CRB High Throughput Lab in Riverside For HLB Diagnostics



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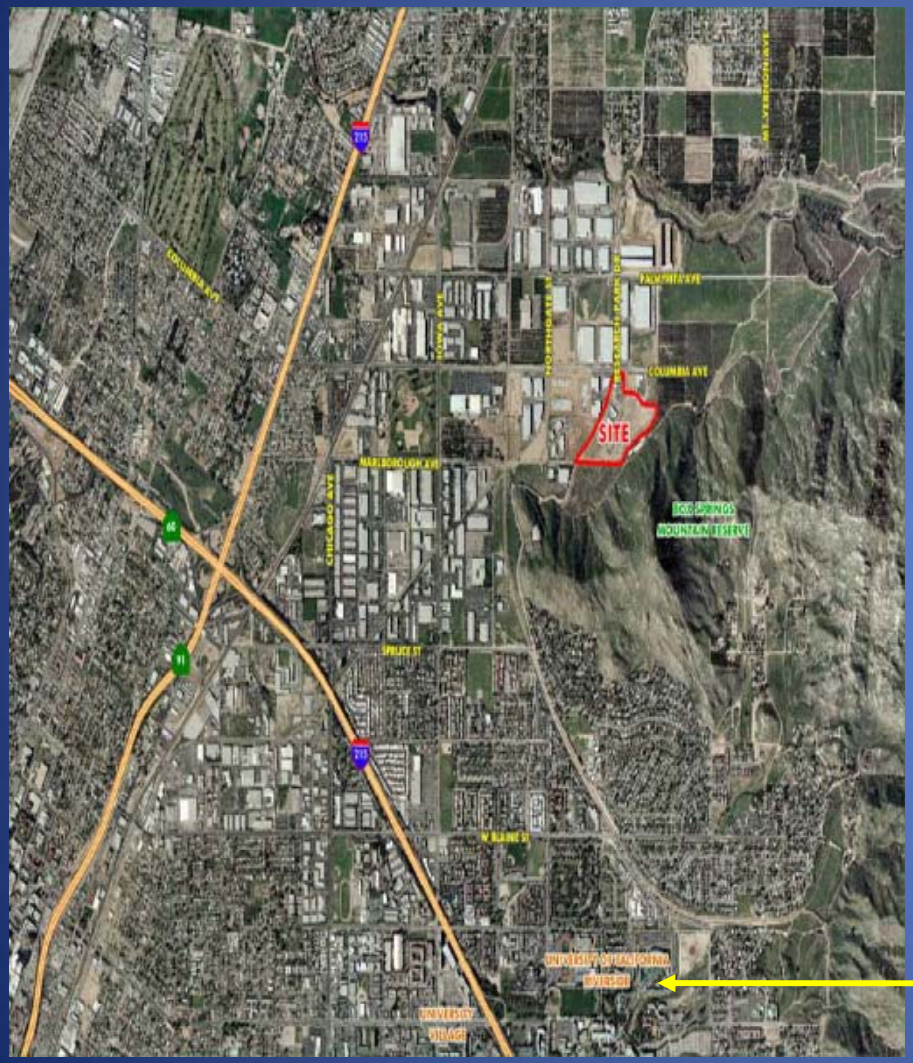


Lab Location

- Suite 500, 1201 Research Park Drive – corner of Columbia and Research Park Drive
- UC Riverside campus 5 minutes away

Laboratory space was a single large room.

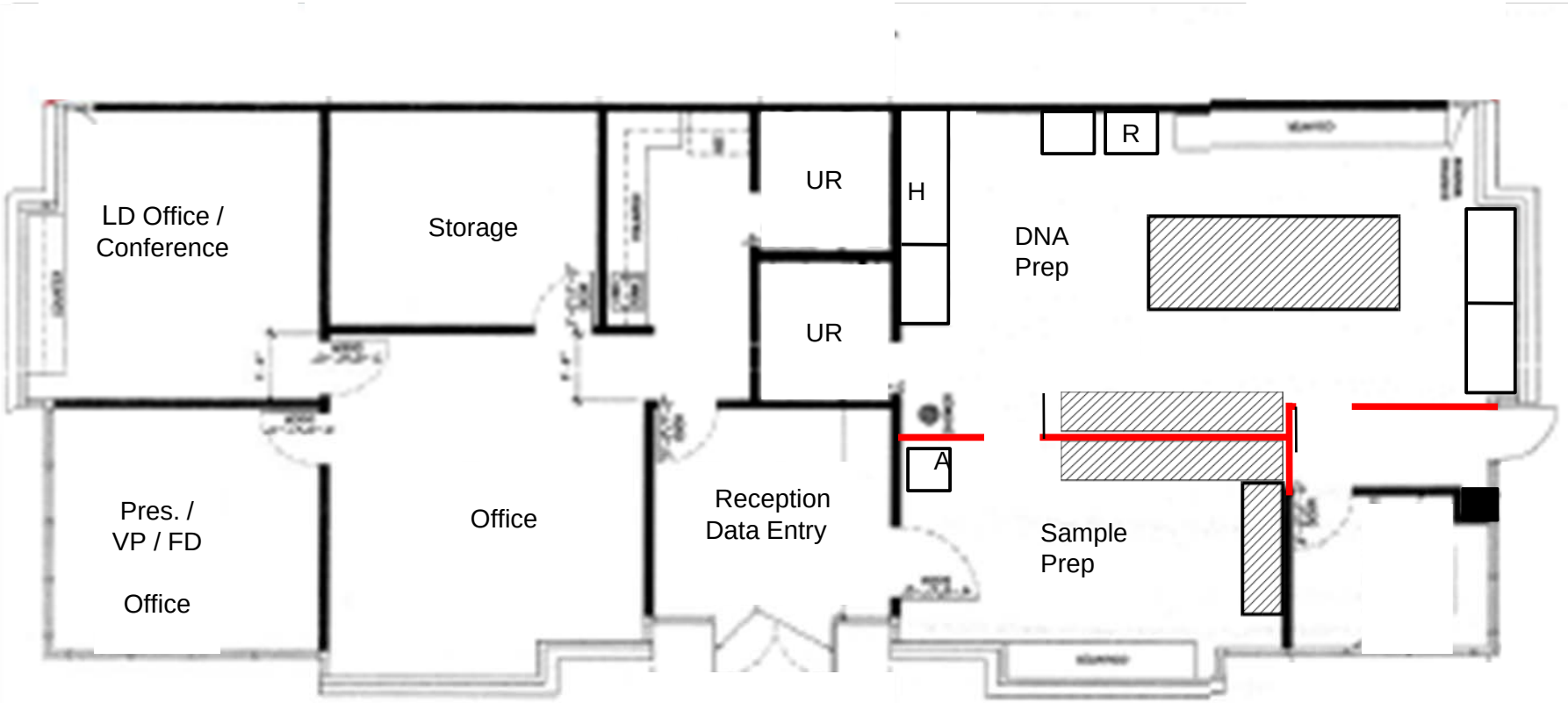
Has been divided into:

- Sample processing
- DNA extraction / PCR set up rooms

Small PCR instrument room.

Has been:

- Isolated by a hallway from other lab areas
- Entered only by lab personnel



UR = unisex restroom
H = hood
S = sink
A = autoclave

FH = flow hood
= new benches
= new walls
= new doors

Lab renovation: 1st steps

1 Framing the hallway

2 Framing the sample lab

4 Preparing for “mud” application

3 Dry wall installation

Lab renovation: relocation of the fume hood and installation of the ice maker

1 Hood moved for larger sink and ice maker

3 Ice maker installed and fittings for
RO water system

2 Installation of new counter next to sink

Lab renovation: Installation of counters and cabinets

Sample Flow

Reception

- Download from data logger / data entry from data forms
  - Associates samples with:
    - Tree bar code
    - GPS coordinates
    - Field photographs

- Scan data forms
  - Electronically captures paper forms
- Photograph samples
  - Accumulation of symptom library associated with test results

- Bar code sample
  - Associates sample with tree
  - Associates RT-PCR results with sample

Sample Flow

Sample Prep Room

- Dissect plant tissue into 1 ml 96 well plate
  - 200 mg of leaf midvein and petiole
- Autoclave remaining plant tissue
  - Eliminates possibility of contamination from plant material
- Freeze dry plant samples
  - Stabilizes sample at room temperature
- Collect live insect samples
  - Open sample with insects inside insect cages
  - Remove pysllid sample into EtOH




- Remove insects from sticky traps using Histoclear
- Freeze all remaining tissue to kill insects

Sample Flow

DNA / PCR Prep Room

- Pulverize freeze dried plant tissue
  - 96 well format bead dispenser and beater
- Extract DNA
  - Initially will use USDA certified procedure using DNeasy Plant Kit (Quiagen, Valencia, CA)
  - Evaluating 96 well format robot for DNA extraction, normalization, and RT-PCR set up
- Set up RT-PCR reactions in high performance laminar flow hood

Instrument Room

- Run RT-PCR on ABI 7500 Fast system using USDA certified primers and parameters
  - Run samples will not re-enter the DNA / PCR Prep Room
  - Samples will be bagged for biohazard waste disposal

Currently

- Construction completed, city certifications in progress
- All equipment in place by the end of June
- ABI 7500 Fast installation scheduled for June 26th
- Ready to perform DNA extraction of citrus and insect samples
- Running RT-PCR reactions by mid July
- Will seek USDA certification by the end of the summer