Tn-seq & SorTn-seq

#E036 #approach

Reference

> Smith, Leah M., Simon A. Jackson, Paul P. Gardner, and Peter C. Fineran. 2021. “SorTn-Seq: A High-Throughput Functional Genomics Approach to Discovering Regulators of Bacterial Gene Expression.” Nature Protocols 16 (9): 4382–4418. https://doi.org/10.1038/s41596-021-00582-6.

SorTn-seq experimental design

- sort ~2.0 x 10^7 cells (binned) based on fluorescence
  - three populations: low or high expression and the middle bin that is "depleted" of potential regulators of the reporter expression
- FACS enrichment of mutant library aliquots is performed in **triplicate**
- DNA is extracted from the different bins, as well as from an **unsorted control sample (input)**
  - this provides information on gene essentiality and library diversity, but is not required
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Adapter, Splinkerette

Reference

> Devon, Rebecca S., David J. Porteous, and Anthony J. Brookes. 1995. “Splinkerettes—Improved Vectorettes for Greater Efficiency in PCR Walking.” *Nucleic Acids Research* 23 (9): 1644–45. https://doi.org/10.1093/nar/23.9.1644.

Notes

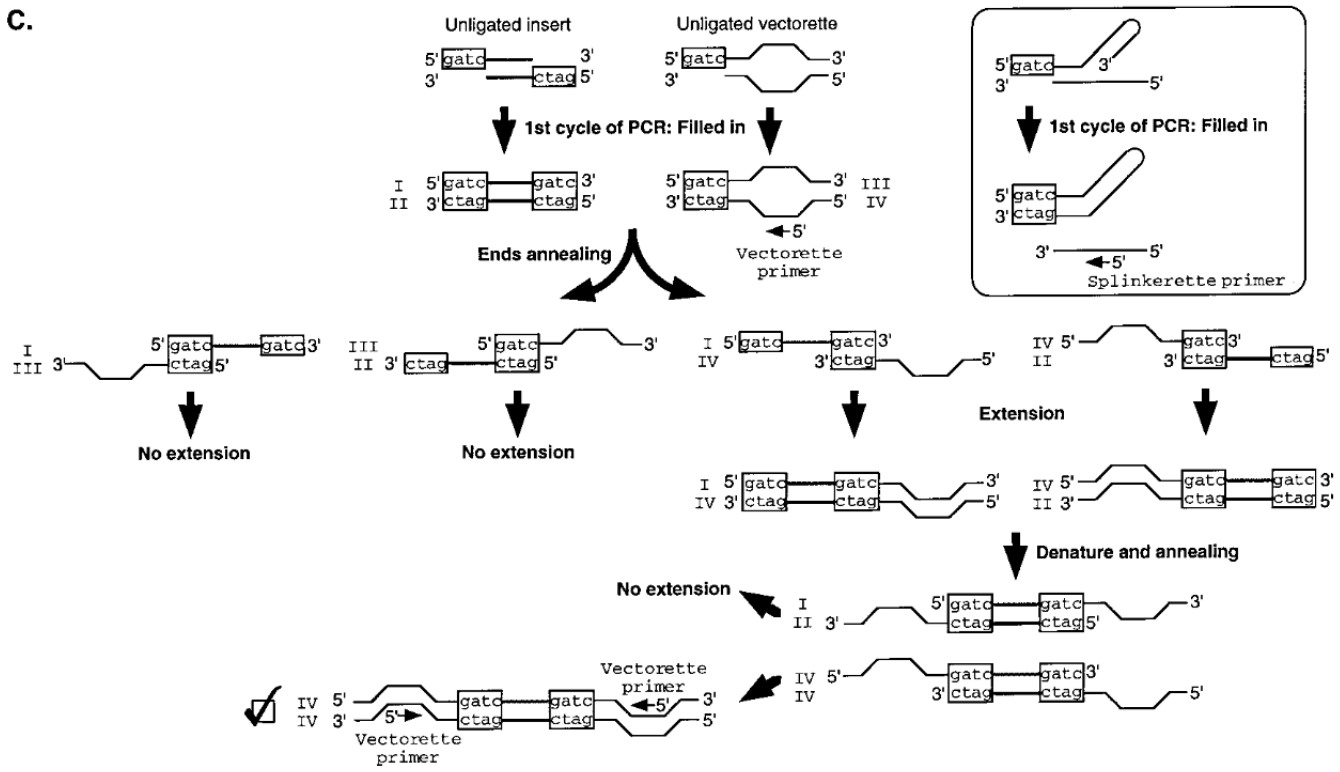
- the hairpin design helps minimize "end-repair" mis-priming products. details aside, it basically reduces non-specific products.
- an important feature of the splinkerette and its predecessor "vectorette" design is the mismatched region in the annealed adapter (dsDNA). with one of the primers used for the first round of PCR matching the top strand inside the mismatched region of the adapter, exponential amplification cannot happen until the transposon specific primer first

synthesize the first strand, ensuring amplification only happens on the gDNA fragments containing the transposon.

end-repair priming

Reference

> Hui, E.K.-W., P.-C. Wang, and S.J. Lo. 1998. “Strategies for Cloning Unknown Cellular Flanking DNA Sequences from Foreign Integrants.” *Cellular and Molecular Life Sciences* 54 (12): 1403–11. https://doi.org/10.1007/s000180050262.



Illumina Y-shaped adapter design

Reference

- SEQanswers post on the purpose of the Y-shaped adapter
- SEQanswers post on what happens during the PCR amplification of the adapter ligated fragments
  - contains a useful diagram visualizing what happens during the PCR step. I downloaded it to the `RDSS/Shared/Protocols/Illumina`
- Youtube video explaining Illumina library prep

Why use a Y-shaped adapter?

- In Illumina library prep, the addition of adapters to fragmented sequences uses TA ligation, resulting in the **same adapter** ligated to both ends of a fragment.
- If one uses a fully complementary adapter design, the result is a product with inverted repeats on both ends. Each single stranded DNA would have fully complementary ends that can self-anneal and form a hairpin.
- After any denaturation step (amplification, flow cell loading, cluster formation), complementary ends of single-stranded DNA would self-anneal to form a loop. Self-annealing, being unimolecular, is an efficient process, and those ends would no longer be substrates for the desired reaction.

TraDIS library prep

Reference

- Barquist, Lars, Matthew Mayho, Carla Cummins, Amy K. Cain, Christine J. Boinett, Andrew J. Page, Gemma C. Langridge, Michael A. Quail, Jacqueline A. Keane, and Julian Parkhill. 2016. “The TraDIS Toolkit: Sequencing and Analysis for Dense Transposon Mutant Libraries.” *Bioinformatics* 32 (7): 1109–11. https://doi.org/10.1093/bioinformatics/btw022.
- Bronner, Iraad F., Thomas D. Otto, Min Zhang, Kenneth Udenze, Chengqi Wang, Michael A. Quail, Rays H. Y. Jiang, John H. Adams, and Julian C. Rayner. 2016. “Quantitative Insertion-Site Sequencing (QIseq) for High Throughput Phenotyping of Transposon Mutants.” *Genome Research* 26 (7): 980–89. https://doi.org/10.1101/gr.200279.115.

Notes

- this and a later paper both mentioned the need for a modified sequencing cycle incorporating "dark cycles" to accommodate the special feature in this library prep, where a true integration event would always have the same bp in the beginning, causing issues with the Illumina sequencer, which uses the first few bases to gauge library diversity.
- Do we need to do this?
- How does the individual PCR steps work in the QI-seq library prep?
- In the Cunningham protocol, what is the sequencing primer?