# Sequence Capture Experiment Design

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# Sequence Capture

- Essentially reducing genomic datasets from entire genomes to smaller subsets, for many individuals
- Can be used for any scale genomic question
- Decreases cost for many individuals (as compared with shotgun sequencing)
- Better for use on degraded samples than some HTS methods (RAD-seq)

# Project Goals

- Large Scale Phylogenies:
  - ‘Universal markers’ eg. UCE’s
  - Exons/Introns
  - Chromosomes or genes etc.
- Small Scale/ population studies:
  - SNP’s
  - Species ID’s
  - Etc.

# A priori Information

- Genomic resources available
  - Previous genetic studies done?
    - All published information can be used
  - Markers found un/informative?
    - Metrosideros plants: ended up generating similar results to microsatellites from ~5,000 SNPs
    - Introns and Exons with previous knowledge useful

# Practical Concerns

- How many samples total for project?
- What sequencing platform and indices?
- Will this tool be used for additional projects?
- Which kit best suits your needs?
  - Example calculation:

    (based on MYbaits 20,000 probe kit for 12 samples-smallest kit available)

    Kit cost: $2400 + S&H = $2,450

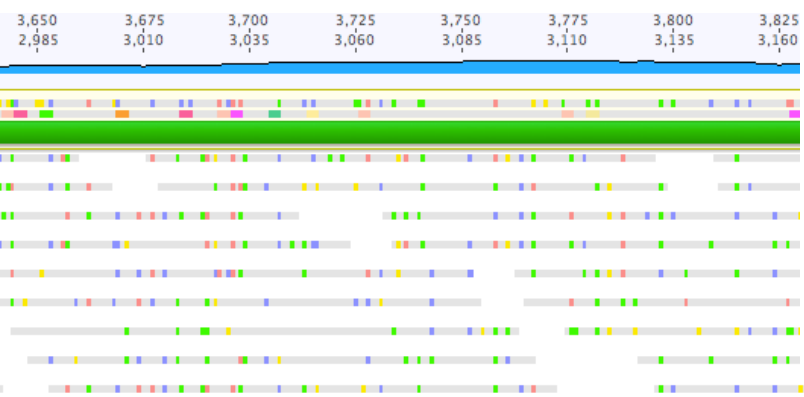
    If 5,000 SNPs included then during synthesis of a 20k kit repeated many times (concentration of 500ng/µl)

    Dilute probe set 1:10 stretches from 12 'samples' (can also multiplex) to 12*10=120 samples ($20/sample)

    If multiplexed with just 2 individuals = 120 * 2 = 240 individuals per kit or $10 per sample (2400/240) versus $200/sample as published on website

# In-Solution Design Steps

- Tiling
  - 2x usually plenty
- Number of probes
  - 4,000 or 40,000?
- Dilution of probes
  - Can dilute up to 1:15 but unstable after further dilution
  - must dilute at each enrichm




| | Pricing for Custom Kits | | | |
|---|---|---|---|---|
| Kit Name (Maximum # of bait sequences) | MYbaits-1 (20,000) | MYbaits-2 (40,000) | MYbaits-3 (60,000) | MYbaits-10 (200,000) |
| # of Captures | Price per Kit (USD) | | | |
| 12 | $2,400 | $3,000 | $3,600 | $7,200 |
| 24 | $3,600 | $4,500 | $5,400 | $10,800 |
| 48 | $5,760 | $7,200 | $8,640 | $17,280 |
| 96 | $8,640 | $10,800 | $12,960 | $25,920 |
| 192 | $13,440 | $16,800 | $20,160 | $40,320 |
| 384 | $23,040 | $28,800 | $34,560 | $69,120 |
| 768 | $38,400 | $48,000 | $57,600 | $115,200 |
| # of Captures | Effective Price Per Capture (USD) | | | |
| 12 | $200 | $250 | $300 | $600 |
| 24 | $150 | $187.5 | $225 | $450 |
| 48 | $120 | $150 | $180 | $360 |
| 96 | $90 | $112.5 | $135 | $270 |
| 192 | $70 | $87.5 | $105 | $210 |
| 384 | $60 | $75 | $90 | $180 |
| 768 | $50 | $62.5 | $75 | $150 |

Target size per 20,000 baits depends on the bait length, tiling density and number of target loci. Please consult with us for more details.

# Practical: EctoBaits

## Simultaneous identification of host, vector and pathogen DNA via in-solution capture

**Melissa T. R. Hawkins*, Michael G. Campana***, Kristin Stewardson, Justin Lock, Kristofer M. Helgen, Hillary Young, Leah Card Jesús E. Maldonado, William J. McShea, Robert C. Fleischer

*In Prep for Molecular Ecology Resources*

- Project Goal: ID of host, vector and pathogen from tick samples
- Workflow:
  - Used mostly published Genbank sequences
  - Align sequences
  - Reduce redundancy (if necessary)
  - Split reads to probe lengths
  - Spend some time explaining project to Tech Support and send files for QC
    - Will test for base composition, strings of nucleotides and ambiguities etc.
  - Wait 6-8 weeks and test array!

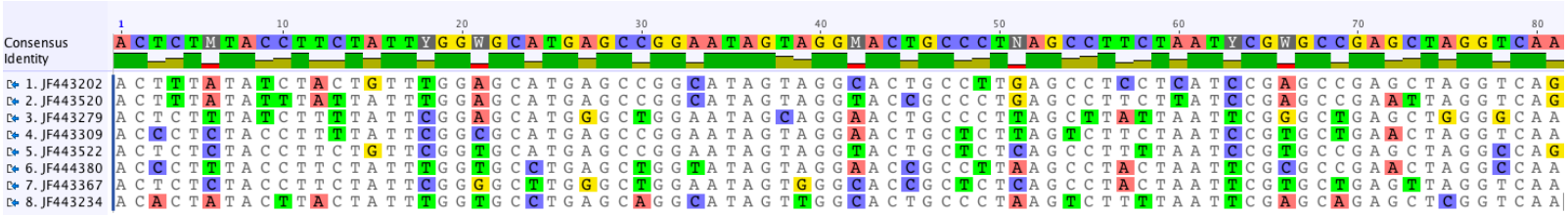
# Download Genbank Sequences

- This project included African and American Mammals, Birds, Ticks, Pathogens
  - This study used primarily CO1 and Cyt *b*

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| • | AB004237 | Felis catus mitochondrial DNA for cytochrome... | Felis catus | 1,140 | AB004237 | 2575782 | DNA |
| • | AB015077 | Sus scrofa domesticus mitochondrial cytb gen... | Sus scrofa | 1,140 | AB015077 | 3241885 | DNA |
| • | AB015081 | Sus scrofa domesticus mitochondrial cytb gen... | Sus scrofa | 1,140 | AB015081 | 3241893 | DNA |
| • | AB462161 | Procyon lotor mitochondrial CYTB gene for cyt... | Procyon lotor | 1,140 | AB462161 | 347582092 | DNA |
| • | AF007908 | Ursus americanus cinnamomum cytochrome b... | Ursus amer... | 719 | AF007908 | 2305025 | DNA |
| • | AF007934 | Ursus americanus americanus cytochrome b (c... | Ursus amer... | 719 | AF007934 | 2305077 | DNA |
| • | AF028140 | Canis latrans cytochrome b (cytb) gene, mitoch... | Canis latrans | 396 | AF028140 | 2826650 | DNA |
| • | AF028156 | Urocyon cinereoargenteus cytochrome b (cytb)... | Urocyon cin... | 396 | AF028156 | 2826682 | DNA |
| • | AF057121 | Lontra canadensis cytochrome b (cytb) gene, m... | Lontra cana... | 1,140 | AF057121 | 3511089 | DNA |
| • | AF068548 | Mustela vison cytochrome b (cytb) gene, partia... | Neovison vi... | 337 | AF068548 | 3273799 | DNA |

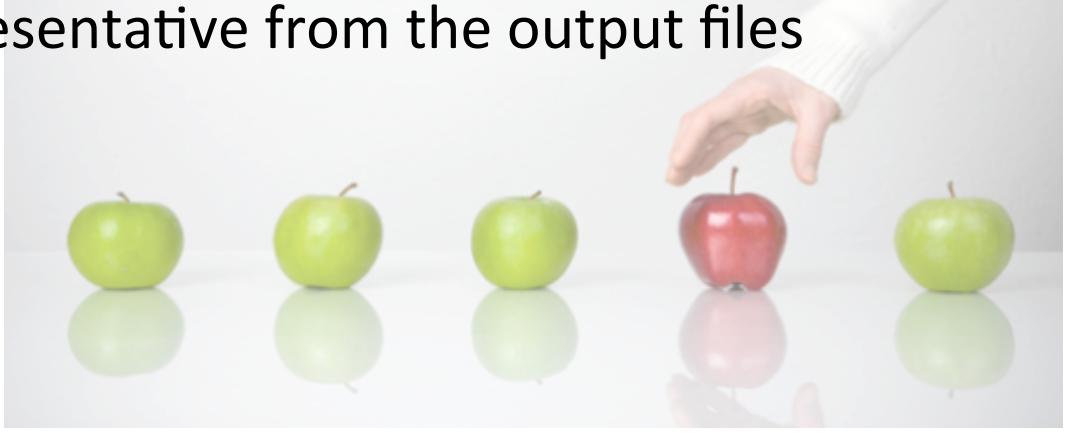
# Combine and align with any unpublished data

- Alignment for orientation (5’-3’ direction)
- Determine if you need to reduce redundancy
  - All exact matches should be reduced to single sequence



# Reducing redundancy

- Probes can anneal to sequences up to 20% (Hawkins et al. 2015 *in revision*) divergent, but better to estimate 10-15% divergence to avoid losing molecules
- Can cluster through CD-HIT-EST to remove overly-similar reads
  - Upload fasta file, determine threshold, use a single representative from the output files



# CD-HIT Suite: Biological Sequence Clustering and Comparison

Server home | cd-hit | cd-hit-est | h-cd-hit | h-cd-hit-est | cd-hit-2d | cd-hit-est-2d | result

**Sequence file and databases**

Load Query Fasta file from your computer: Choose File No file chosen

☐ Incorporate annotation info at header line

**Sequence Identity Parameters**

Sequence identity cut-off 0.9

**Algorithm Parameters**

-r: comparing both strands ◉ No ○ Yes

-G: use global sequence identity ○ No ◉ Yes

-g: sequence is clustered to the best cluster that meet the threshold ○ No ◉ Yes

-b: bandwidth of alignment 20

**Alignment Coverage Parameters**

-aL: minimal alignment coverage (fraction) for the longer sequence 0.0

-AL: maximum unaligned part (amino acids/bases) for the longer sequence unlimitec

-aS: minimal alignment coverage (fraction) for the shorter sequence 0.0

-AS: maximum unaligned part (amino acids/bases) for the shorter sequence unlimitec

-s: minimal length similarity (fraction) 0.0

-S: maximum length difference in amino acids/bases(-S) unlimitec

**Mail address for job checking**

Give your mail address:

Submit Clear

Upload aligned or unaligned files

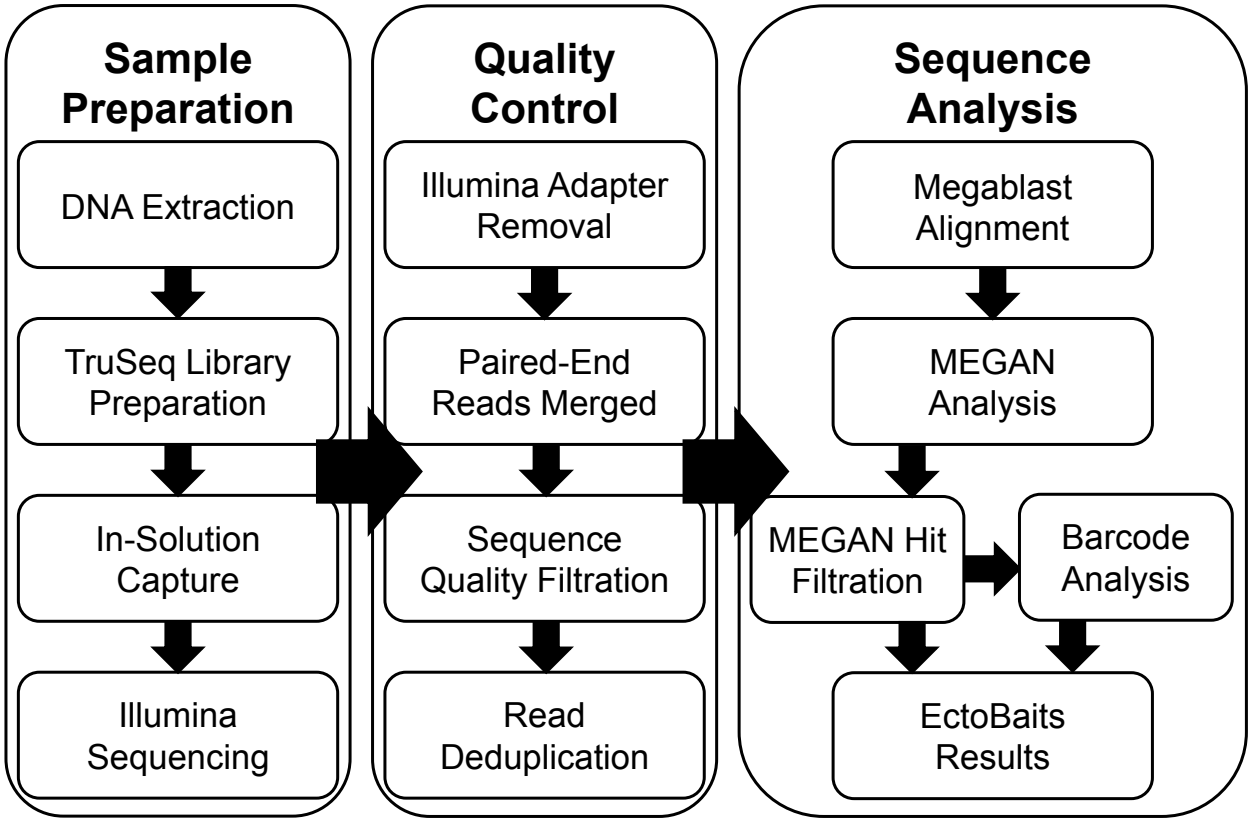
Set identity parameter

Enter email address

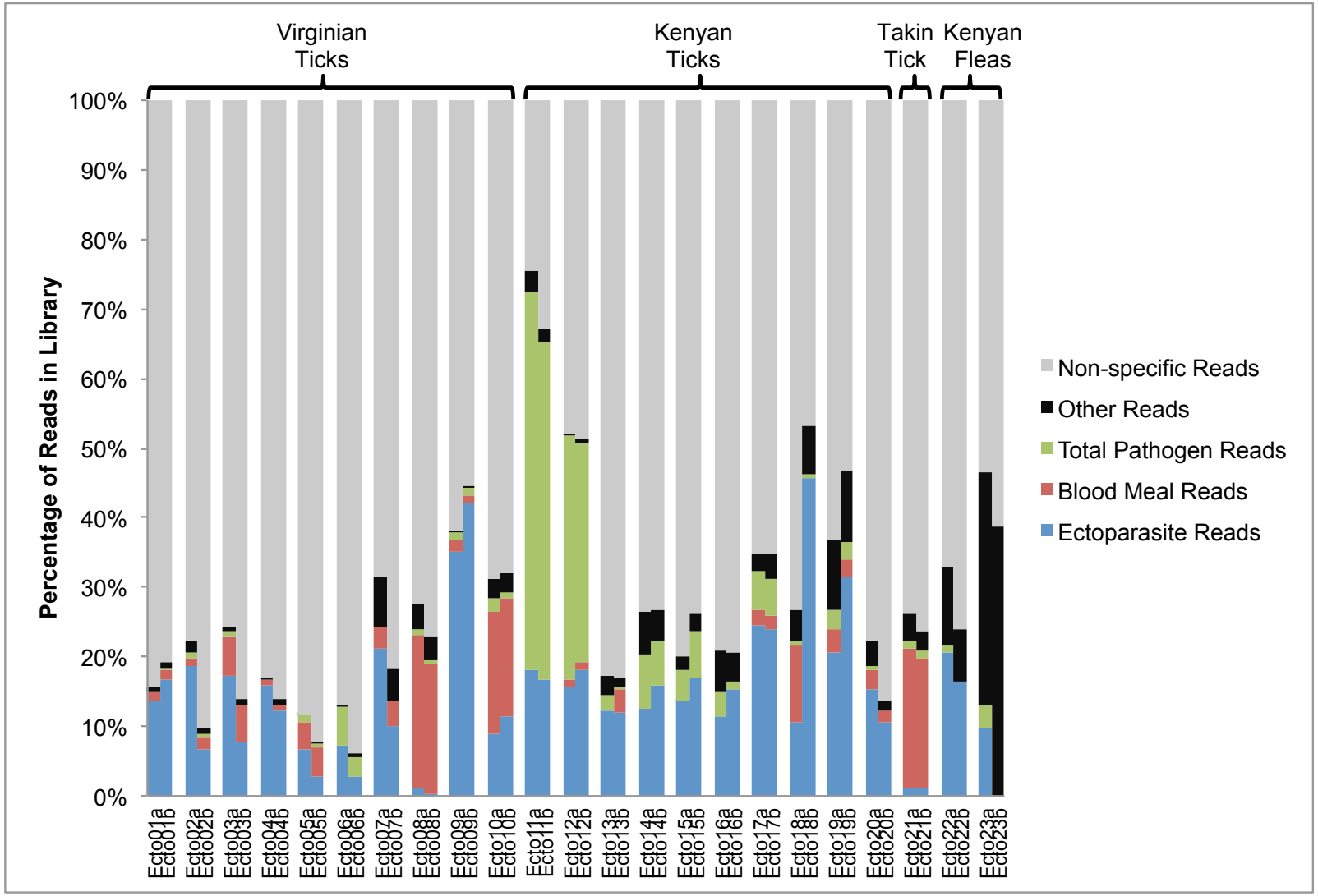
# Split reads to probe length

- ‘split’ command
- EctoBaits: 100bp fragments
  - Mybaits can be from 80-120bp long
- Combine to single fasta file
- Email files to Tech Support
  - Will perform additional QC, replace ambiguous sites
  - Synthesis takes approximately 6-8 weeks

# EctoBaits Analysis:



# EctoBaits Results:



# Other Capture Array Designs:

- Mammal mitogenome array 'MMA'
- Bacterial Genomes (homologous regions)
- Ultraconserved Elements
- RAD-seq SNP ID'd contigs
- Limited only by creativity

# Questions?