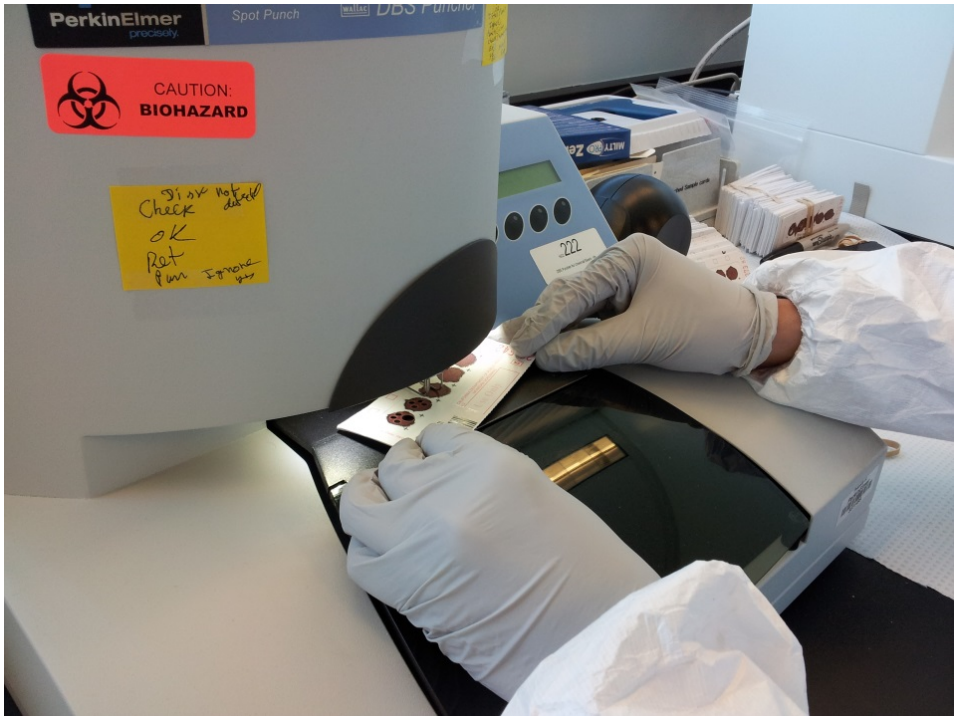
UCSF NSIGHT Project: Newborn Sequencing, NBSeq



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11/18/15

UCSF NBSeq

Explore potential applications of whole exome sequencing (WES) to public health newborn screening (NBS).

- Develop WES from archived dried blood spots (DBS); apply to currently screened metabolic disorders and to primary immunodeficiencies.
- Build on our experience with California screening for severe combined immunodeficiency (SCID), which has identified infants with low T lymphocytes, many of whom had a gene diagnosis made by WES.
- Study parents' views and values regarding NBS with WES—Barbara Koenig's presentation.

Methodologic Questions

- What are the sensitivity and specificity of WES sequencing for metabolic disorders?
- Can WES replace tandem mass spectroscopy now used?
- Can sequence analysis augment the information derived from current metabolic disease screening?
- Would addition of deep sequencing shorten the time to definitive diagnosis of “parents in waiting”?
- For infants and children with immune system defects early diagnosis is essential for optimum treatment and outcome. Can WES detect pre-symptomatic immune disorders that would otherwise not be found before onset of serious infectious complications?

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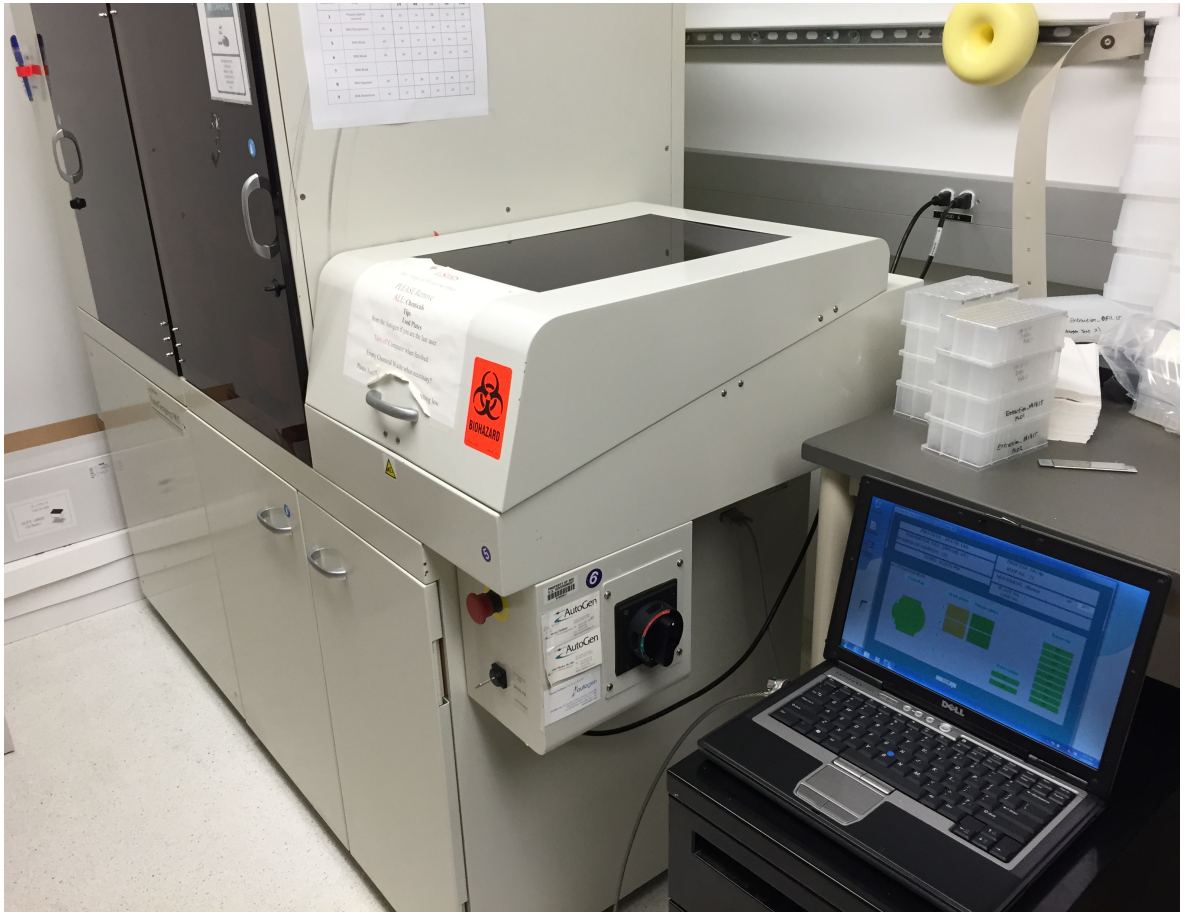
NBSeq Study Samples and Methods

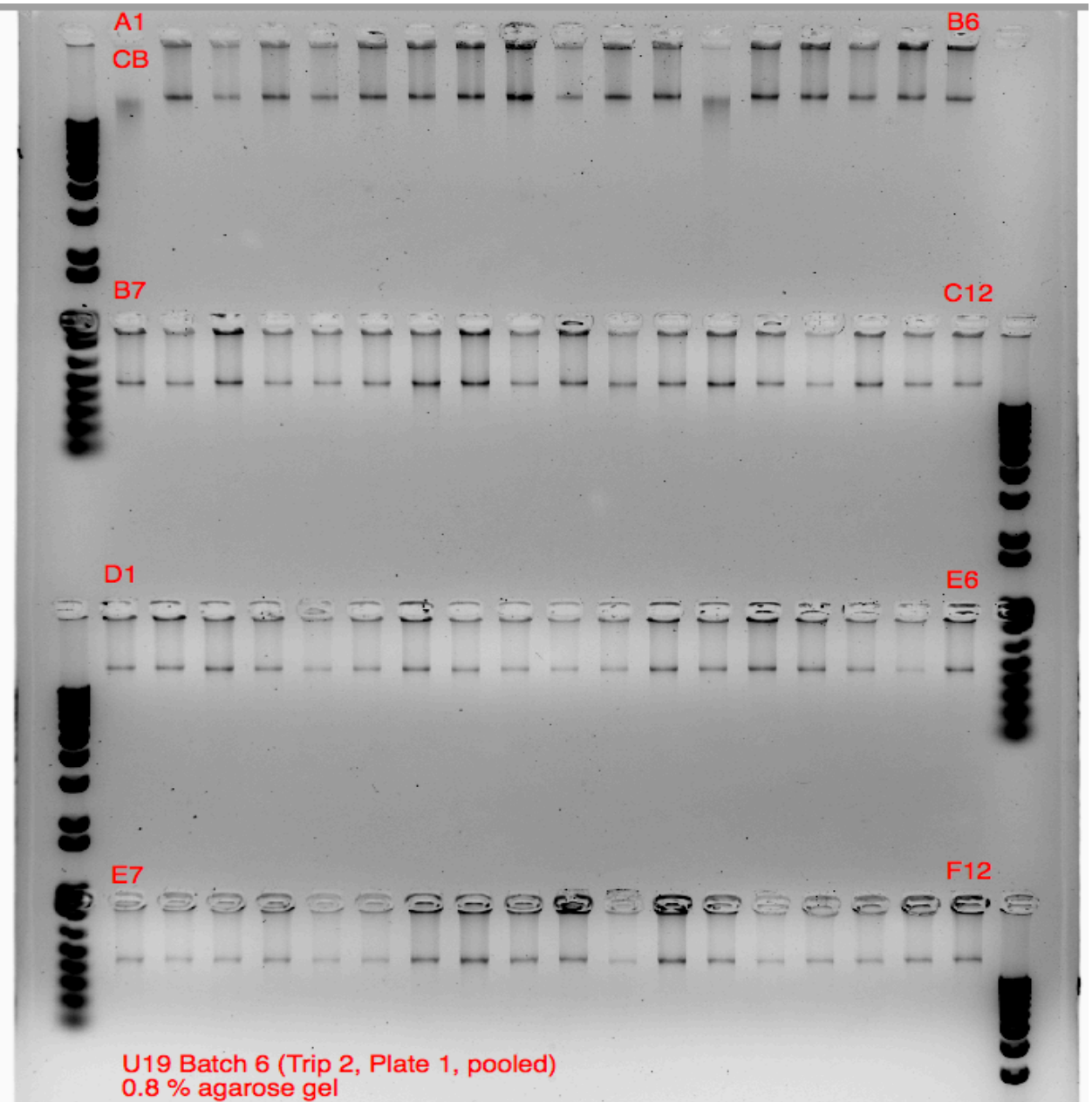
- IRB approved collaboration with CA Department of Public Health Genetic Disease Laboratory to obtain:
  - Archived DBS from which all identifying information has been removed, but phenotype data regarding metabolic diagnosis are available.
  - Archived DBS from identified subjects with known or suspected inherited immune disorders, following written informed consent.
- Pilot DNA extraction, WES, and analysis optimized with pre-1982 anonymous and immune deficient pt DNA.
  - Sequence annotation and variation for metabolic disorders, restricted to a metabolic gene list; informatics custom pipeline developed by Berkeley and TCS.
  - Immunodeficiency variant calling using the whole exome.

DNA Isolation Using Autogen965 DNA Extractor



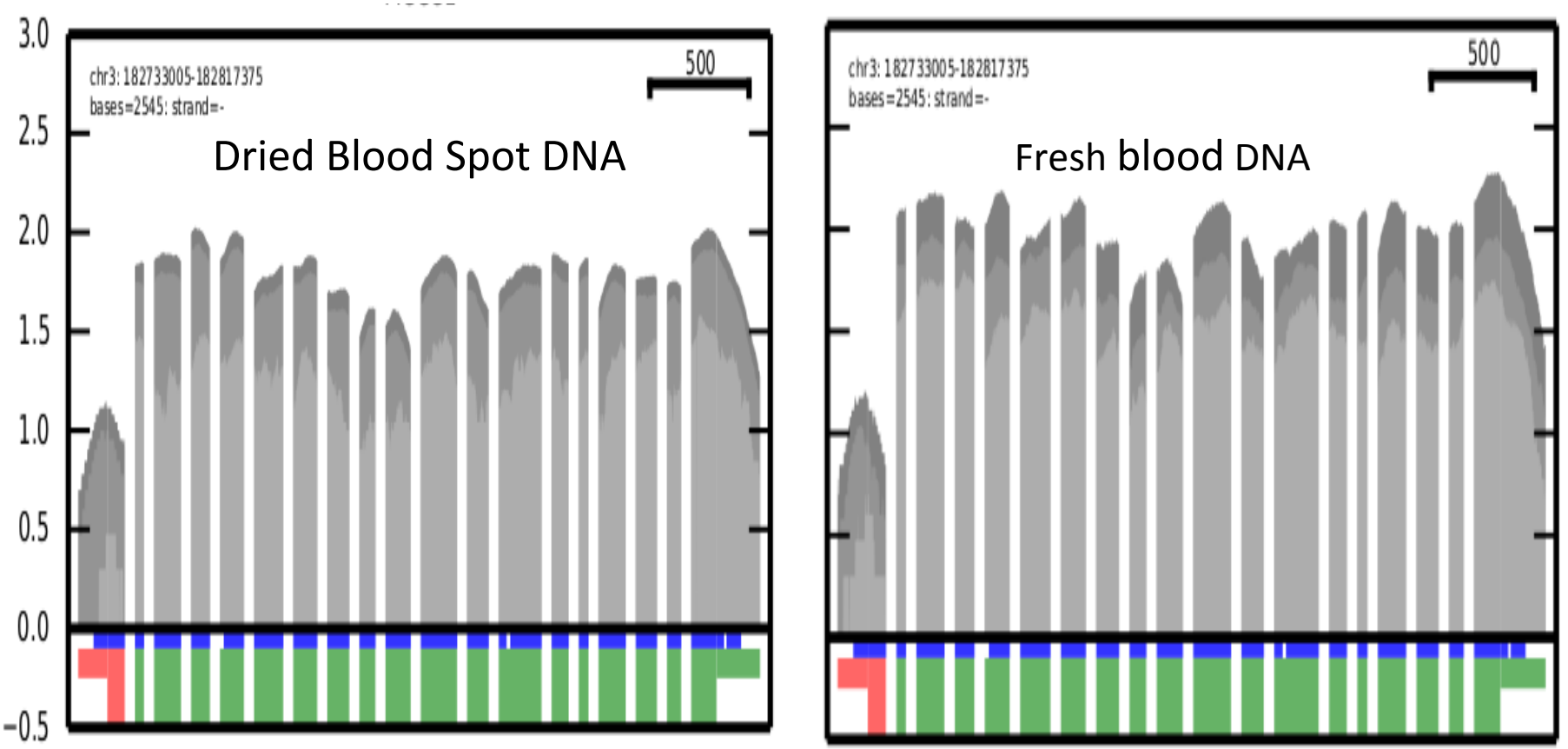
- 96-well deep plates
- One 3 mm DBS punch per well, 2 punches per sample
- Method: ProK digestion, organic extraction, alcohol ppt, resuspension
- Yield 100 – 1000 ng DNA per punch
- 260/280 mean 1.8

Extracted DNA Quality and Quantity



Gel electrophoresis:
5% of the 100 µL DNA from 2 pooled DBS, visualized on agarose gel after extraction, prior to fragmentation for WES.
High molecular wt. DNA is obtained from >98% of the DBS.

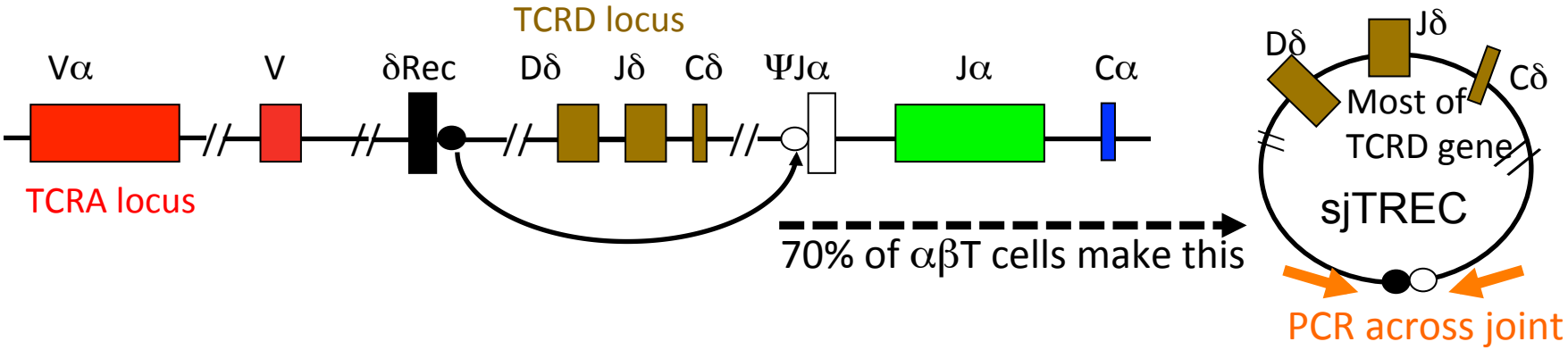
Gene Coverage for MCCC1



DBS sequence data quality comparable to that from fresh blood

UCSF Results with WES to Diagnose Primary Immunodeficiency Disorders

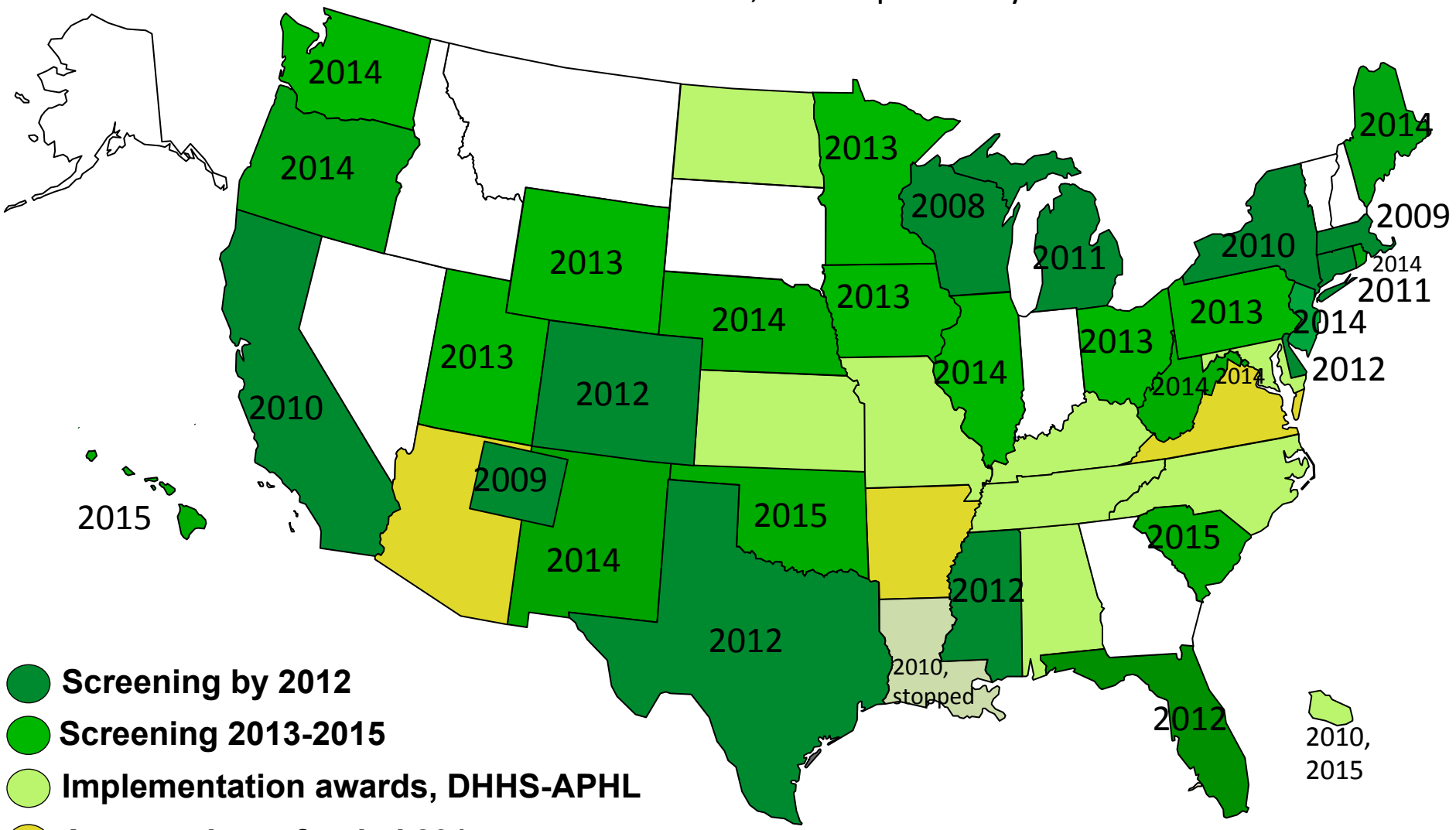
- Built on success of newborn screening with the T cell receptor excision circle (TREC) assay to detect SCID:



- SCID cases and older children enrolled before U19 had phenotypic information and parents to inform exome analysis.
- Of 34 completed WES cases, 13 had diagnoses confirmed after functional study of variants (38%), with 3 more under study to confirm (47%). S. Brenner and TCS analysis essential.

SCID Newborn Screening, 2015

>75% of births; 93% expected by 2016



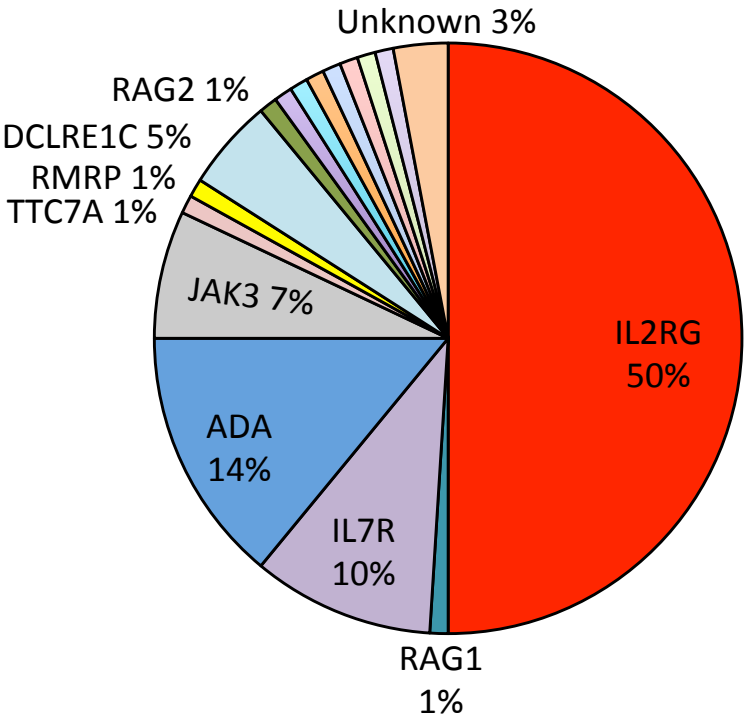
- **Screening by 2012**
- **Screening 2013-2015**
- **Implementation awards, DHHS-APHL**
- **Approved, not funded 2015**
- **Not screening**

Thanks to A. Brower, NBSTRN

Genotypes of Typical and Leaky SCID

Reports from Transplant Centers, no Screening

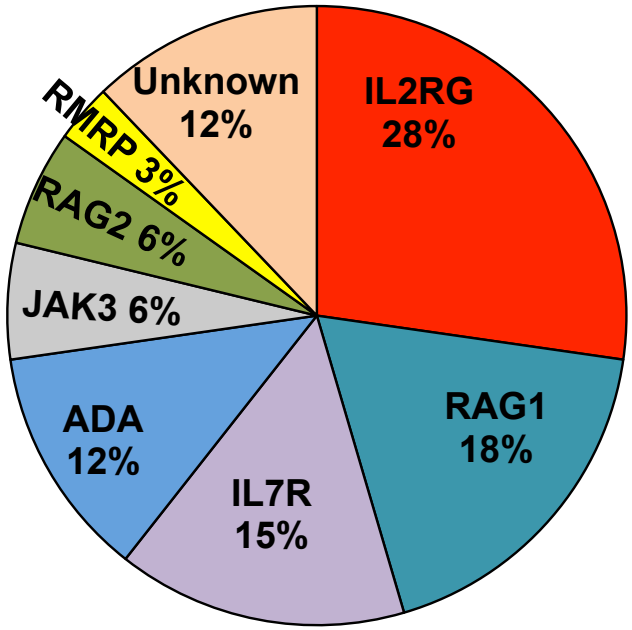
Duke University, European centers (estimates)



Overall Survival ~74% or lower

California, with TREC Screening

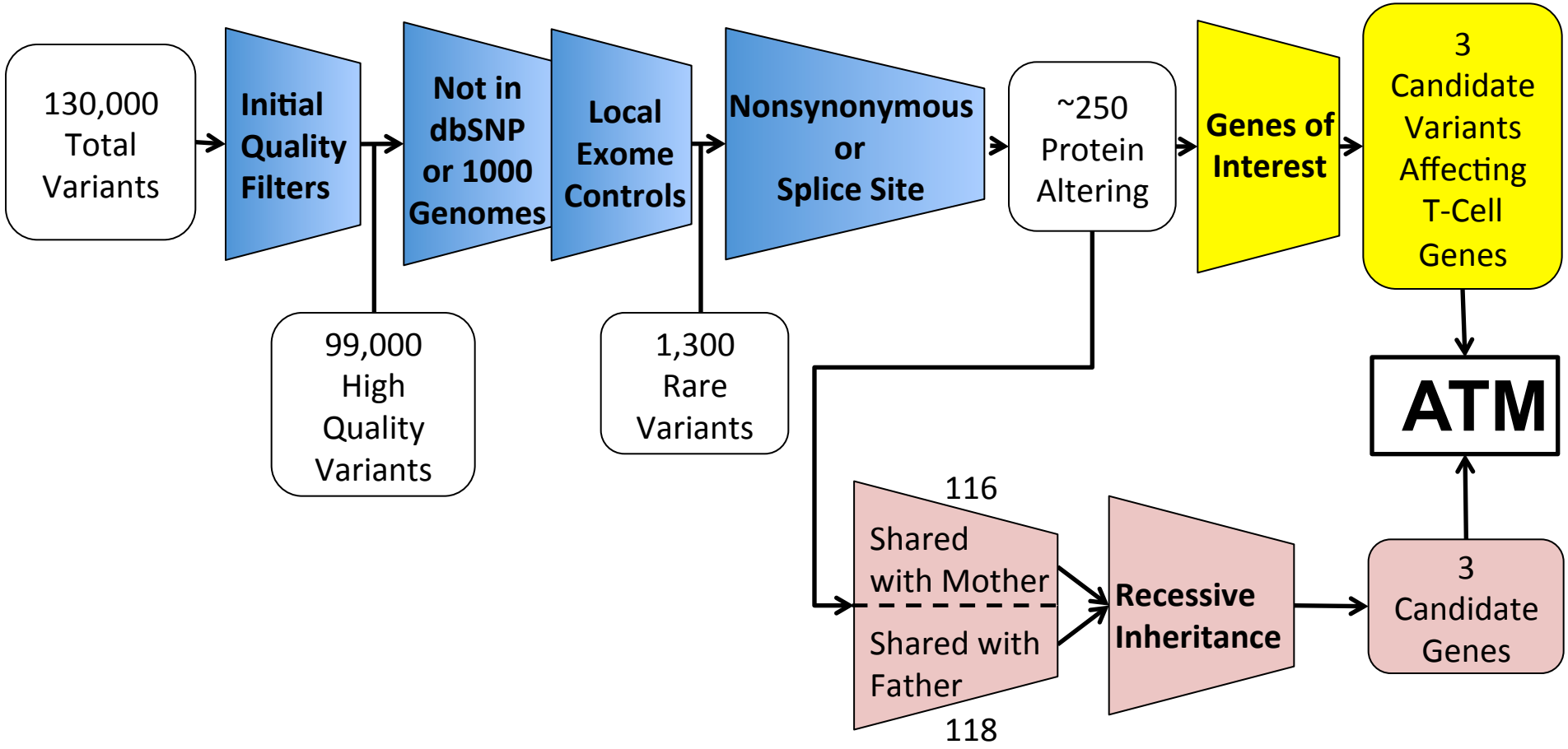
4 years, ~2 million infants



Overall Survival 95%

Kwan and Puck, Sem Perinatol 2015;39:194-205

WES Analysis of a Case with Low TRECs on NBS, Low T cells, but No Diagnosis: Filtering of Variants



Mallott J et al., *J Clin Immunol* 2013;33:540-49

TREC Screen Does Identify non-SCID Immune Defects

- **For defects after TCR recombination in thymus, T cell number or diversity are not reduced, TRECs are present.**
  - CD40L deficiency, Hyper-IgM syndrome
  - MHC II deficiency
  - ZAP70 deficiency
- **Syndromes with variable T cell deficiency, but with enough T cells to have TRECs above the screen cutoff.**
- **Immune defects not involving T cells.**
  - X-Linked agammaglobulinemia, Chronic granulomatous disease, etc. There are now >250 known primary immune disease genes (*C. Picard et al, J Clin Imm, published online Oct 19, 2015; open access*)

UCSF NBSeq Plan for Immune Deficiencies

- Non-SCID immune disorders would benefit from early diagnosis and treatment.
- With many known and additional unknown primary immunodeficiency genes, but no biomarkers comparable to TRECs for non-SCID PI's, a sequencing approach is needed.
- **Could newborn DBS WES identify actionable PI conditions prior to onset of severe infectious complications?**

UCSF NBSeq Plan for Immune Deficiencies

- This part of the project enrolls individuals with suspected primary immune defects, but no gene diagnosis, who were born in CA in 1982 or later.
- With informed parental consent, archived residual newborn DBS are retrieved for DNA extraction, WES and analysis by the NBSeq team of collaborators, including Steven Brenner and members of TCS.
- 10 individuals have enrolled to date (plan to enroll 50), with sequencing and analysis under way.

Thanks to Our Collaborators and Funders

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