Nanopore sequencing of single individual *Oikopleura dioica*

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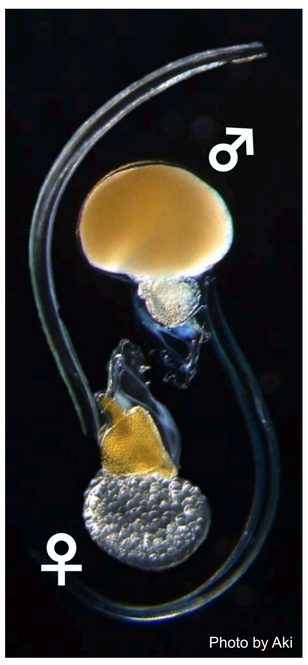
BACKGROUND



Why *Oikopleura dioica*?

Biological Feature:

- Globally distribution
- Short life cycle (5 days at 20 ℃)[1],
- Chordate body plan,
- Transparent embryonic development
- Separate sexes

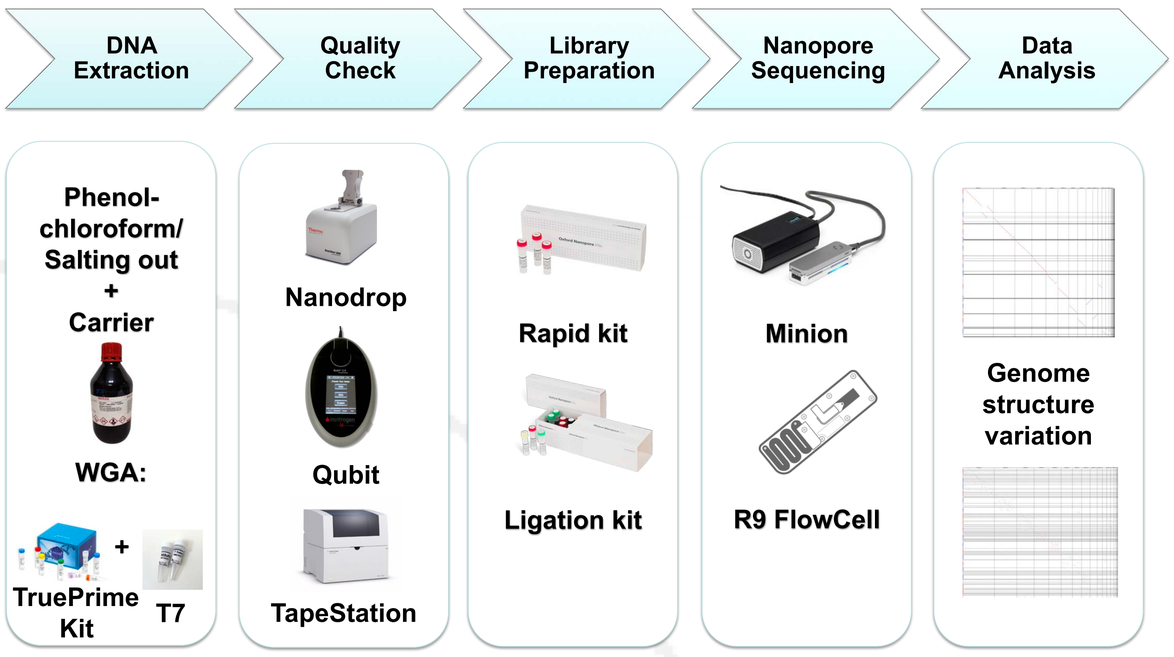
Genetical Feature:

- Most compact genome identified in any chordate, only 70Mb[2]
- >18000 predicted genes[2, 3]
- Small introns (peak at **47** base pairs, **2.4**% > **1**kb)

Why Nanopore sequencing?

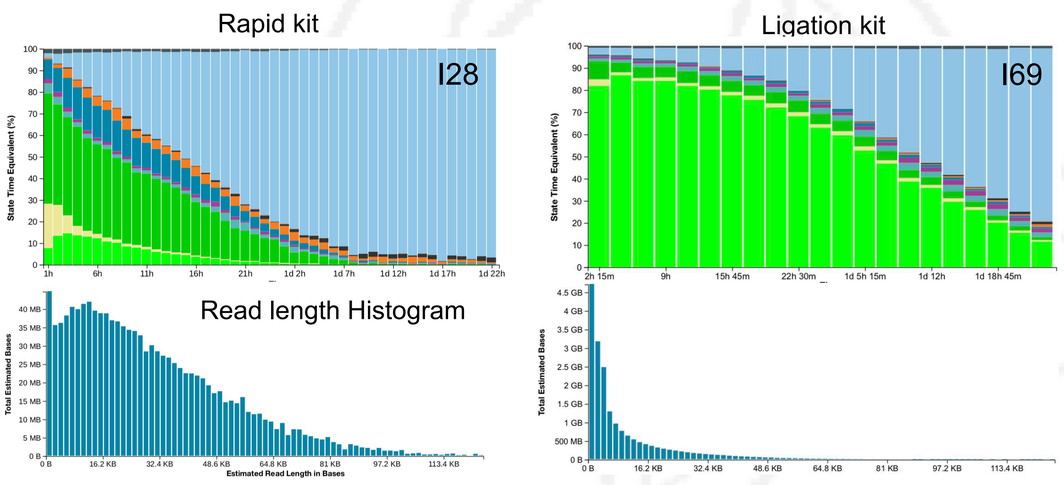
- Easy library preparation
- Sequencing in real time
- Long reads
- Accessible

METHODS



RESULT

1. Native genome DNA sequencing: Comparation between Rapid and Ligation kit

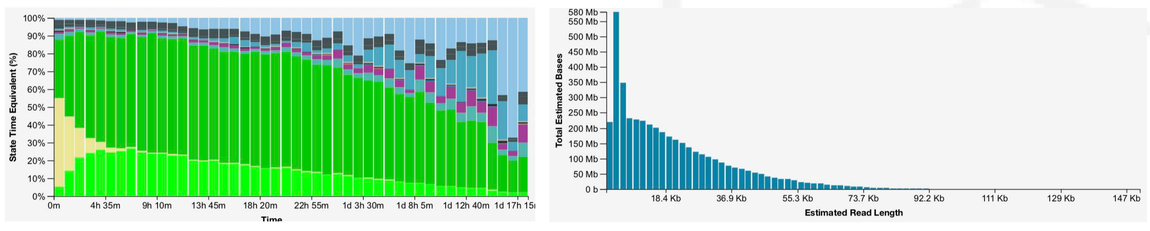


	Rapid kit			Ligation kit		
Sample No.	I28	I93	I32	O9	I69	O3
Input DNA(ng)	300	400	200	400	400	300
Yield(Gb)	0.95	0.4	0.13	17.6	16.8	8.05

2. WGA DNA sequencing performance: Ligation kit

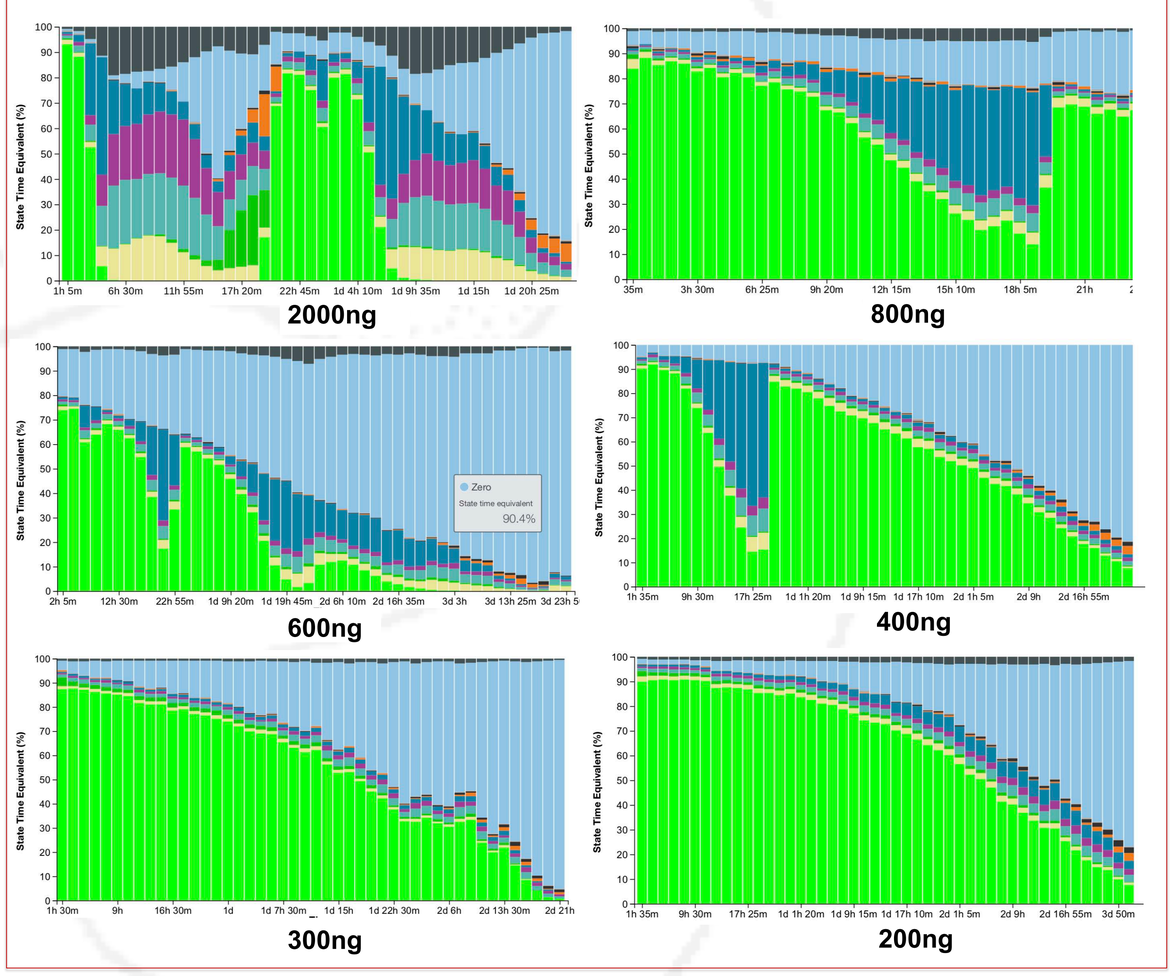
WGA DNA					
Sample No.	Bar1	KUM-F04	Yon1	KUM-F02	Bar3
Input DNA(ng)	500	200	300	300	600
Yield(Gb)	25.3	11.8	24.7	30.1	25.4
N50(kb)	3.47	2.4	3.2	3.39	1.91
Maximum Read length(kb)	479	129	166	203	82.9

3. Low input Native genome DNA sequencing performance: Ligation kit



Low input Native genome DNA					
Sample No.	AMA15	AMA17	Ala-R77	KUM-M1	KUM-M3
Input DNA(ng)	76.7	100	94	100	194
Loaded DNA(ng)	12	17	40	30	100
Yield(Gb)	4.3	2.2	9.1	19.4	16.6
N50(kb)	13.67	15.87	3.86	5.07	21.72
Maximum Read length(kb)	147	166	369	442	663

4. Sequencing buffer reloading: WGA DNA



Other founds in our Nanopore sequencing

- Salting out DNA extraction method can promise longer read
- T7 digestion is needed after WGA
- WGA DNA can also apply on Rapid kit, with low yield(1-4Gb) and short read length

CONCLUSION

- **Ligation kit is more stable than Rapid kit**
- **WGA DNA(Ligation kit) can generate higher yield, but shorter read length(N50:2-3kb)**
- **Low input DNA can also yield useful data**
- **200-300ng WGA DNA is enough for sequencing, if input is over 400ng, sequencing buffer reloading may be required**

FUTURE WORK

- **Barcoding sequencing**
- **Can we freeze Ligation kit library?**
- **Nanopore RNA sequencing**

REFERENCES

1. **Nishida, H**. (2008) Development of the appendicularian *Oikopleura dioica*: Culture, genome, and cell lineages. *Develop. Growth Differ* 50:S239-S256.
2. **Denoeud, F.** *et al.* (2010) Plasticity of animal genome architecture unmasked by rapid evolution of a pelagic tunicate. *Science* 330:1381- 1385.
3. **Seo,HC**,*et al*.(2001) Miniature genome in the marine chordate Oikopleura dioica. *Science* 294:2506.

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