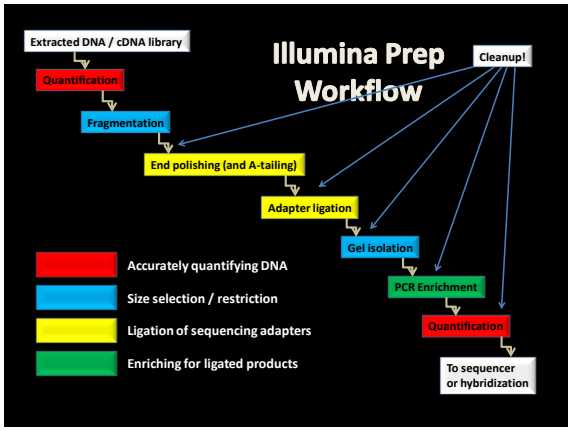
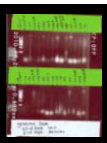
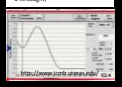
Library Prep & Multiplexing



Quantification Methods:

- Gel electrophoresis
- Nanodrop - spectrophotometry
- BioAnalyzer




Qubit - fluorometry

Benefits
- pretty cheap
  - ~$1000 initial investment
  - ~$0.55 / sample
- fairly quick
- (usually) low sample consumption (1-2 ul)
- minimal contamination issues
- highly accurate readings

Drawbacks
- a little slower, more consumables, than Nanodrop
- should have ballpark [DNA] before running

Fragmentation Methods:

Sonication:

Pros:
- Can be high-throughput (8-24 samples)
- Very little sample loss
- $0 / sample

Cons:
- Can be slow
- High initial cost ($19-40,000)

Nebulization:

Pros:
- Low initial investment: $20 / chamber

Cons:
- Typically lose ~1/2 sample
- Can be slow (6 min / sample)
- Costs add up $20 / sample


Cleaning Methods:

Qiagen column-based cleanups

Benefits
- fairly rapid cleanup per sample
- affordable (~$2 / cleanup)
- ~ required for gel isolation
- range of cleanup products
  - PCR cleanup kit
  - Gel Extraction kit

Drawbacks
- reliably mediocre
  - yield often on low side
- columns not amenable to high-throughput
  - high-throughput option available, but expensive

Cleaning Methods:

AMPure paramagnetic beads (SPRI-beads)


Benefits
- fairly rapid cleanup per sample at high sample numbers
  - very amenable to high-throughput
- affordable ($0.20-0.60 / cleanup)
- high yield
- preferentially binds fragments >200 bp

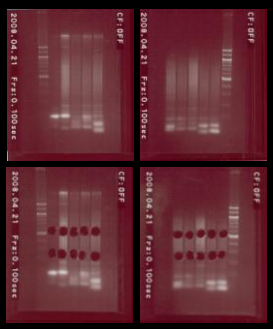
Drawbacks
- slower than Qiagen for low sample numbers
- technically a little more challenging
- initial cost (magnet) a little high ($250 - >$500)
- short shelf life (6 months to 1 year)
- Less efficient with very low [DNA]

Size Selection:

Gel Isolation – required step(?)

Procedure
- ≥2% agarose gel
- 1 hour run time
- UV illumination
- cut out gel pieces of selected size
- Qiagen gel cleanup

Tips?
- use shortened pipette tips to cut gel plugs out
- melt gel directly out of tips with buffer QG



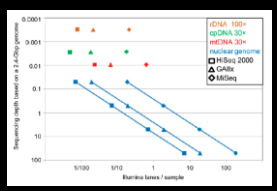
Size Selection:

Alternatives?

A couple of possibilities
- alter AMPure buffer salt concentration through dilution
  - allows selection of variable size classes
- SKIP IT!
  - can be ok for single end preps

Multiplexing

Addition of a unique sequence identifier (barcode) allowing multiple samples to be run together on a single flow cell lane.



$$\text{Multiplex level} = \frac{(\text{Lane Capacity}) \bullet (\text{Correction}) \bullet (\%\text{ hitting target})}{(\text{Depth Desired}) \bullet (\text{Target Genome Size})}$$

Straub et al 2012

Multiplexing

Extracted DNA / cDNA library
→ Quantification
→ Fragmentation
→ End polishing (and A-tailing)
→ Adapter ligation
→ Gel isolation
→ PCR Enrichment
→ Quantification
→ To sequencer or hybridization

Quantify and pool after adapter ligation

HOWEVER:

Maintenance of about equal [DNA] is strongly recommended.

Possible to pool before gel size selection:

Quantify → Pool Subset → Gel → PCR → Quantify → Final pool

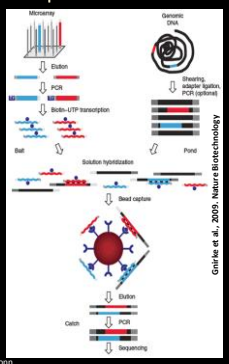
Hyb-Seq

Targeted Sequencing: HybSeq Enrichment

Solution Phase Hybridization

Examples: Agilent SureSelect, NimbleGen SeqCap EZ, Mycroarray MyBait, etc

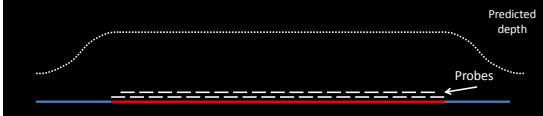
- 'Baits' synthesized on arrays
- 120 bp RNA probes.
- Hybridization in solution
- Immobilization via biotin-streptavidin capture
- 1 – 5 ug DNA of input library.
- Scalable to large samples, targets



Slide courtesy of Rich Cronn

Targeted Sequencing: HybSeq Enrichment

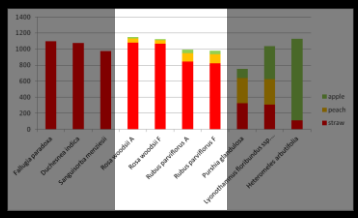
Multi –Locus Sequencing

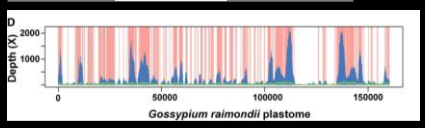


SNP Genotyping

Targeted Sequencing: HybSeq Enrichment

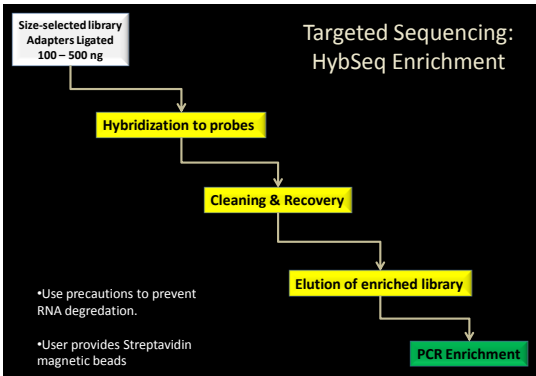
Probe specificity allows enrichment in distantly related organisms:





Cronn et al 2012

Targeted Sequencing: HybSeq Enrichment



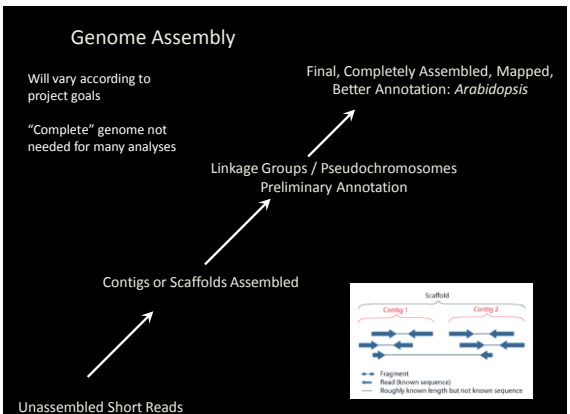
- Use precautions to prevent RNA degredation.
- User provides Streptavidin magnetic beads

Genome Assembly

Genome Assembly

Will vary according to project goals

"Complete" genome not needed for many analyses



Assembly metrics:

What is a good assembly?

- Coverage: Portion of the target genome represented in the assembly.
- N50: Contig size where the sum of all equal and greater contigs equals 50% the sum of all contigs.
- Number of contigs or scaffolds: A lower number indicates better success. *
- Depth: Number of reads hitting a particular position (often averaged across a region).

Data Preparation

- Quality Control
  - Trim low quality bases at the ends of reads
  - Removal of adapters
- Removal of high-copy regions
  - Chloroplast
  - Mitochondria
  - rDNA

Kmer selection

- Kmer – A substring, with length K, of a read.
  - A read of length L has (L – K +1) Kmers.
- Optimal value of K needs to be found for each assembly.
- K too low: Increase of spurious Kmer overlap.
- K too high: Kmers will not overlap with enough neighbors.
- Depends on sequencing depth, error rate, genome complexity

Programs

- Velvet
- ABySS
- SOAPdenovo
- ALLPATHS-LG

- Major considerations (beyond quality)
  - Memory Use
  - Time
- Comparisons:
  - Assemblathon, GAGE