



Laboratory Robotics Interest Group,
Seventh Annual Sample Management Conference

Increasing DNA Extraction Capacity
Technology Overview

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Bob Corr
Principal Scientist
Pfizer Inc.



Setting the Scene:

- ◆ **Current State:**
 - ◆ Existing process has been operational since June 2006
 - ◆ 1-9 ml extractions, x-250 μg yields, 11.5 hr process per 96 samples
 - ◆ New protocols, extensive multiplexing, increased sensitivity of detection, and advances in miniaturization and automation have substantially reduced the amount of DNA required for genomic assays.

- ◆ **Process refinement and optimization:**
 - ◆ Target yield: 6-10 μg
 - ◆ Decrease extraction volume and bankable DNA
 - ◆ Increase throughput

- ◆ **Goal:**
 - ◆ Increase extraction capacity to 1,000 samples per day
 - ◆ Transition from tube-based to plate-based extraction
 - ◆ Maintain quality
 - ◆ Reduce operating cost



Major Players in the Field Include:

◆ Chemistry

- ◆ Agencourt, Agowa, AutoGen, Invitex, Invitrogen, Macherey-Nagel, Omega BioTek, Promega, Qiagen, Scigenix

◆ Automation

- ◆ AutoGen, Beckman Coulter, Caliper, Covaris, Hamilton, RTS, Tecan, Thermo Fisher Scientific, Qiagen

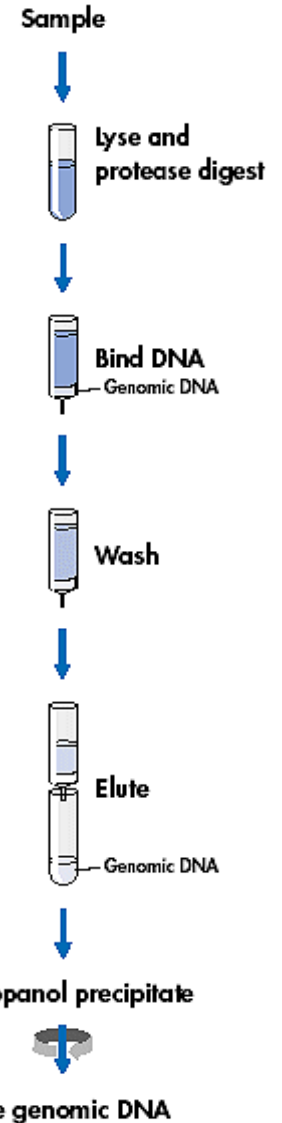
◆ Outsourcing Vendors

- ◆ Fisher BioServices, Qiagen

Chemistry Options (1)

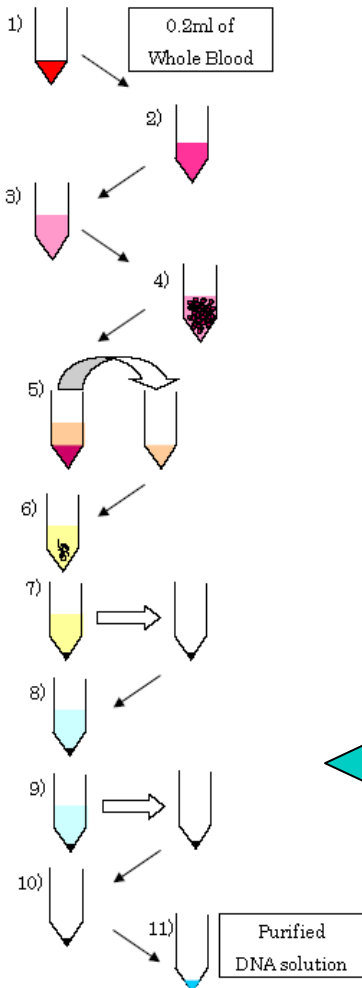
◆ Solid-Phase, Anion-exchange chromatography

- ◆ Delivers ultrapure transfection grade DNA
- ◆ Easy to automate – expensive – \$5.35 per prep



◆ Precipitation – solution phase

- ◆ Delivers lower-purity DNA
- ◆ May require additional cleanup



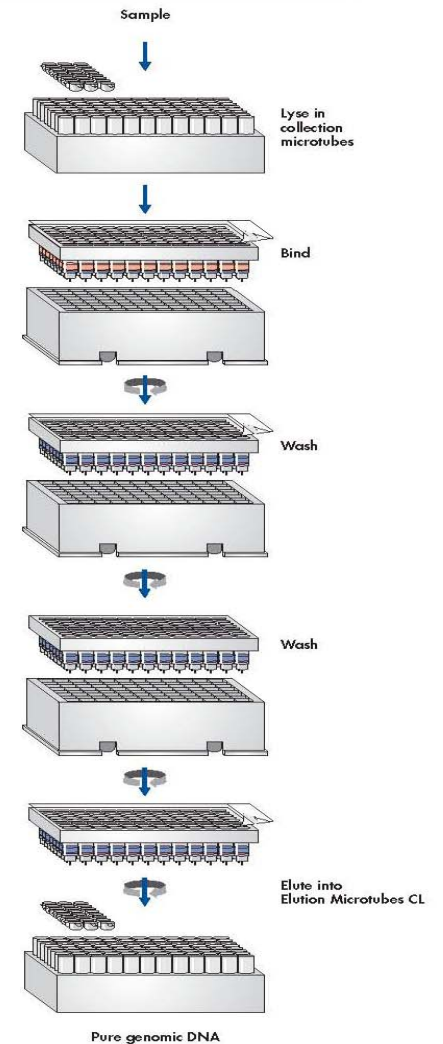
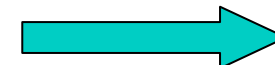
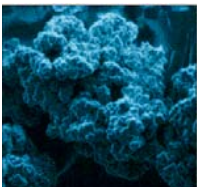
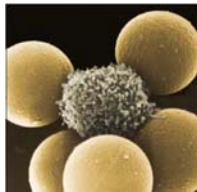
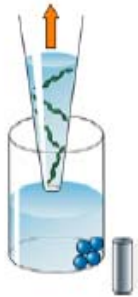
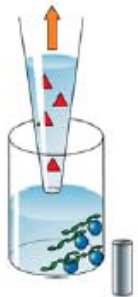
Chemistry Options (2)

- ◆ **Silica Technology – selective adsorption to silica under controlled ionic conditions**

- ◆ Delivers high-purity DNA
- ◆ Well established – consistent results
- ◆ Used by outsourcing vendors
- ◆ Vacuum causes shearing – shorter DNA strand length

- ◆ **Magnetic Bead Technology – binding to magnetic silica particles**

- ◆ Delivers high-purity DNA
- ◆ Higher binding capacity/less shear than silica
- ◆ Fast, inexpensive, easy to automate
- ◆ The Industry is concentrating new development efforts here





Volume Options

- ◆ **Chemistry kits available in 3 volume ranges for DNA extraction from whole blood**
 - ◆ **Large**
 - ◆ 1 – 10 ml (tube based)
 - ◆ **Medium**
 - ◆ 100 μ l – 350 μ l (96 well plates)
 - ◆ 400 μ l – 1 ml (24 well plates)
 - ◆ **Small (Forensic)**
 - ◆ 10 – 20 μ l

Automation Options: Generic Pros and Cons

- ◆ **Semi Automated Bench Top Instruments**
 - ◆ Run 1 plate at a time - prepare and load plates manually
 - ◆ Fast process times – many chemistries validated
 - ◆ Can Integrate with liquid handling instruments
 - ◆ Relatively inexpensive instruments – use lots of consumables

- ◆ **Fully Automated - DNA Extraction Specific**
 - ◆ Chemistry companies team up with Swiss automation companies
 - ◆ Instruments designed and built around specific chemistries
 - ◆ Validated protocols – no integration time
 - ◆ Locked into vender chemistry and consumables - \$\$\$
 - ◆ Loose the ability to repurpose instruments

- ◆ **Fully Automated (generic) Liquid Handling Instruments**
 - ◆ Versatile instruments
 - ◆ Considerable configuration, integration, and chemistry validation time
 - ◆ Only consider companies with DNA extraction experience
 - ◆ Instruments can be repurposed as business needs change



Data Viewing for Decision Making:

- ◆ **Data Assessment Tools**
 - ◆ Custom tools built by Research Informatics
 - ◆ Stored data is retrieved for easy viewing and comparison

UV Concentration Reporter

Source Container Barcode: GLEG00001256 [Retrieve] [Save]

Well	Volume ul	Conc ng/ul	Conc CV	A260/280	Amount ug
A1	147.54	84.54	0.32	1.74	12.47
B1	149.33	81.16	1.05	1.78	12.12
C1	149.15	56.94	0.53	1.71	8.49
D1	148.37	65.18	2.92	1.79	9.67
E1	151.19	92.82	0.22	1.71	14.03
F1	150.72	77.05	2.21	1.70	11.61
G1	148.31	73.89	2.37	1.70	10.96
H1	148.42	53.35	1.44	1.78	7.92
A2	149.76	90.08	2.99	1.75	13.49
B2	152.32	62.60	0.62	1.78	9.54
C2	151.87	95.42	2.20	1.71	14.49
D2	149.73	55.93	0.96	1.71	8.37
E2	150.85	67.08	1.04	1.76	10.12
F2	149.25	67.20	2.48	1.86	10.03
G2	152.31	77.88	0.67	1.88	11.86
H2	151.28	54.73	1.82	1.76	8.28
A3	149.49	74.65	2.02	1.75	11.16
B3	148.70	82.84	2.54	1.81	12.32
C3	151.87	64.03	1.47	1.74	9.72
D3	150.19	64.28	1.99	1.79	9.65
E3	147.83	52.86	1.12	1.84	7.81
F3	152.29	67.42	0.55	1.88	10.27



Chemistry Cost Considerations:

◆ **Current:**

- ◆ 9 ml extractions
- ◆ 200-250 μg yields
- ◆ Magnetic bead chemistry
- ◆ \$8.34 per prep

◆ **Outsourcing:**

- ◆ 200 μl extractions
- ◆ 4-8 μg yields
- ◆ Silica based chemistry
- ◆ Price ranges from \$5.46 to \$9.50 dependent on numbers of samples/ year

◆ **In-House Low Volume Chemistry**

- ◆ 300-350 μl extractions
- ◆ 6-10 μg yields
- ◆ Magnetic bead chemistry
- ◆ \$2.00 list price per prep
- ◆ Cost will be lower after discounts and bulk packaging vs. individual kits



Future Plans:

- ◆ Implement lowest cost option for external reagents and internal resources

- ◆ Continue with magnetic bead technology but miniaturize to adapt to customer needs for:
 - ◆ total amount DNA
 - ◆ purity and concentration
 - ◆ throughput

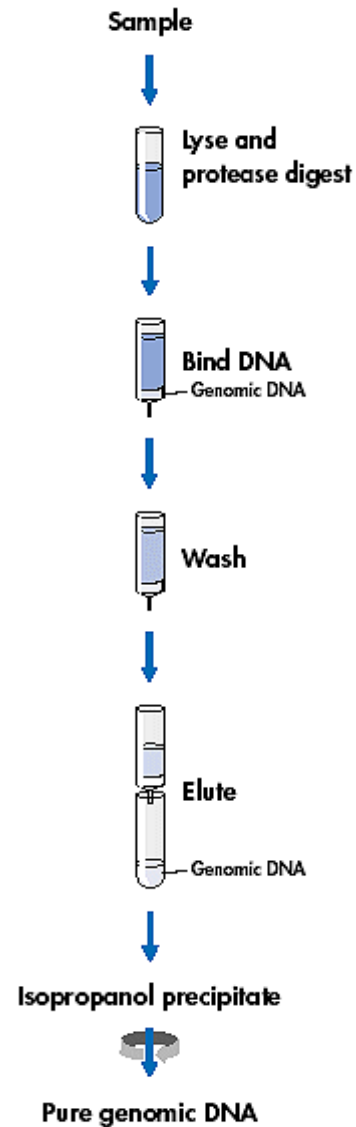
- ◆ Evaluate surplus equipment available internally within Pfizer with a view to implementing fully automated liquid handling and extraction

- ◆ Implement a 2nd generation DNA extraction process to support customer needs in 2Q09



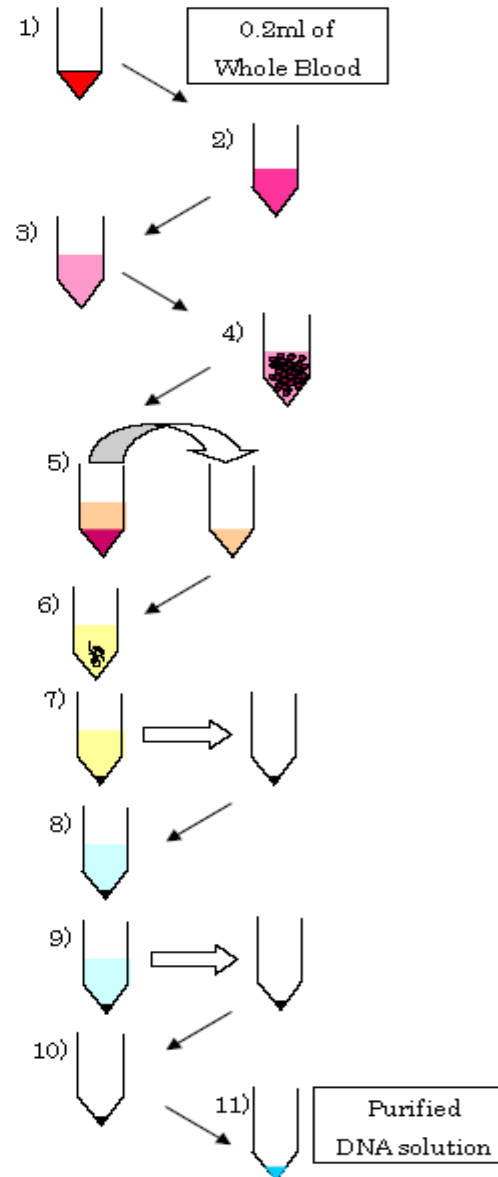
Backup Slides

Solid-Phase, anion-exchange chromatography

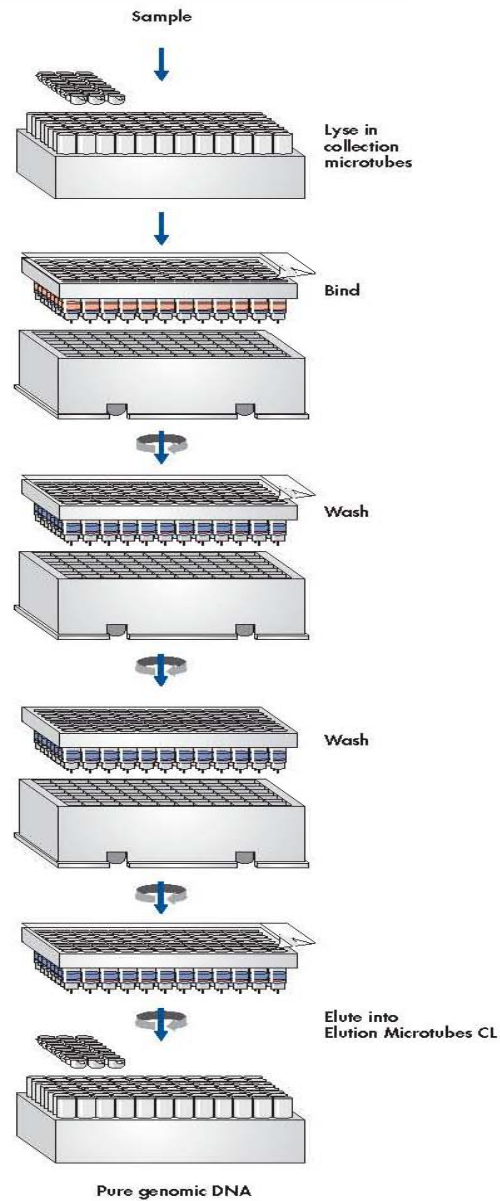


Precipitation (solution phase) Chemistry

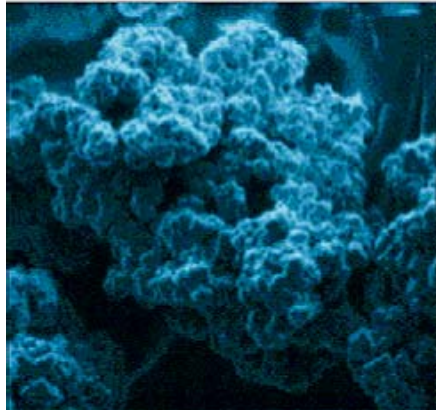
- 1) 0.2ml Blood in 96-deep well plate
- 2) Reagent R1 (Lysis of whole blood)
Agitation
- 3) Reagent R2 (Denature of protein)
Agitation
- 4) Reagent R3 (Precipitation of protein)
Agitation
- 5) Centrifugation (Remove the denatured protein)
Transfer the Supernatant
- 6) Reagent R4 (Precipitation of DNA)
Agitation
- 7) Centrifugation
Recover the Precipitant (Remove the supernatant)
- 8) Reagent R5 (Washing of DNA)
Agitation
- 9) Centrifugation
Recover the Precipitant (Remove the supernatant)
- 10) Evaporate Residual Alcohol
- 11) Reagent R6 (Dissolving of DNA)
Agitation



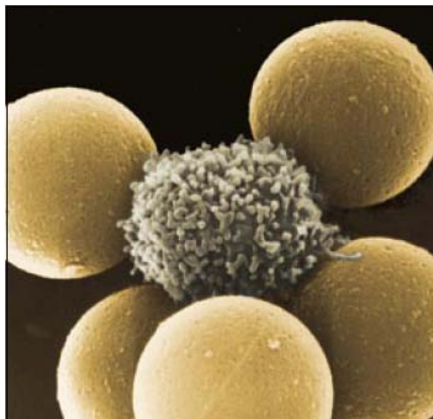
Silica Chemistry – Vacuum or Centrifugation



Magnetic Bead Chemistry



Promega - MagneSil



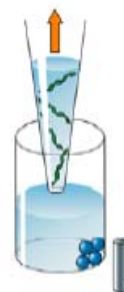
Invitrogen – Dynabeads
more surface area
no aggregation



Whole blood is lysed with Lysis Buffer MB1. Binding conditions are adjusted and the NucleoMag Blood Beads are added to the sample.



DNA is bound to the NucleoMag Blood Beads. Beads are held back in the well while contaminants are washed away.



DNA is eluted from the beads and recovered, while beads are held back in the well by the magnet. DNA is ready-to-use in downstream applications.

- DNA
- Contaminants
- NucleoMag Beads