Wisconsin Newborn Screening Laboratory

Molecular Technology in Newborn Screening: SCID and Beyond

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History of NBS Molecular Testing

- **1994**
  - Washington: hemoglobin second tier testing (Hb S, C, and E) by RELP
  - Wisconsin: *CFTR* mutation analysis for F508del
- **1998**
  - New England: 2 GALT mutations (Q & N) by RFLP
- **1999**
  - New England: MCADD (c.985A>G) by RFLP
- **2005**
  - Wisconsin: MSUD (p.Y438N) by Tetra-primer ARMS-PCR

History of NBS Molecular Testing

- **2006**
  - New York: Krabbe disease (3 polymorphisms & 5 mutations) by DNA sequencing
- **2008**
  - Wisconsin: TREC assay for SCID screening by Real-time PCR
    - *1st use of molecular test as a primary test for population screen*
- **2010**
  - 36 NBSPs in US use molecular testing for CF

Severe Combined Immunodeficiency (SCID)

Then…

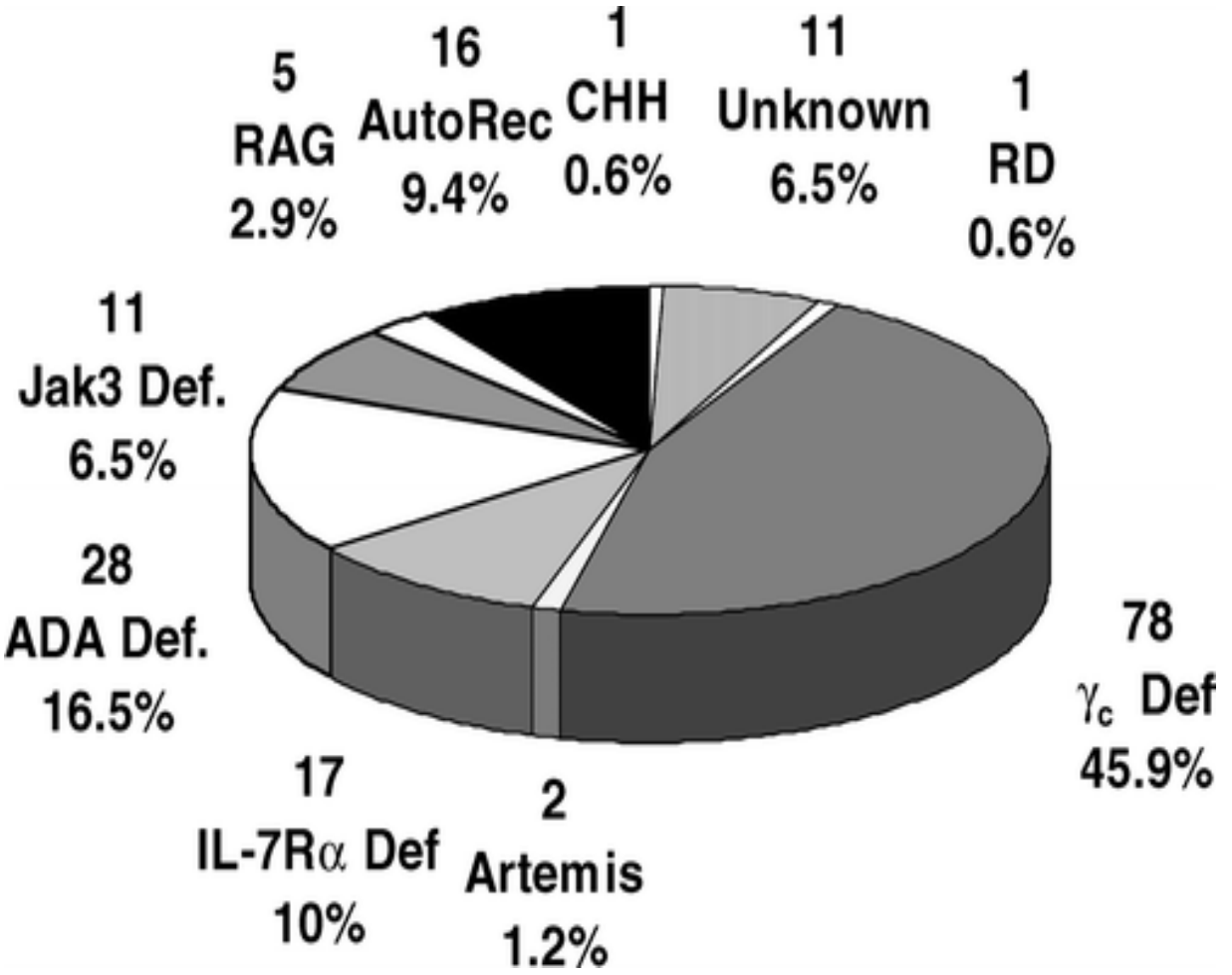
Now…

Severe Combined Immunodeficiency (SCID)

- **Infections in first year of life**
  - recurrent, etiology bacterial, viral and fungal
  - persistent despite routine treatment
  - severe--including sepsis, meningitis
  - opportunistic pathogens, such as PCP (pneumonia)
- **Failure to thrive, chronic diarrhea**
- **T cells decreased or absent**
  - poor proliferation *in vitro* to mitogens
- **B cells absent or non-functional**
  - low Ig's after maternal IgG wanes; no specific antibody responses
- **Fatal without immune reconstitution**

SCID Genetic Analysis

- X-linked SCID is most common form (males)
- Specific gene defect can be found in 80% of cases (15 genes known)
- Clinical applications:
  - Carrier and prenatal dx
  - Predict response to BMT
  - Gene therapy



Buckley Ann. Rev Imm 2004

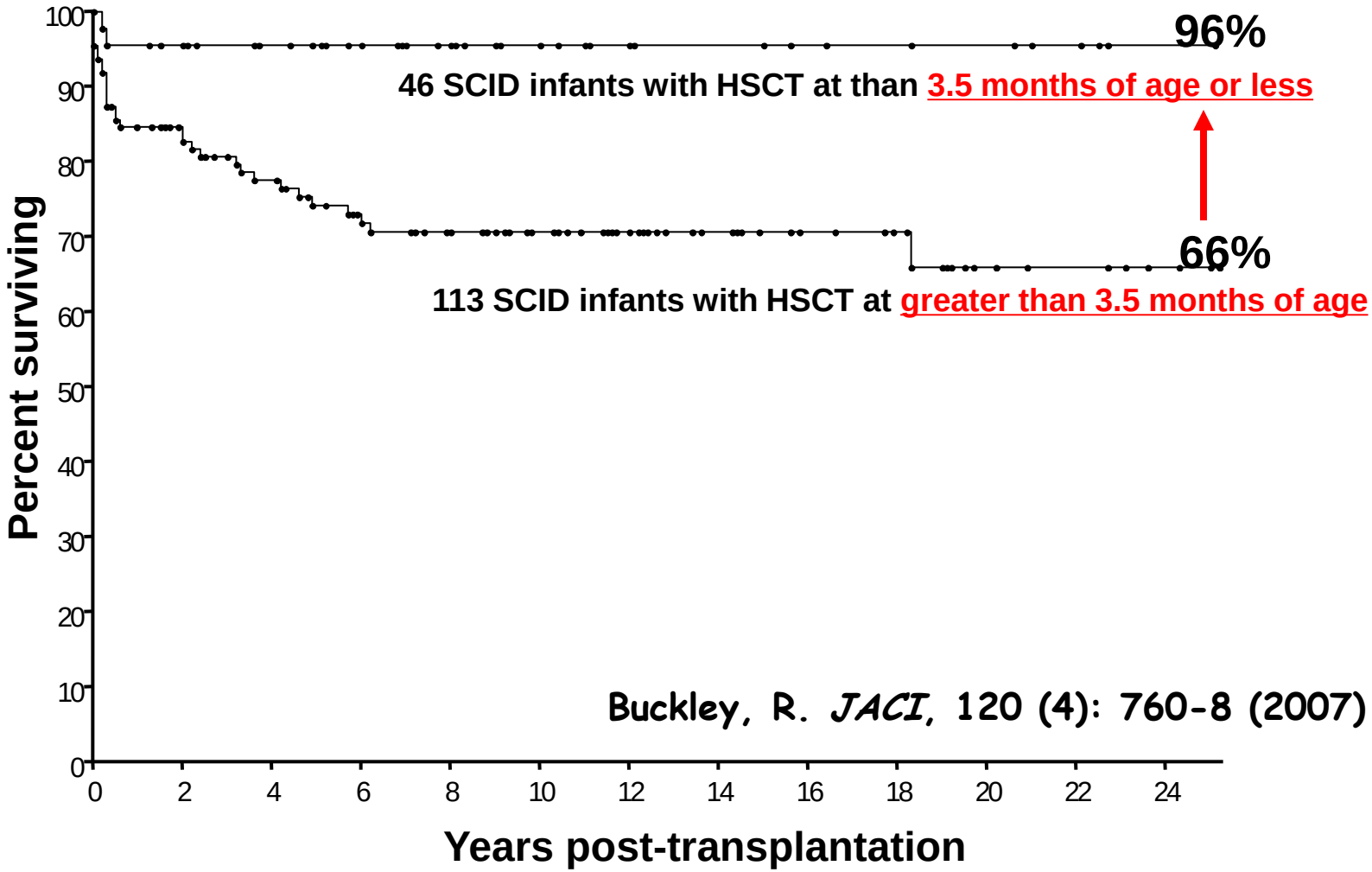
Available Curative Treatment Modalities for SCID

- Bone Marrow Transplantation
- Gene Therapy (X-linked and adenosine deaminase deficiency SCID)

Does SCID fulfill NBS criteria?

- Prevalence of the disease (1:100,000 or greater)
  - SCID: 1:66,000 (conservative estimate)
- Can the disorder be detected by routine physical exam?
  - SCID: No, SCID baby appears normal at birth.
- Does the disorder have a short asymptomatic period after birth?
  - SCID: Yes, SCID baby can be protected by passive maternal immunity.
- Does the disease cause serious medical complications?
  - SCID: Yes, universally fatal within the first year of life
- Is there potential for successful treatment?
  - SCID: Yes, hematopoietic stem cell transplantation
- Is there a confirmatory test?
  - SCID: Yes, lymphocyte subpopulation analysis (flow cytometry)
- Does early intervention leads better outcome?
  - SCID: Yes!
- Is there a screening test?
  - SCID: Yes, measurement of TRECs using real-time qPCR

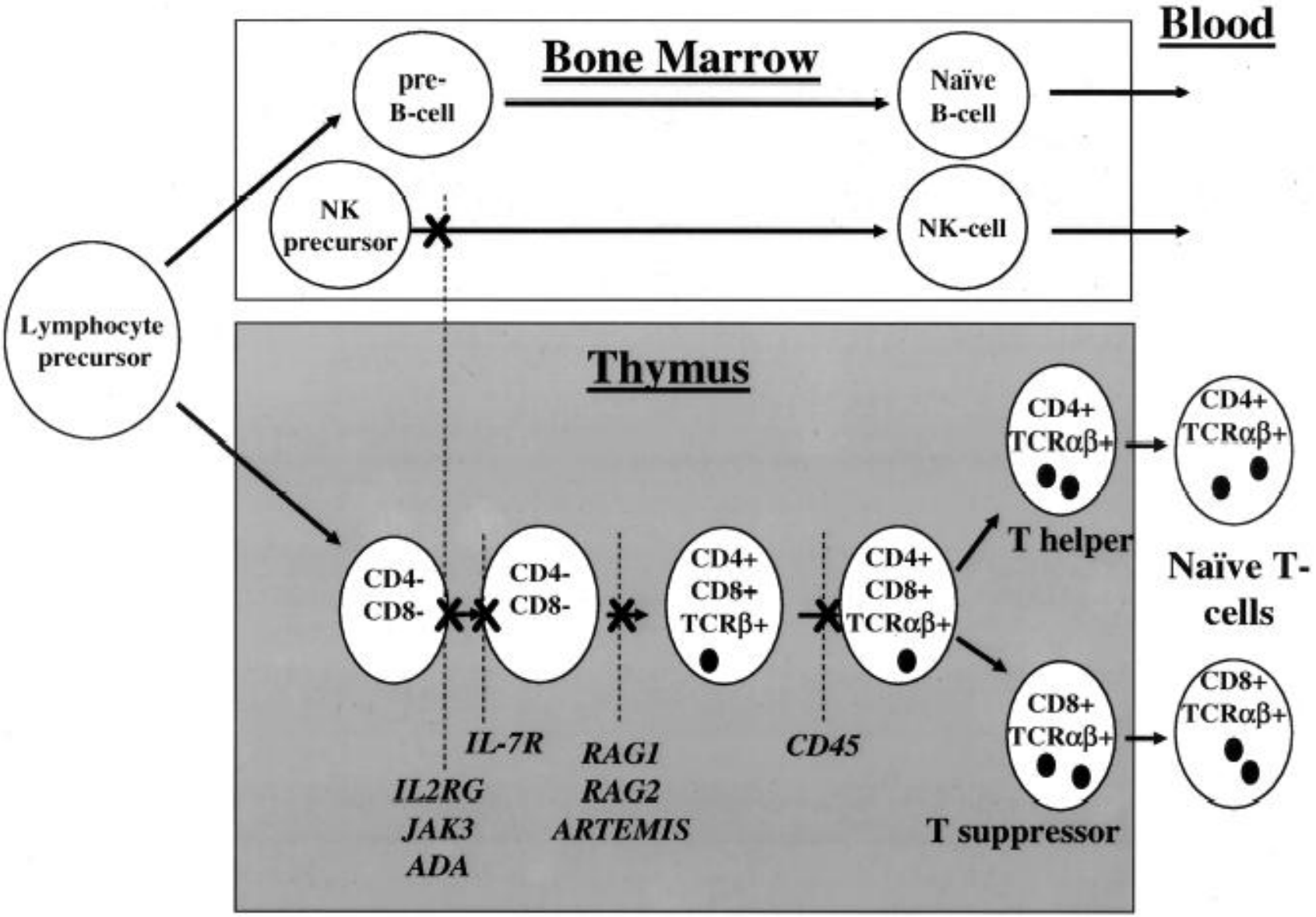
SCID: Benefits of Early Diagnosis



Screening for SCID in Newborns Considerations

- Many genes
- Many mutations in each known gene
- Some genotypes still not known

TRECs are reduced in nearly ALL forms of SCID



● = T-cell receptor excision circle (TREC)

Genet Med 2004:6(1):16–26.

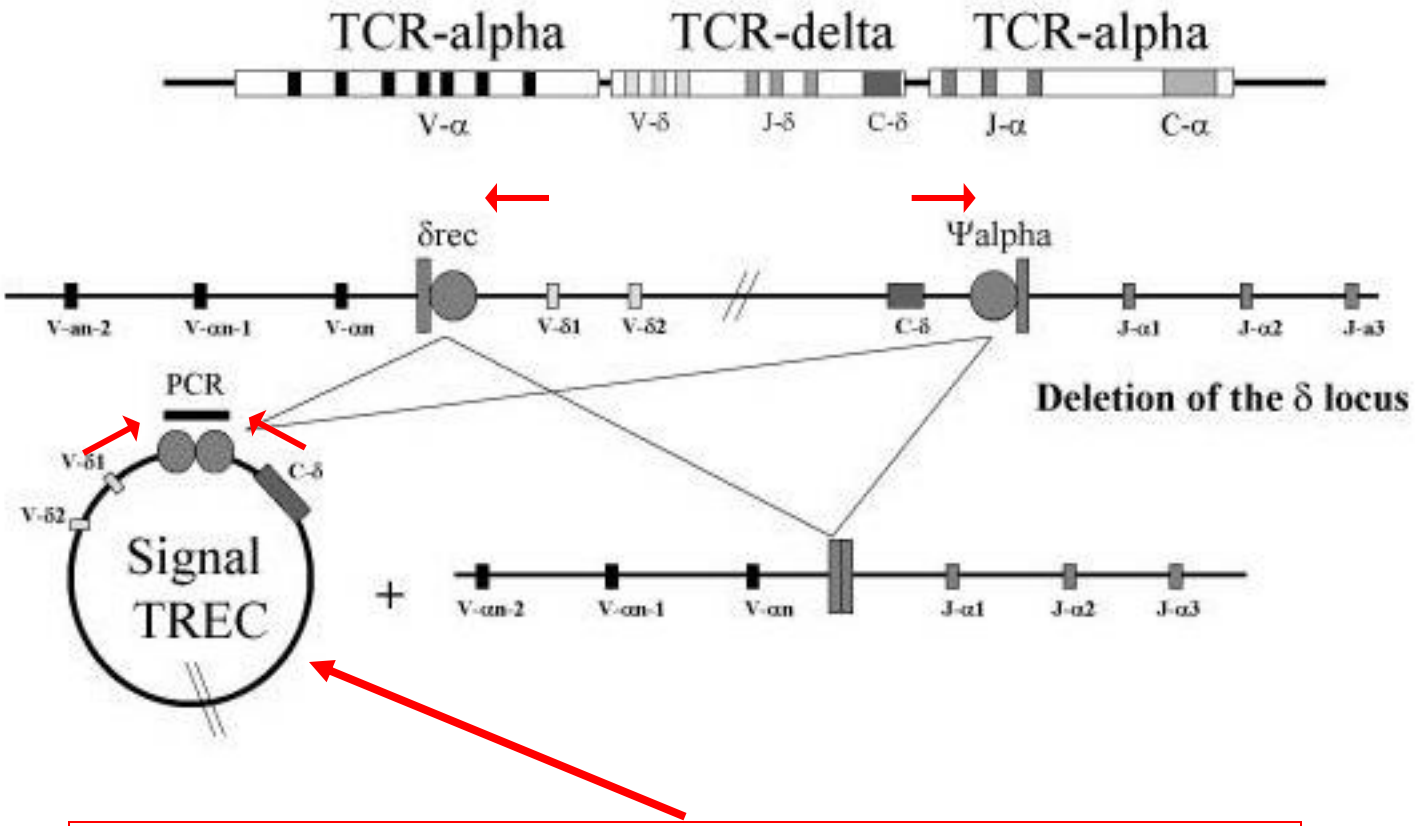
T-cell Generation in Newborns

- **Two mechanisms:**
  - Thymic output
  - Postthymic T-cell proliferation

- **Consequences:**
  - Majority of T-cells are naïve T cells in newborns.
  - TREC s are diluted out, and 10% T cells contain TRECs in newborns.

Schönland et al. *Blood*.2003; 102: 1428-1434
Gent et al, *Clinical Immunology.* 2009; 133: 95–107

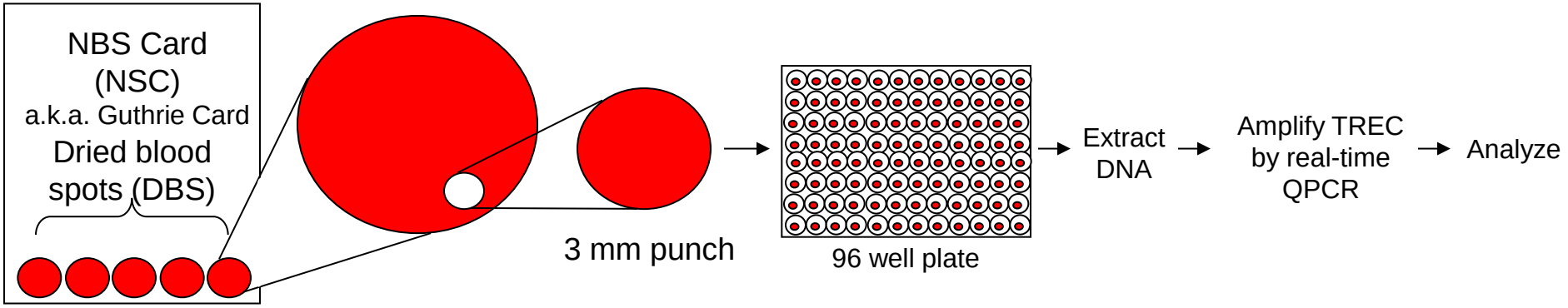
T Cell Receptor Recombination During Development in the Thymus

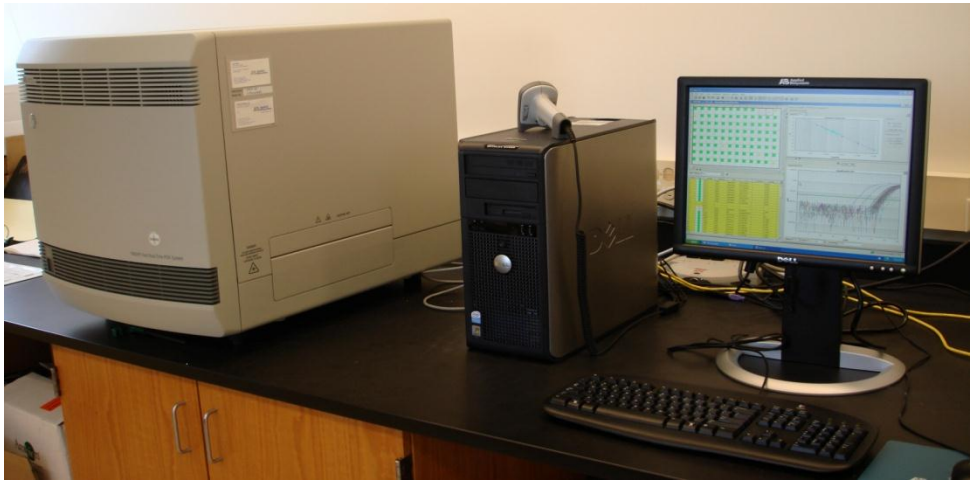


Generation of T cell receptor excision circles (TRECs) occur in >70% of all new (naïve) T cells and can be detected by PCR

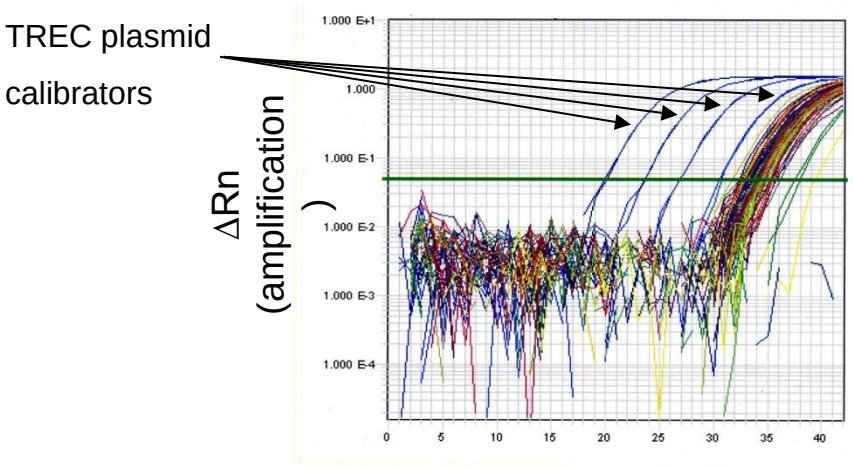
Ponchel *et al. BMC Biotechnology* 2003 **3**:18 doi:10.1186/1472-6750-3-18

Overall Analysis Scheme

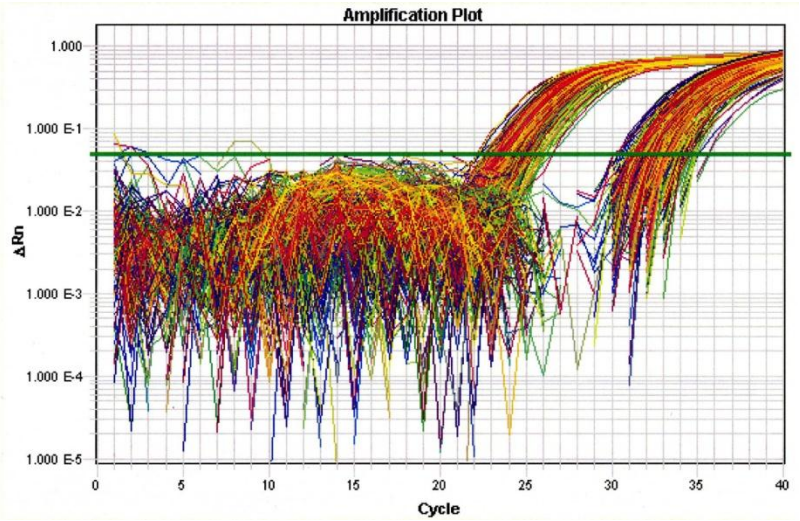


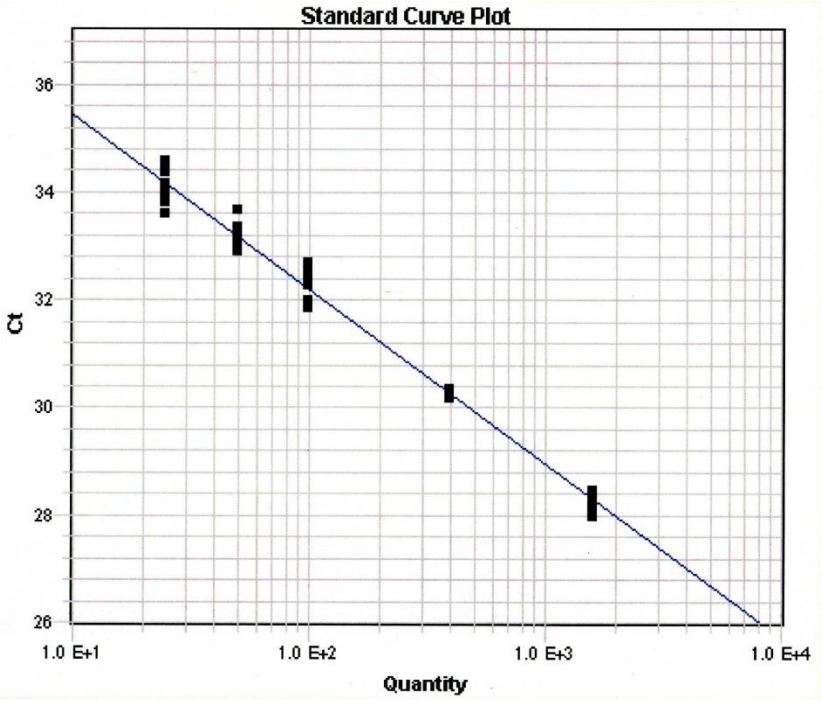


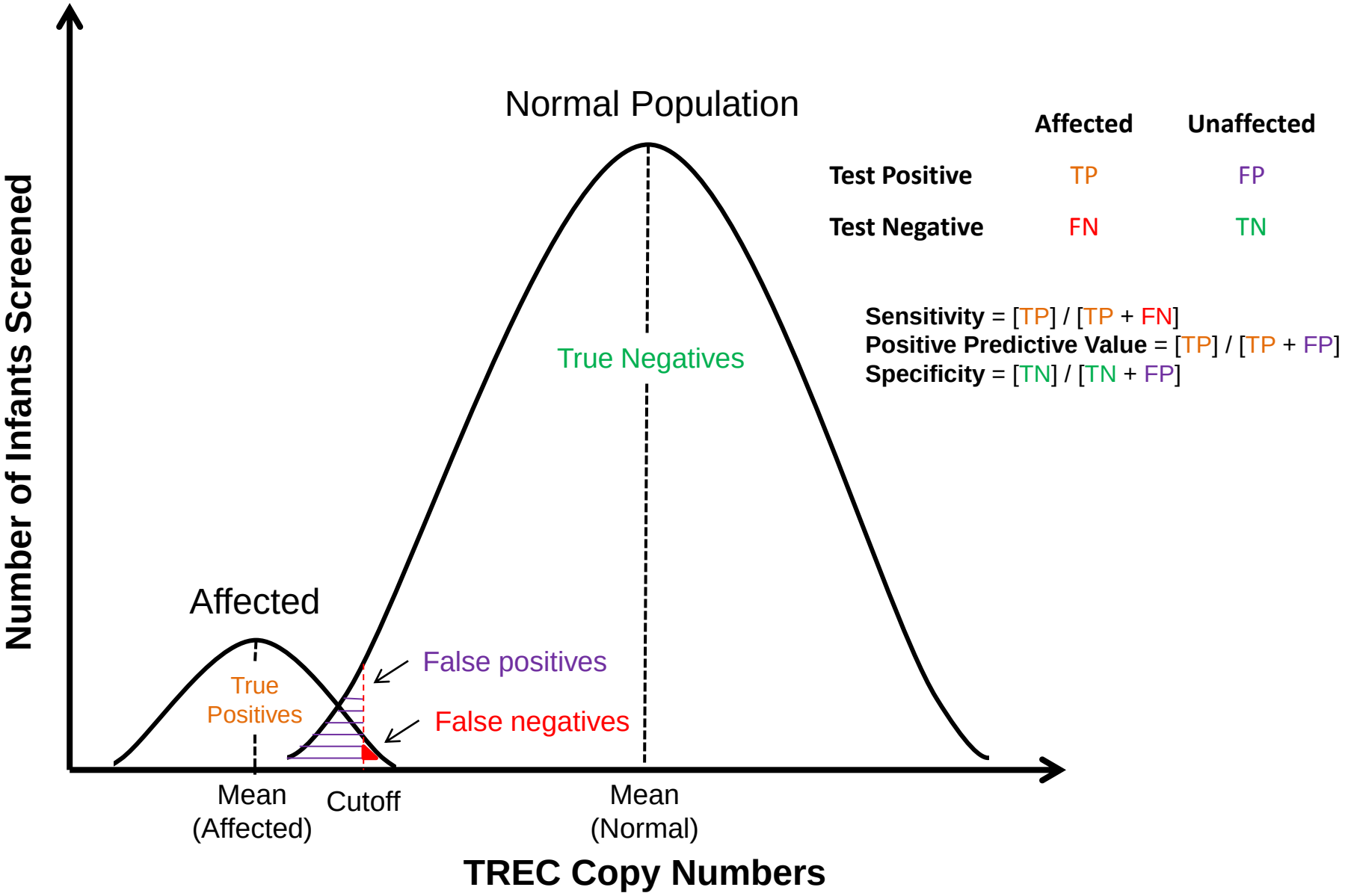
ABI 7900HT Fast Real-Time PCR System



Multiplexing _384-well Plate



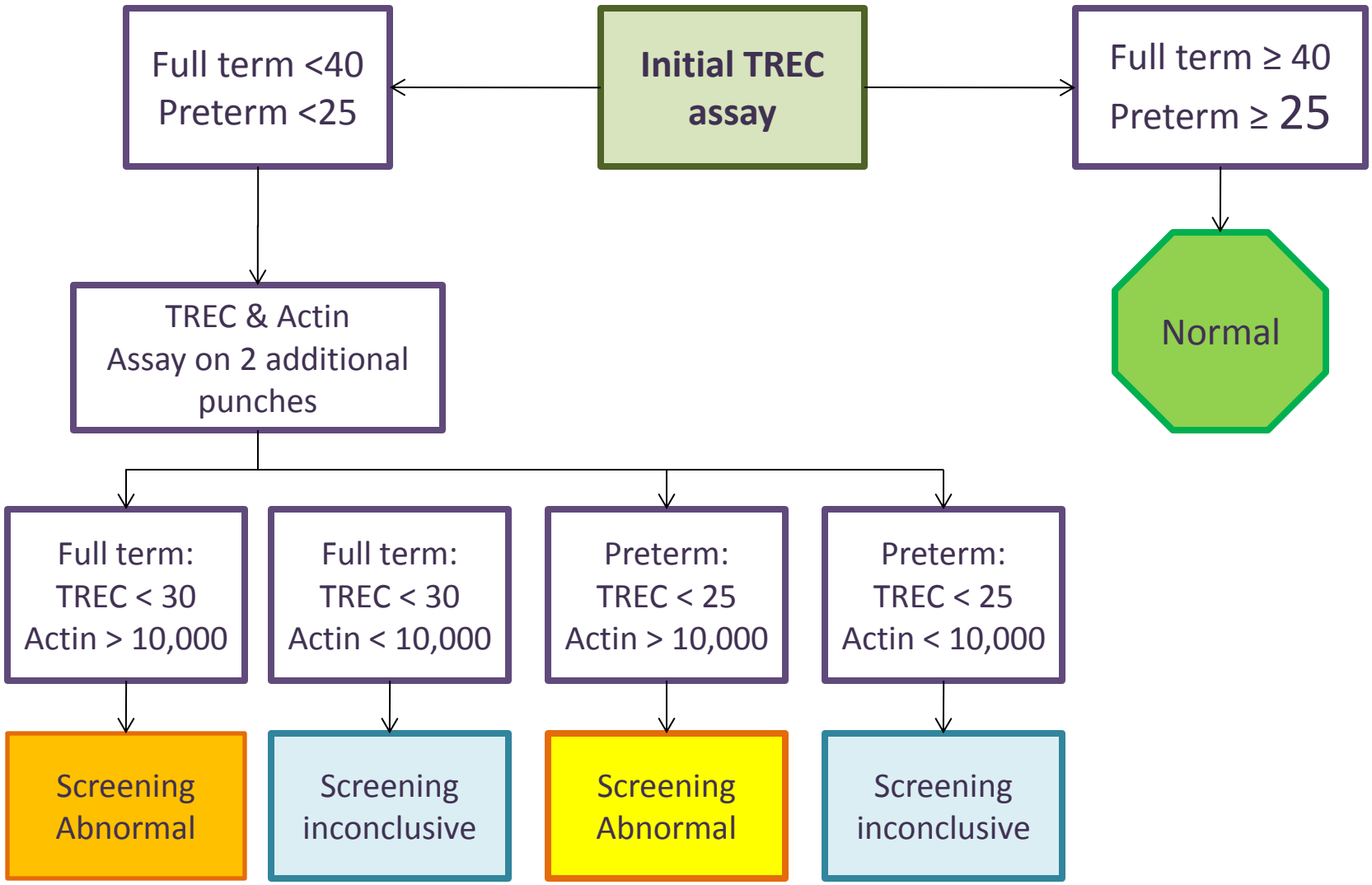




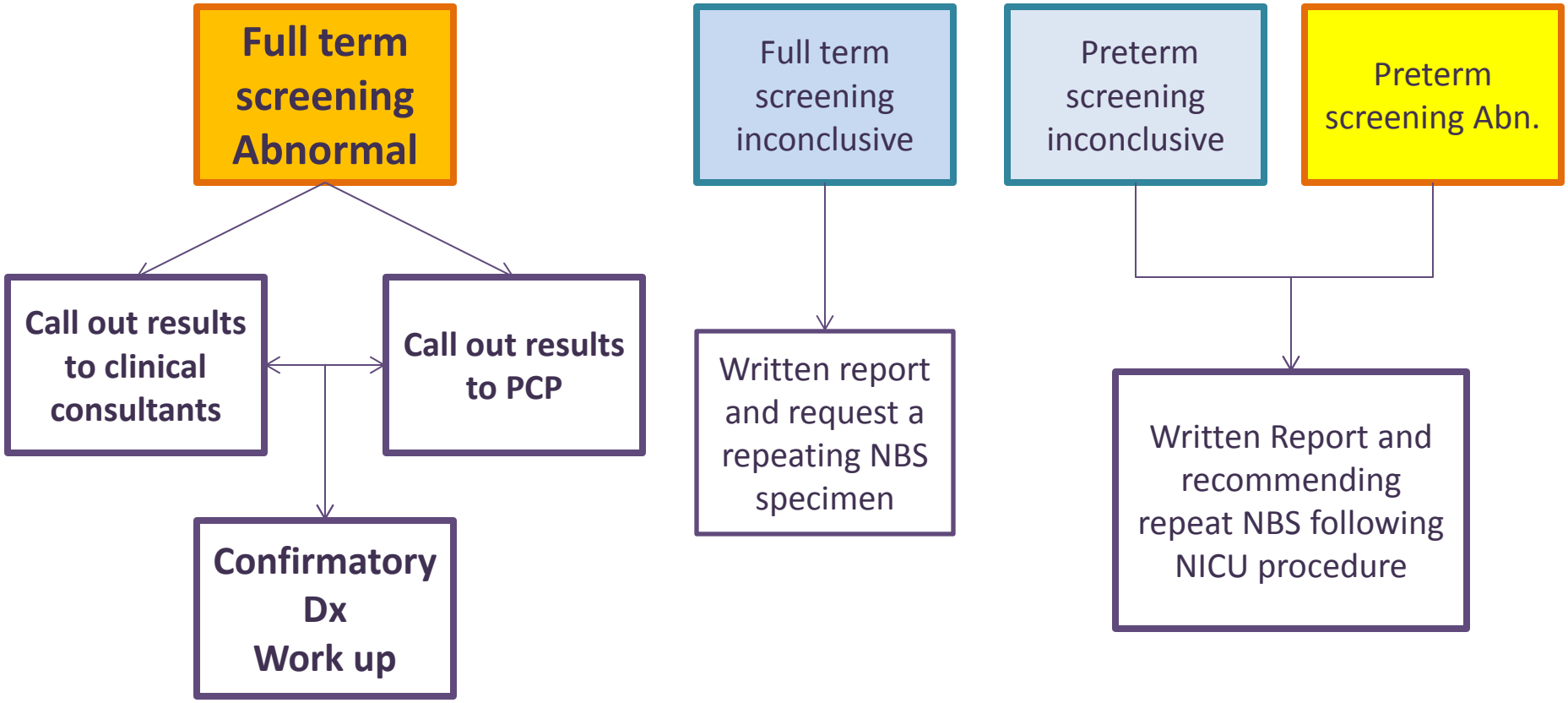
	Affected	Unaffected
Test Positive	TP	FP
Test Negative	FN	TN

Sensitivity = [TP] / [TP + FN]
Positive Predictive Value = [TP] / [TP + FP]
Specificity = [TN] / [TN + FP]

SCID Testing Algorithm



SCID Reporting Algorithm



Confirmatory testing

- Flow cytometry
  lymphocyte subset enumeration for T , B and NK cell quantitation
- Lymphocyte (T and/or B) proliferation tests
- Quantitative immunoglobulin assessment (IgG, IgA, IgM and IgE)
- HIV testing (to rule out secondary causes of T-cell lymphopenia)
- Genetics testing
- Others: enzymes, Fluorescence *in situ* hybridization (FISH)

Special Considerations

- TREC copy numbers
  - Measurement units
  - DNA extraction
  - Calibrators
- TREC assay platform
  - Multiplexing vs. single target
  - 384-well vs. 96-well
- Automation
- QA/QC issues
- Premature Newborns

Wisconsin Experience

(January 1, 2008– December 31, 2012)

Infants Screened:	**340,037**
- Premature (< 37 wks)	30,664
- Full term	309,373
Abnormal results:	**246**
- Premature (<37 wks)	147 (0.04%)
- Full term	99 (0.03%)
Inconclusive Results:	**472**
- Premature (<37 wks)	382 (0.11%)
- Full term	90 (0.03%)

Total number of flow cytometry referral: 108

Wisconsin Experience

(January 1, 2008– December 31, 2012)

Severe T cell Lymphopenia Cases

- Rac 2 mutation
- ADA SCID
- T-B-NK+ SCID
- T-B+NK+ (3)
- RAG 1 SCID

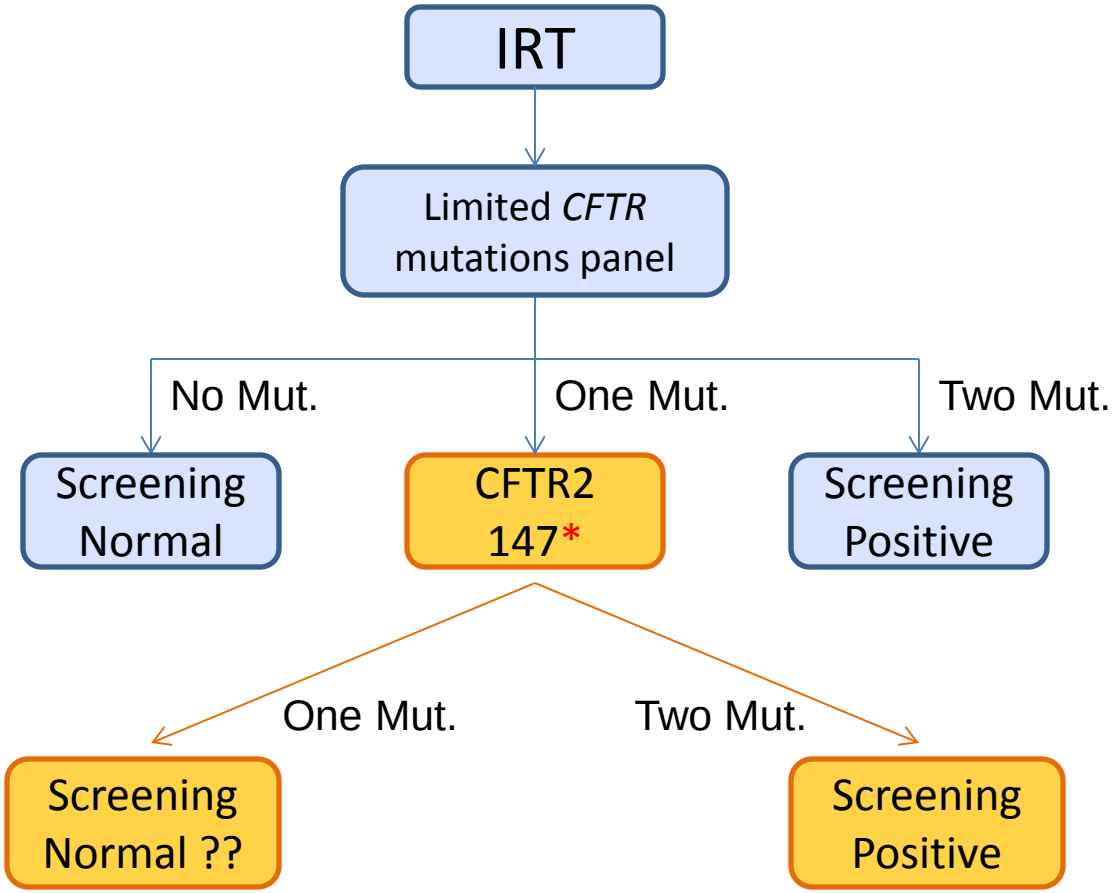
Wisconsin Experience

(January 1, 2008– December 31, 2012)

Other T cell Lymphopenia Cases

- Chromosomal abnormalities
  - 22q11.2 deletion (11)
  - Trisomy 21
- Syndromes with T cell impairment
  - Jacobsen syndrome
  - Tar syndrome
  - Ectrodactyly ectodermic dysplasia
  - Ataxia Telangiectasia
- Idopathic T-cell lymphopenia

Improving NBS for CF to Reduce Screening false positives using Next Generation sequencing Technology



*Disease-causing mutations and mutations with varying consequences. (Sosnay et al, *Nature Genetics,* 2013)

Specific Aims

1. Establish a method of simultaneously detecting 162 *CFTR* mutations/gene variants ***using dried blood spot routine newborn screening specimens to create IRT/DNA/DNA CF screening opportunity.***

2. Demonstrate that the three-tier IRT/DNA/DNA CF screening protocol would significantly reduce false positive screening results caused by identification of CF heterozygote carrier infants.

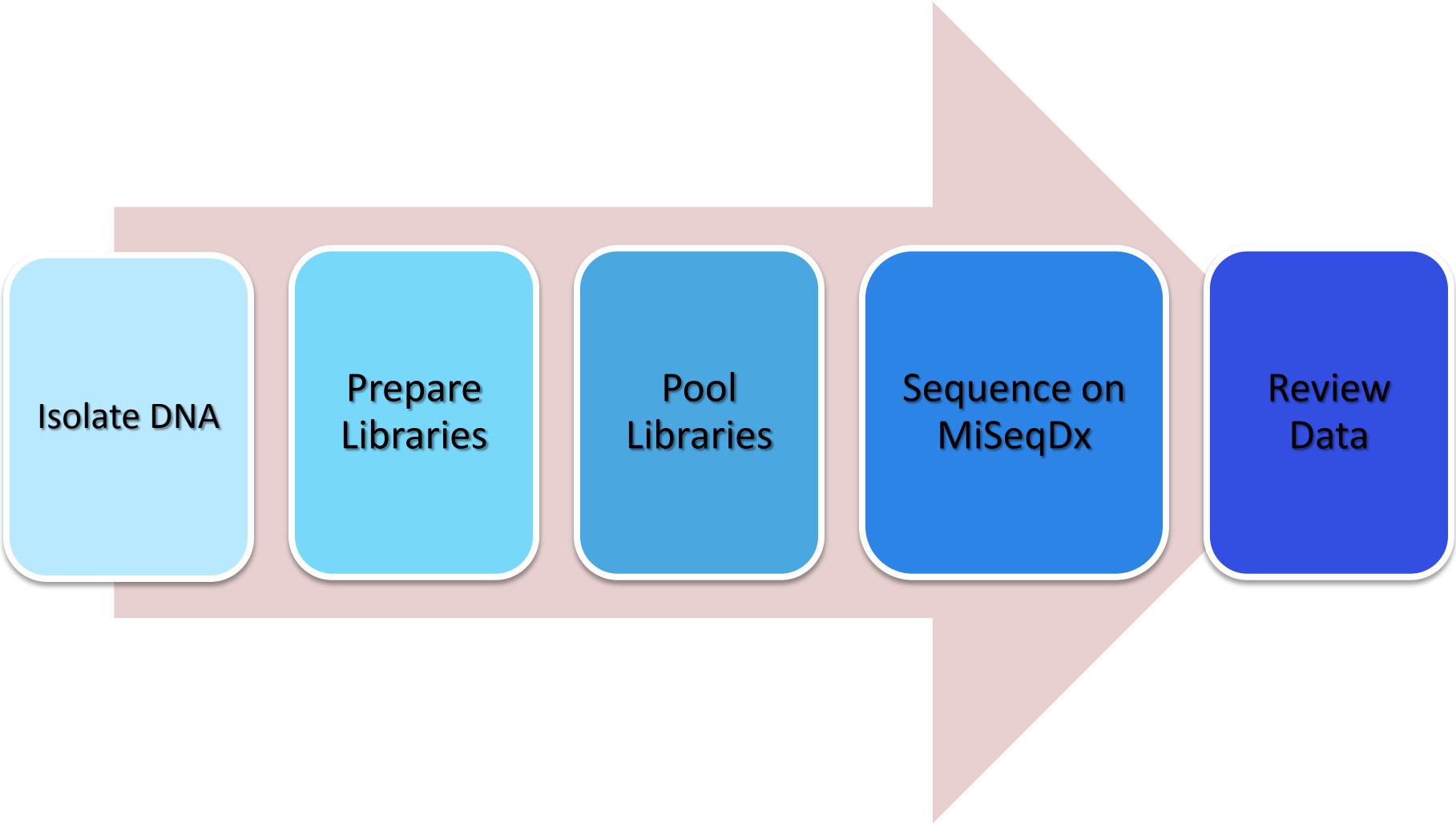
3. Demonstrate that it is cost effective to implement the three-tier IRT/DNA/DNA CF screening protocol into routine NBS for CF.

MiSeqDx Cystic Fibrosis System

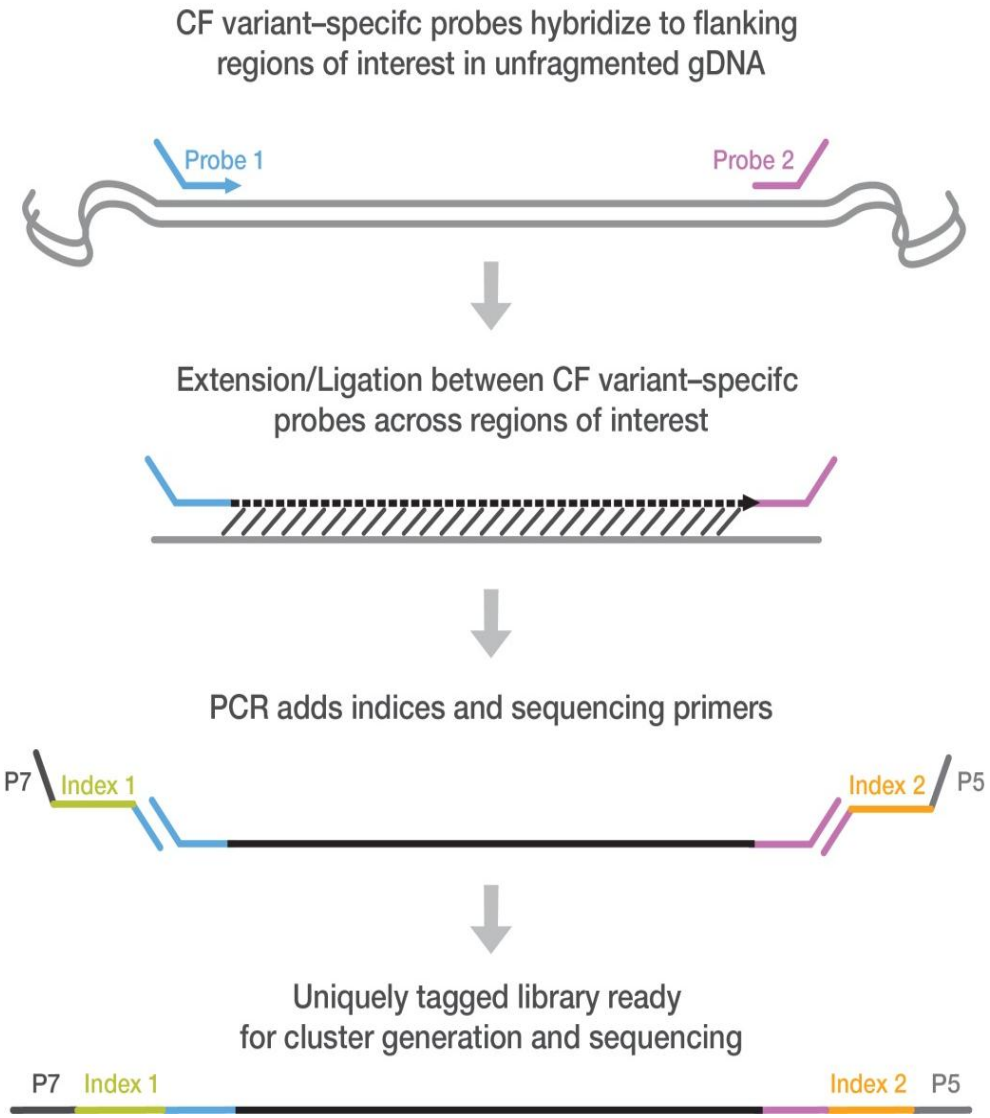
- 162 *CFTR* mutations/variants (IUO version*)
  - 127 single nucleotide mutations/variants
  - 32 insertion/deletion mutations
  - 2 large deletions
  - PolyTG/PolyT region

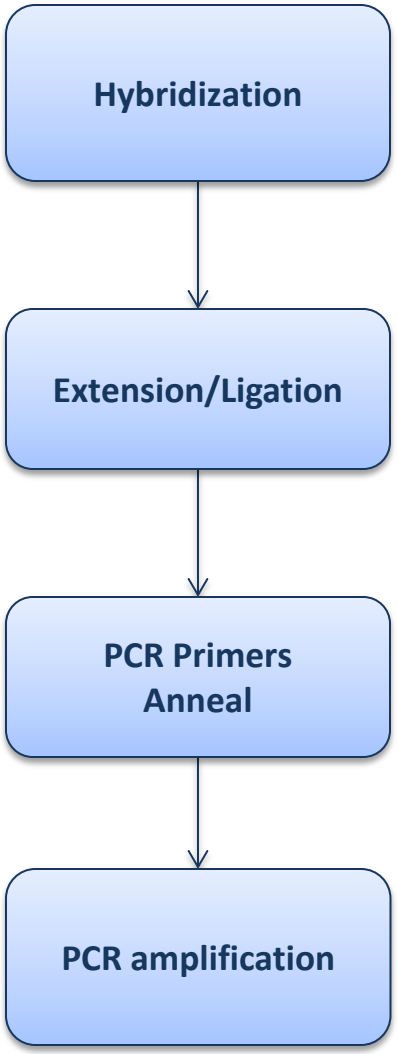
*Product is currently under FDA review.

Cystic Fibrosis System Workflow

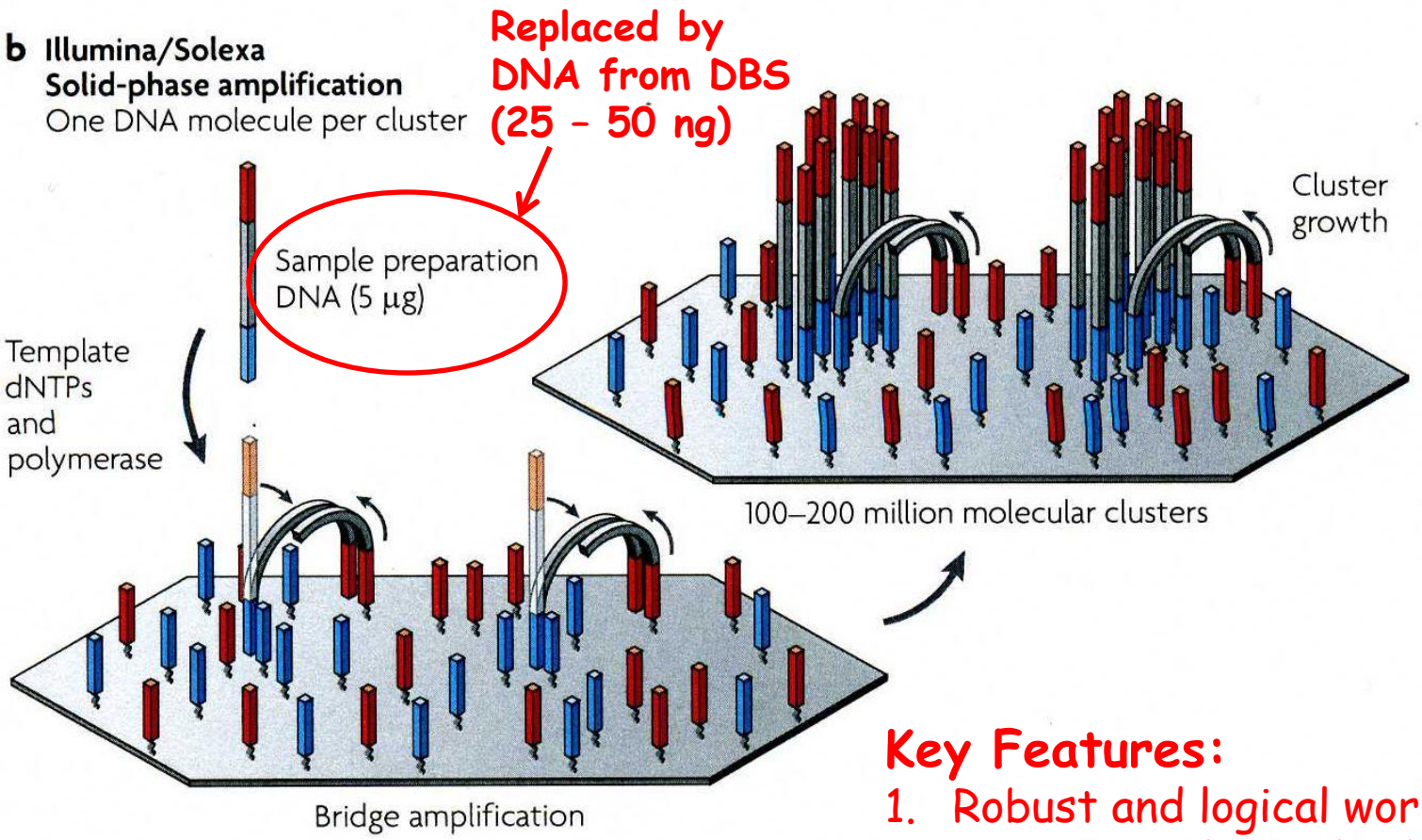


Sequencing Library Generation





Genotyping-by-sequencing



Key Features:

1. Robust and logical work flow
2. 46 + 2 samples multiplexing platform
3. Dual indexing identification
4. Immediate result w/o additional informatics requirements

Michael L. Metzker, *Nature Review Genetics*, 2010

Funding Support for SCID

Wisconsin Newborn Screening Laboratory

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the legacy of angels foundation

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