## Nøvogene

### Isoform Sequencing (Full-length Transcript Sequencing)

### 1. Sample Requirements

Libray Type	Sample Type	Amount	Volume	Concentration	RIN (Agilent 2100)	Purity (Nanodrop <sup>™</sup> /Agarose Gel)
PacBio sequel II/ IIe RNA Library	Total RNA	≥ 800 ng	≥ 15 μL	≥ 60 ng/μL	≥ 6.5	A260/280=1.8-2.2; A260/230=1.3-2.5; NC/QC ≤ 2.5

\*RIN: RNA Integrity Number

#### 2. Sequencing Parameters

Platform	PacBio Sequel system
Recommended sequencing depth	≥ 15 G bases pair per sample
**Turnaround time	Within 7 weeks from project verification to data releasing without bioinformatic analysis

\*\*Turnaround time varies depending on the project volume.

### 3. Data Analysis Contents

Standard Analysis (for Species with Reference)						
(1) Data quality control						
(2) Identification, clustering and correction of full-length transcripts						
(3) Mapping reads to reference genome						
(4) Prediction and annotation (GO, Swiss-Prot) of novel transcripts						
	Fusion transcript analysis					
(E) Structural Analysis of Isoforms	Alternative splicing					
(5) Structural Analysis of Isoforms	Alternative polyadenylation					
	TTS & TSS prediction					
(6) Transcription factor analysis						
(7) IncRNA prediction						
	Isoform quantification and differential expression analysis					
(8) Differential expression analysis (Only for Compared Groupings)	GO enrichment					
	KEGG enrichment					

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#### Standard analysis (for Species without Reference)

(1) Data quality control

(2) Identification, cluster and correction of full-length transcripts

(3) Simple Sequence Repeat (SSR) analysis

(4) Functional annotation (via Nr, Nt, KEGG, GO, KOG, Swiss-Prot and Pfam databases)

(5) Transcription factor analysis (only for plants and animals species)

Isoform quantification and differential expression analysis

(6) Differential expression analysis (Only for Compared Groupings) GO enrichment

KEGG enrichment