Buffer (SFB) is used to enrich fragments of all sizes after adapter ligation.
  - Long Fragment Buffer (LFB) is used to enrich fragments of ≥3 kb after adapter ligation.
- **Ligation Buffer (LNB)**
  - This buffer is used to aid ligation of the sequencing adapters.
- **Elution Buffer (EB)**
  - This buffer is optimised to keep the integrity of the motor protein on the Y-shaped adapter.
- **Loading Beads (LB)**
  - These beads, included in the SQK-LSK109 kit, help reduce the frequency of blocking.
- **Loading Beads II (LBII)** and **Loading Solution (LS)**
  - These are upgraded components in the SQK-LSK110 kit. Loading Solution has been included to ease loading viscous libraries without beads.
- **Library Beads (LIB)** and **Library Solution (LIS)**
  - These reagents are upgraded components from LB and LS in the SQK-LSK114 kit to be compatible for our Kit 14 chemistry.
- **Flush Buffer (FB) and Flush Tether (FLT)**
  - For SQK-LSK109 kit, these components are in the Flow Cell Priming Kit but have been included in the SQK-LSK110 kit.
- **Flow Cell Flush (FCF)** and **Flow Cell Tether (FCT)**
  - These reagents are upgrades of the FB and FLT to be compatible for our Kit 14 chemistry.
  - The tethers are found in FLT of the Flow Cell Priming Kit and FCT of our Kit 14. The tethers bring the DNA strand to the membrane of the flow cell. This improves DNA capture by approximately 10,000 fold compared to capture without the tethers.
  - FCT in Kit 14 has had the tether optimised for duplex sequencing and to be compatible with the new sequencing adapter.
- **Adapter Mix (AMX)**
  - The Adapter Mix, included in the SQK-LSK109 kit, are the adapters which are ligated to DNA fragments for movement through the nanopore.
- **Adapter Mix F (AMX F)**
  - This is a new component included in the SQK-LSK110 kit which is an upgraded sequencing adapter. This component significantly reduced the consumption of ATP, leading to improved maintenance of enzyme speed and higher sequencing output.
- **Adapter Mix H (AMX H)**
  - This is a new adapter included in the SQK-LSK112 kit which is loaded with an updated sequencing enzyme for sequencing accuracies of over 99% (Q20+). It is also has a higher capture rate and contains a fuel fix technology for improved maintenance of enzyme speed without the need for fuel addition during the run.
- **Ligation Adapter (LA)**
  - This new sequencing adapter included in the SQK-LSK114 kit has been optimised for improved duplex rates and to increase output as well as further increased sequencing accuracies of over 99% (Q20+). This sequencing adapter also includes the improvements from the last upgrade, such as high capture rate and the fuel fix technology.
  - For all sequencing adapters (AMX, AMX F, AMX H and LA), they are ligated to the dA-tailed DNA fragments during the adapter ligation step. This ligation is assisted by the hybridisation of the A and T overhangs of the DNA fragments and adapters, respectively. The aim of this step is to prepare fragments which are adapted at both ends.
  - The Y-adapter is loaded with a processive enzyme, the motor protein, which regulates the DNA passage through the nanopore. The enzyme is active in solution and specialised bases in the adapter prevent it from contacting the rest of the DNA before loading onto the nanopore.

3. Ligation Sequencing Kit XL

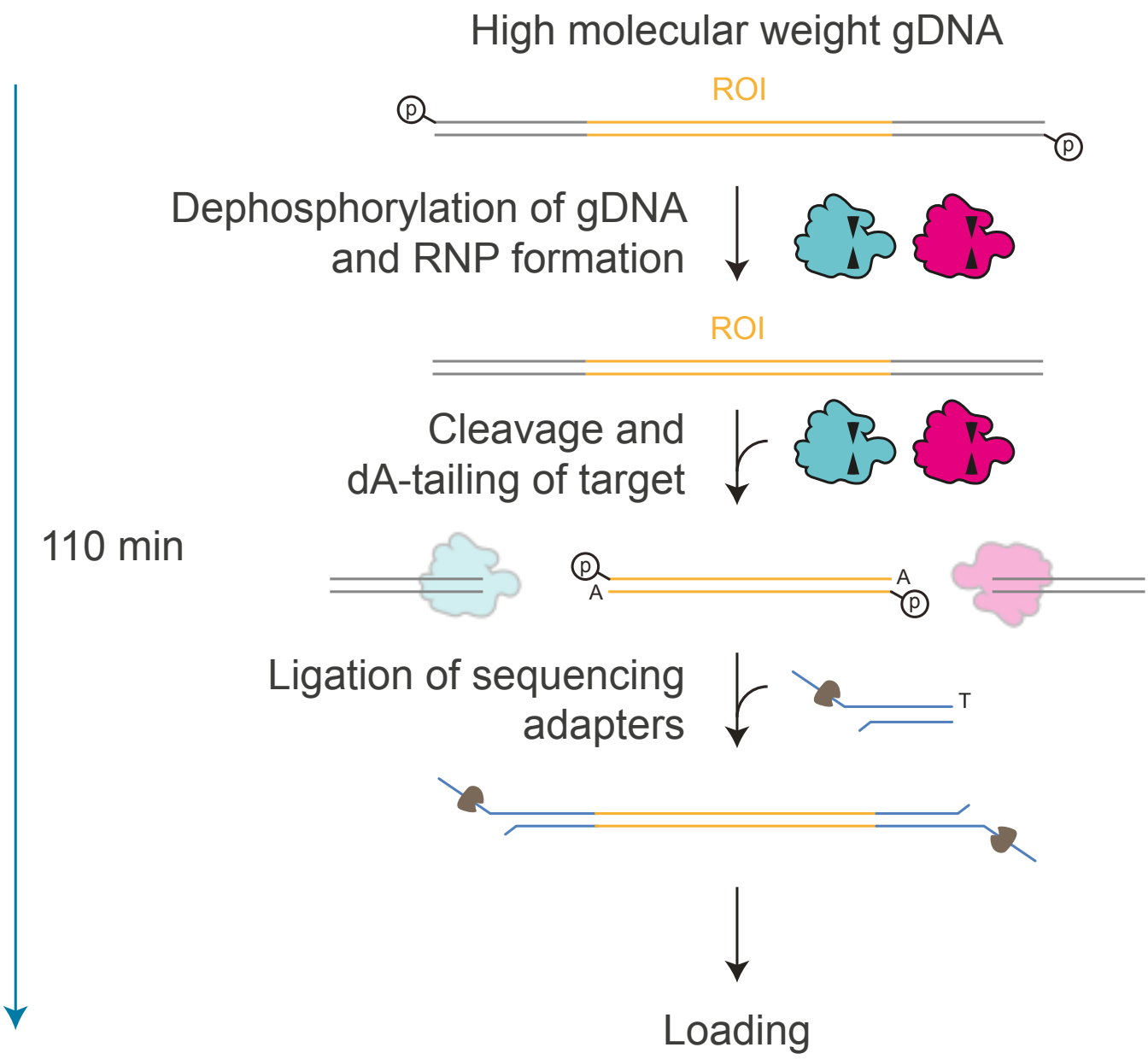
   The Ligation Sequencing Kit XL (SQK-LSK109-XL, SQK-LSK110-XL, SQK-LSK112-XL and SQK-LSK114-XL) is a scaled-up version of the Ligation Sequencing Kit and contains larger quantities of the same components.

   In this kit, there are sufficient reagents to generate 48 sequencing libraries and is recommended for users who would like to process multiple samples simultaneously, either with a multichannel pipette or a liquid handling robot.

4. Cas9 Sequencing Kit (SQK-CS0109)

   The Cas9 Sequencing Kit (SQK-CS9109) uses ligation-based chemistry and is a fast and flexible way to target specific regions of interest within a genome without amplification, increasing the depth of coverage. This is particularly useful for areas not amenable to PCR, “dark” areas of the genome, or for users wishing to target specific regions but maintain DNA modifications. The Targeted, amplification-free DNA sequencing using CRISPR/Cas document explains how to design and order crRNA probes and shares best practices for performing targeted sequencing.

   Using the recommended 5 μg high molecular weight gDNA, the DNA is dephosphorylated before adding Cas9 ribonucleoprotein particles (RNPs) with bound crRNA and tracrRNA to the gDNA for binding and cleavage of the Region of Interest (ROI). The cleavage by Cas9 reveals blunt ends with 5’ phosphates which are dA-tailed to prepare the blunt ends for barcode ligation. Sequencing adapters are ligated to the Cas9 cut sides. Finally, there is a clean-up step to remove excess adapters and the library is sequenced.



5. Related expansion packs:

There are expansion packs available to work alongside the Ligation Sequencing Kits to enable users to barcode their samples for more efficient use of the kits. For more information on barcoding samples and the kits available, please see the Barcoding and the available kits section.

Barcoding expansion packs available for the Ligation Sequencing Kits:

- Compatible with Ligation Sequencing Kit (SQK-LSK109):
  - Native Barcoding Expansion 1-12 (EXP-NBD104)
  - Native Barcoding Expansion 13-24 (EXP-NBD114)
  - Native Barcoding Expansion 96 (EXP-NBD196)

- Compatible with the following Ligation Sequencing Kits (SQK-LSK109, SQK-LSK110, SQK-LSK112 and SQK-LSK114):
  - PCR Barcoding Expansion 1-12 (EXP-PBC001)
  - PCR Barcoding Expansion 96 (EXP-PBC096)

For multiplexing samples using ligation-based Kit 14 chemistry, we recommend using our native barcoding kits:

- Native Barcoding Kit 24 V14 (SQK-NBD114.24)
- Native Barcoding Kit 96 V14 (SQK-NBD114.96)

Favourite

Chemistry Technical Document

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  - Rapid Sequencing Kit family
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  - RNA and cDNA sequencing and kits
  - Expansion packs

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