BioVendor Group NGS

epicGEN Quick Start Protocol

Prepare before start

	Reagents		Equipment
☐	Absolute Ethanol (100%)	☐	Digital capillary electrophoresis
☐	SPRIselect™ purification beads	☐	DNA quantification kit
☐	Nuclease-Free water	☐	PCR tubes, 0.2 ml
☐		☐	96-well, low-bind PCR plates
☐		☐	Low-bind DNA tubes, 1.5 ml
☐		☐	Filter tips with range 2–1000 µl
☐		☐	Magnetic stand
☐		☐	2 PCR thermal cyclers
☐		☐	Microcentrifuge
☐		☐	Vortex
☐		☐	Microseal® B PCR Plate Sealing Film, adhesive, optical

epicGEN Quick Protocol

1. ☐ **Enzymatic fragmentation**

Pre-cool PCR cycler to 4°C

☐ 19,5 µl DNA sample (1-1000 ng)
☐ 10,5 µl Fragmentation Master Mix

Reagent	1X reaction	… x reactions
epicGEN Fragmentation Buffer	3 µl	
epicGEN Fragmentation Enhancer	1,5 µl	
epicGEN Fragmentation Enzyme	6 µl	

Run Fragmentation Program (pg. 10)

2. ☐ Ligation of adaptors

- ☐ 30 µl fragmented DNA (from prev. step)
- ☐ 30 µl Ligation Master Mix:

Reagent	1X reaction	… x reactions
epicGEN Ligation Buffer	12 µl	
epicGEN Ligation Enzyme	4 µl	
epicGEN Adaptors (diluted according DNA input)	5 µl	
TE Buffer	9 µl	

Run Adaptor Ligation Program (pg. 11)

3. ☐ Post-Ligation Clean-up (SPRIselect™ purification beads)

- ☐ Add 48 µl of SPRIselect™ purification beads
- ☐ 5 minutes incubation at RT
- ☐ Discard supernatant
- ☐ Add 180 µl of 80% Ethanol (30 s incubation)
- ☐ Discard supernatant
- ☐ Add 180 µl of 80% Ethanol (30 s incubation)
- ☐ Discard supernatant and dry the beads
- ☐ Elution in 20 µl of TE Buffer
- ☐ Transfer sample to the new tube

4. ☐ PCR Amplification 1

- ☐ 20 µl DNA (after clean-up)
- ☐ 5 µl epicGEN UDI primers (Unique primer pair for each sample)
- ☐ 25 µl epicGEN PCR1 Master Mix

Run PCR1 Amplification Program (pg. 13)

5. ☐ Post-PCR Purification (SPRIselect™ purification beads)

- ☐ Add 90 µl of SPRIselect™ Beads
- ☐ 5 minutes incubation at RT
- ☐ Discard supernatant
- ☐ Add 180 µl of 80% Ethanol (30 s incubation)
- ☐ Discard supernatant
- ☐ Add 180 µl of 80% Ethanol (30 s incubation)
- ☐ Discard supernatant and dry the beads
- ☐ Elution in 20 µl of TE Buffer
- ☐ Transfer 20 µl of sample to the new tube

6. ☐ QC assessment

- ☐ Fluorometric quantification
- ☐ Fragment length distribution

7. ☐ Hybridization

- ☐ Prepare Hybridization Master Mix and leave on ice

Reagent	1X reaction	… x reactions
epicGEN Hybridization Buffer	9,5 µl	
epicGEN Hybridization Buffer Enhancer	3 µl	
epicGEN Blockers TS	2 µl	
epicGEN Solid Cancer & MSI panel	4,5 µl	

- ☐ Pool 500 ng DNA from each sample (8 samples per pool)
- ☐ Add 7,5 µl Human Cot DNA

Concentration of DNA on SPRIselect™ purification beads

- ☐ Add SPRIselect™ Beads. Calculate volume to to achieve a 1,8X sample-to-bead ratio

	Volume of pooled samples	Volume of beads (1,8X)
Pool 1		
Pool 2		
Pool 3		
Pool 4		

- ☐ 10 minutes incubation at RT
- ☐ Place on magnet and discard supernatant
- ☐ Add 80% Ethanol to cover the bead pellet (30 s incubation)
- ☐ Place on magnet and discard supernatant
- ☐ Add 80% Ethanol to cover the bead pellet (30 s incubation)
- ☐ Place on magnet, discard supernatant and dry the beads
- ☐ Elution in 19 µl of Hybridization Master Mix (5 min incubation)
- ☐ Transfer 17 µl of sample to the new tube

Run Hybridization Program overnight (pg. 15)

End of Day 1

8. ☐ Preparation of 1X Hybridization buffers

Reagent	Stock Buffer (1 reaction + 10%)	NF Water (1 reaction + 10%)	Final volume of 1X Buffer
epicGEN 2X Bead Wash Buffer	165 µl	165 µl	330 µl
epicGEN 10X Wash Buffer 1	27,5 µl	247,5 µl	275 µl
epicGEN 10X Wash Buffer 2	16,5 µl	148,5 µl	165 µl
epicGEN 10X Wash Buffer 3	16,5 µl	148,5 µl	165 µl
epicGEN 10X Stringent Wash Buffer	33 µl	297 µl	330 µl

9. ☐ Preparation of Streptavidin Beads

- ☐ Prepare Resuspension Master Mix

Reagent	1X reaction	… x reactions
epicGEN Hybridization Buffer	8,5 µl	
epicGEN Hybridization Buffer Enhancer	2,7 µl	
NF Water	5,8 µl	

10. ☐ Wash Streptavidin beads

- ☐ Add 100 µl of 1X Bead Wash Buffer into the 50 µl Streptavidin beads
- ☐ Place on magnet and discard supernatant
- ☐ Add 100 µl of 1X Bead Wash Buffer into the Streptavidin beads
- ☐ Place on magnet and discard supernatant
- ☐ Add 100 µl of 1X Bead Wash Buffer into the Streptavidin beads
- ☐ Place on magnet and discard supernatant
- ☐ Add 17 µl of Resuspension Master Mix
- ☐ Add the Streptavidin Beads to the sample

11. ☐ Capture of hybridized targets

12. ☐ Run Wash Program for 45 min (pg. 15)

13. ☐ Heated Wash

- ☐ Add 100 µl 1X Wash Buffer 1
- ☐ Place on magnet and discard supernatant
- ☐ Add 150 µl 1X Stringent Wash Buffer
- ☐ 5 min incubation in cycler
- ☐ Place on magnet and discard supernatant
- ☐ Add 150 µl 1X Stringent Wash Buffer
- ☐ 5 min incubation in cycler
- ☐ Place on magnet and discard supernatant

14. ☐ Room Temperature Wash

- ☐ Add 150 µl 1X Wash Buffer 1
- ☐ 2 min incubation
- ☐ Place on magnet and discard supernatant
- ☐ Add 150 µl 1X Wash Buffer 2
- ☐ 2 min incubation
- ☐ Place on magnet and discard supernatant
- ☐ Add 150 µl 1X Wash Buffer 3
- ☐ 2 min incubation
- ☐ Place on magnet and discard supernatant
- ☐ Add 20 µl NF Water

15. ☐ PCR Amplification 2

- ☐ 20 µl DNA
- ☐ 30 µl PCR2 Amplification Master Mix

Reagent	1X reaction	… x reactions
epicGEN PCR2 Master Mix	25µl	
epicGEN Primer Mix	1,25 µl	
NF Water	3,75 µl	

Run PCR2 Amplification Program (pg. 20)

16. ☐ Final Clean-up (SPRIselect™ purification beads)

- ☐ Add 75 µl of SPRIselect™ Beads
- ☐ 5 minutes incubation at RT
- ☐ Discard supernatant
- ☐ Add 125 µl of 80% Ethanol (30 s incubation)
- ☐ Discard supernatant
- ☐ Add 125 µl of 80% Ethanol (30 s incubation)
- ☐ Discard supernatant and dry the beads
- ☐ Elution in 22 µl of TE Buffer
- ☐ Transfer 20 µl of sample to the new tube

17. ☐ QC assessment

- ☐ Fluorometric quantification
- ☐ Fragment length distribution

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