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BGISEQ-500 WGS library construction

GigaByte

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

Created: April 27, 2018

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Protocol Integer ID: 11837

Abstract

BGISEQ-500 is a desktop sequencer developed by BGI. Using DNA nanoball and combinational probe anchor synthesis developed from Complete Genomics™ sequencing technologies, it generates short reads at a large scale. Library construction on the platform includes fragmentation, size selection, end repair and A-tailing, adaptor ligation, PCR amplification, and splint circularization.

Materials

MATERIALS

- Fresh 80% ethanol **XILONG SCIENTIFIC**
- ERAT Buffer
- ERAT Enzyme
- Adapter Mix
- Ligation Buffer
- Ligation Enzyme
- TE buffer **Ambion**
- PCR Enzyme Mix
- Splint Buffer
- Digestion Buffer
- Digestion Enzyme

STEP MATERIALS

- Fresh 80% ethanol **XILONG SCIENTIFIC**
- ERAT Buffer
- ERAT Enzyme
- Adapter Mix
- Ligation Buffer
- Ligation Enzyme
- TE buffer **Ambion**
- PCR Enzyme Mix
- Splint Buffer
- Digestion Buffer
- Digestion Enzyme
- Fresh 80% ethanol **XILONG SCIENTIFIC**
- ERAT Buffer
- ERAT Enzyme
- Adapter Mix
- Ligation Buffer
- Ligation Enzyme

TE buffer **Ambion**

PCR Enzyme Mix

Splint Buffer

Digestion Buffer

Digestion Enzyme

Protocol materials

- Splint Buffer
- Ligation Buffer
- Ligation Enzyme
- PCR Enzyme Mix
- Digestion Enzyme
- Ligation Buffer
- Digestion Buffer
- Digestion Buffer
- Ligation Enzyme
- Adapter Mix
- Adapter Mix
- Splint Buffer
- Ligation Enzyme
- Fresh 80% ethanol **XILONG SCIENTIFIC**
- Digestion Enzyme
- ERAT Enzyme
- Fresh 80% ethanol **XILONG SCIENTIFIC**
- ERAT Buffer
- ERAT Buffer
- ERAT Buffer
- TE buffer **Ambion**
- Splint Buffer
- Fresh 80% ethanol **XILONG SCIENTIFIC**
- Adapter Mix
- Ligation Buffer
- TE buffer **Ambion**
- Digestion Enzyme
- ERAT Enzyme
- PCR Enzyme Mix
- ERAT Enzyme

Digestion Buffer

TE buffer **Ambion**

PCR Enzyme Mix

Fresh 80% ethanol **XILONG SCIENTIFIC**

ERAT Buffer

ERAT Enzyme

Adapter Mix

Ligation Buffer

Ligation Enzyme

TE buffer **Ambion**

PCR Enzyme Mix

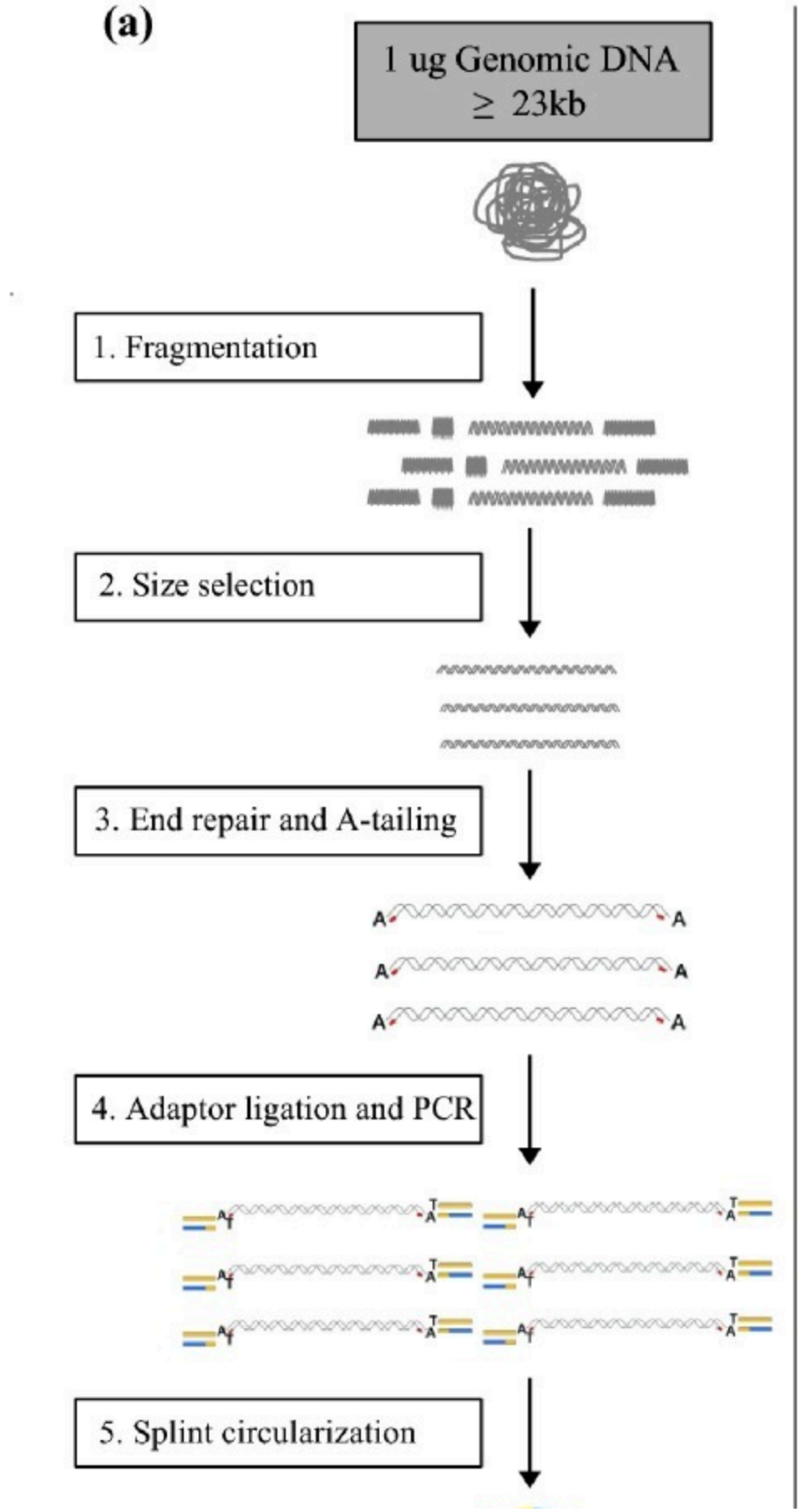
Splint Buffer

Digestion Enzyme

Digestion Buffer

Overview

1


(a)
1 ug Genomic DNA
≥ 23kb
1. Fragmentation
2. Size selection
3. End repair and A-tailing
A
A
A
A
A
A
4. Adaptor ligation and PCR
5. Splint circularization

DNA fragmentation

2 **1) Input genomic DNA sample**

Genomic DNA Sample Recommendation

	Nucleic Acid	*High-quality genomic DNA
	Molecular Weight	>23k bp
	Amount	1μg
	Concentration	≥12.5ng/μL
	Purity	OD260/OD280=1.8~2.0

*High-quality genomic DNA should be free of salt or organics. It could run as an intact band with DNA length >23kb during 1% agarose gel electrophoresis

2) Fragmentation

Use the Covaris Focused-ultrasonicator for genomic DNA fragmentation following the instructions of the instrument. Optimisation should be performed on DNA prior to experiment and analyzed with agarose electrophoresis or an Agilent 2100 BioAnalyzer

	Sequencing with	Input Amount	Reaction Volume	Derived Fragments
	PE 100	1μg	80μL	100-700 bp (main band≈200-300 bp)
	PE 50	1μg	80μL	100-500 bp (main band≈200 bp)
	PE 150	1μg	80μL	100-700 bp (main band≈400 bp)

3) Bead-based Cleanup

1. Place **AMPure XP magnetic beads** at RT for 30 min, fully thaw before use.
2. **PE100:**Pipette 48 μL AMPure XP magnetic beads to 80 μL shearing product, and mix well by gently pipetting 10 times, incubate for 5min at room temperature.
   (**PE50:**Pipette 80 μL AMPure XP magnetic beads to 80 μL shearing product, and mix well by gently pipetting 10 times, incubate for 5min at room temperature.**PE150:**Pipette 44 μL AMPure XP magnetic beads to 80 μL shearing product, and mix well by gently pipetting 10 times, incubate for 5min at room temperature.)
3. After brief centrifugation, place the non-stick tube on the magnet for 2min until the liquid clears, carefully transfer the supernatant to a new non-stick tube with a pipette.
4. **PE100:** Pipette 16 μL AMPure XP magnetic beads to 128 μL supernatant, mix well by gently pipetting 10 times, and incubate at room-temperature for 5 min.

(**PE50:** Pipette 40 µL AMPure XP magnetic beads to 160 µL supernatant, mix well by gently pipetting 10 times, and incubate at room-temperature for 5 min. **PE150:** Pipette 12 µL AMPure XP magnetic beads to 124 µL supernatant, mix well by gently pipetting 10 times, and incubate at room-temperature for 5 min.)

5. After brief centrifugation, place the non-stick tube on the magnet for 2min until the liquid clears, remove and discard the supernatant with a pipette.
6. Add 500 µL of fresh 80% ethanol, while the tube remains on the magnet, then, rotate the tubes in the rack by half turns 4 times to wash the beads then carefully remove and discard the supernatant after 1 min.
7. Repeat step 6 once, and remove all liquid from tube without disrupting the beads.

4) Homogenization

1. Use double-strand DNA quantification kit such as Qubit® dsDNA HS Assay Kit or Quant-iT™ PicoGreen® dsDNA Assay Kit, and quantify the sample as per the instructions of the quantification kit.
2. Remove 50 ng of sample (calculated based on its concentration) to a new 0.2 mL PCR tube, and add NF water to final volume of 40 µL.

00:30:00 3) 1
00:05:00 3) 2
00:02:00 3) 3
00:05:00 3) 4
00:02:00 3) 5
00:01:00 3) 6
00:01:00 3) 7
Fresh 80% ethanol **XILONG SCIENTIFIC**

End Repair and Tailing

3 1) Prepare the mixture as follows in PCR tube (do not vortex enzymes):

	Components	Volume
	DNA	40µL
	ERAT Buffer	7.1µL
	ERAT Enzyme	2.9µL
	Total	50µL

2) Mix well by gently pipetting (Do not mix by vortexing), concentrate the reaction liquid to tube bottom by brief centrifugation.

3) Place the PCR tube containing the reaction mixture of above step in a Thermal Cycler, and initiate the reaction as per the following conditions:

	Temperature	Time
	Heated lid	On
	37°C	30 min
	65°C	15 min
	4°C	Hold

00:45:00 3)

ERAT Buffer

ERAT Enzyme

Ligate Adapters

4 1) Add **5 µL of Adapter Mix** to above PCR tube, and mix well by pipetting. Now 16 Adapter Mix are available, 8 libraries in one lane strategy, every sample with 4 different barcodes.
2) Prepare the following reaction mixture (Note: Ligation Buffer II is viscous, pipette slowly):

	Components	Volume
	Ligation Buffer	23.4 µL
	Ligation Enzyme	1.6 µL
	Total	25 µL

3) Add 25 µL of above reaction mixture to the reaction solution containing adapters from above step.
4) Place the tube in a Thermal Cycler, then initiate reaction as per following condition:

	Temperature	Time
	Heated lid	On
	23°C	30 min
	4°C	Hold

5) After ligation, add **20 µL TE to final volume of 100 µL,** then transfer entire volume to a non-stick tube containing 50 uL of room temperature AMPure beads and mix by slow pipetting 10 times to avoid bubble formation.

00:30:00 4)

Adapter Mix

Ligation Buffer

Ligation Enzyme

TE buffer **Ambion**

Purify Ligated DNA

5 1) Incubate at room temperature for 5 min.
2) After brief centrifugation, place the non-stick tube on the magnet for 2 min until the liquid clears, remove and discard the supernatant with a pipette:
3) Add 500 µL of fresh 80% ethanol, while the tube remains on the magnet, then, rotate the tubes in the rack by half turns 4 times to wash the beads. Carefully remove and discard the supernatant after 1 min.
4) Repeat step 4) once, remove all liquid from tube without disrupting the beads.
5) Open the cap of non-stick tube, while the tube remains on the magnet, and dry at room temperature for 3 min.
6) Remove the non-stick tube from the magnet, add 46 µL of TE for DNA elution, mix well by pipetting and incubate at room temperature for 5 min.
7) After brief centrifugation, place the non-stick tube on the magnet for 2 min until the liquid clears, transfer all 44 µL of supernatant to a new 0.2 mL PCR tube ready for PCR in next step, or store at -20°C.

00:05:00 1)
00:02:00 2)
00:01:00 3)
00:03:00 5)
00:05:00 6)
00:02:00 7)

PCR

6 1)

	Components	Volume
	DNA	44 µL
	PCR Enzyme Mix	50 µL
	PCR Primer Mix	6 µL
	Total	100 µL

2) Place above PCR tube in a Thermal Cycler, and the initiate the reaction as per following conditions:

	Temperature	Time	Cycles
	Heated lid	On	

	95℃	3 min	
	98℃	20 sec	
	60℃	15 sec	8
	72℃	30 sec	
	72℃	10 min	
	4℃	Hold	

00:15:50 2)

PCR Enzyme Mix

Purify PCR Product

7 1) Place AMPure XP magnetic beads at room temperature 30 min in advance, mix well by vortexing before use.
2) Add 100 µL of AMPure XP magnetic beads to 100 µL of PCR product, mix well by gently pipetting 10 times, and incubate at room temperature for 5 min.
3) After brief centrifugation, place the non-stick tube on the magnet for 2 min until the liquid clears, remove and discard the supernatant with a pipette.
4) Add 500 µL of fresh 80% ethanol, while the tube remains on the magnet, then, rotate the tubes in the rack by half turns 4 times to wash the beads. Carefully remove and discard the supernatant after 1 min.
5) Repeat step 4) once, and try to suck up all liquid from tube bottom.
6) Open the cap of non-stick tube, while the tube remains on the magnet, and dry at room temperature for 3 min.
7) Remove the non-stick tube from the magnet, add 32 µL of TE water for DNA elution, mix well by pipetting and incubate at room temperature for 5 min.
8) After brief centrifugation, place the non-stick tube on the magnet for 2 min until the liquid clears, transfer the supernatant to a new non-stick tube. Proceed next step reaction or store at -20℃.

00:30:00 1)

00:05:00 2)

00:02:00 3)

00:01:00 4)

00:01:00 5)

00:03:00 6)

00:05:00 7)

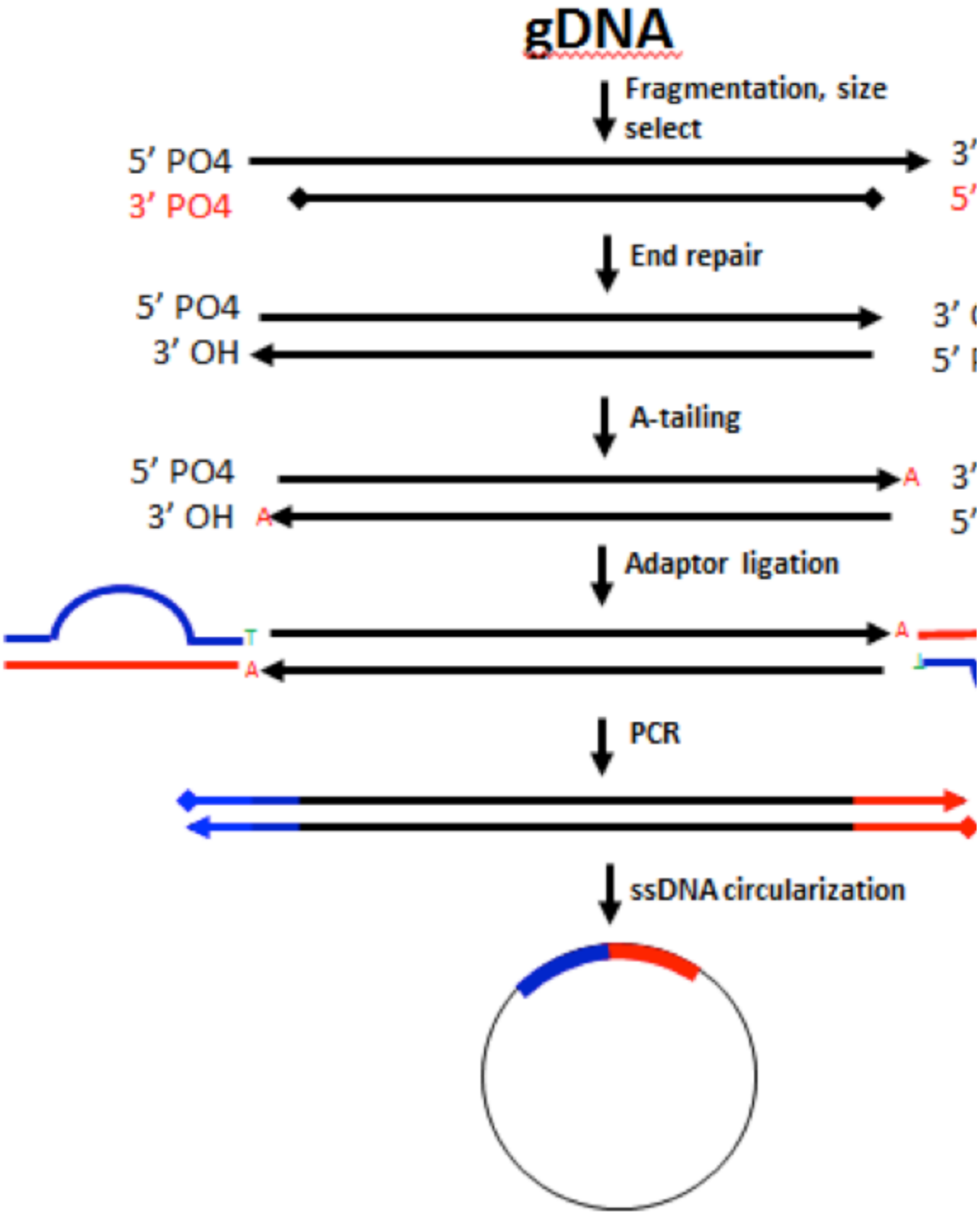
00:02:00 8)

Homogenization

8 1）Use double-strand DNA quantification kit such as Qubit® dsDNA HS Assay Kit or Quant-iT™ PicoGreen® dsDNA Assay Kit, and quantify the sample as per the instructions of the quantification kit.
2）It is recommended to mix samples of different Barcodes here.
3）Add mixed sample (calculated based on its concentration) to a PCR tube, and add NF water to final volume of 48 µL.

Circularization

9



1)Denature the homogenized PCR product on a Thermal Cycler at 95℃ for 3 min, then immediately transfer to ice batch.

2) Prepare reaction mixture on ice as per following system:

	Components	Volume
	Splint Buffer	11.6 µL
	Ligation Enzyme	0.2 µL

Total	11.8 µL

3) Add 11.8 µL of above reaction mixture to 48 µL of denatured DNA.
4) Place above PCR tube in a Thermal Cycler, and initiate the reaction as per following conditions:

Temperature	Time
Heated lid	on
37℃	30 min
4℃	Hold

00:03:00 1)

00:30:00 4)

Splint Buffer

Digestion

10 1) Prepare digestion reaction solution on ice as per following system:

Components	Volume
Digestion Buffer	1.4 µL
Digestion Enzyme	2.6 µL
Total	4 µL

2) After the circularization reaction is finished, directly add 4 µL of digestion reaction solution into circularized DNA solution, mix well and briefly centrifuge, then place the PCR tube in a Thermal Cycler, and initiate the reaction as per following conditions:

Temperature	Time
Heated lid	on
37℃	30 min
4℃	Hold

3) Add 7.5 µL of Digestion Stop Buffer to each reaction, mix well to terminate the reaction.
4) Transfer all the reaction solution to a new non-stick tube, ready for purification.

00:30:00 2)

Digestion Buffer

Digestion Enzyme

Purify Digestion Product

11 1) Place AMPure XP magnetic beads and place at room temperature for 30 min in advance. Mix well by vortexing before use.
2) Pipette 168 µL AMPure XP magnetic beads to digestion product, mix well by pipetting 10 times, and incubate at room temperature for 10 min.
3) After transient centrifugation, place the non-stick tube on the magnet for 2 min until the liquid clears, remove and discard the supernatant with a pipette:
4) Add 500 µL of fresh 80% ethanol, while the tube remains on the magnet, then, rotate the tubes in the rack by half turns 4 times to wash the beads. Carefully remove and discard the supernatant after 1 min.
5) Repeat step 4) once, and try to suck up all liquid from tube bottom.
6) Open the cap of non-stick tube, while the tube remains on the magnet, and dry at room temperature for 3 min.
7) Remove the non-stick tube from the magnet, add 32 µL of TE for DNA elution, mix well by pipetting and incubate at room temperature for 10 min.
8) After brief centrifugation, place the non-stick tube on the magnet for 2 min until the liquid clears, transfer the supernatant to a new non-stick tube. Store at -20℃, ready for preparation of DNB.

00:30:00 1)
00:10:00 2)
00:02:00 3)
00:01:00 4)
00:01:00 5)
00:03:00 6)
00:10:00 7)
00:02:00 8)