

## **Frequently Asked Questions Regarding Sequence Based Typing**

**Q:** What loci should I type?

**A:** Class I is generally the priority for viral studies, such as HIV, while Class II is more popular for autoimmune studies. We suggest adding DRB1 at least to the Class I typing.

**Q:** Why do you suggest adding DRB1?

**A:** The Class II has such a strong Linkage Disequilibrium based off the DRB1 that the DRB345, DQB1, and DQA1 loci will follow with the DRB1 type we provide for the most part.

**Q:** What specimen material and quantity do you need to type ABC DR/DQ?

**A:** Lymphocyte cells are our preferred starting material (3-6 million cells), but 200  $\mu$ l blood is sufficient. We do take DNA specimen with vary results depending on the extraction method. We use the Qiagen Mini Blood Kit with elution of 150  $\mu$ l of water. Elution buffers can create variables we prefer to avoid. The sample rejection rate is considerably higher with DNA specimens.

**Q:** What concentrations of DNA?

**A:** Our method is optimized for 50  $\mu$ l of 50-150 ng/ $\mu$ l DNA. We have been successful with less than 200 ng total DNA of with an OD 260/280 of  $\sim$ 1.8. We are willing to try your specimen. We realize how difficult it is to obtaining new specimen from the patient especially for our international cohorts. We only invoice for sequenced specimens.

**Q:** How do I prepare a shipment?

**A:** International importing requires a CDC permit. We highly recommend pelleting lymphocyte specimens for 4 reasons 1) USDA requires a separate permit for Bovine products 2) we like to visually confirm cell are in each specimen tube 3) to quantify cells to not overload extraction columns 4) sample preservation. Add 50  $\mu$ l of PBS to keep the cells hydrated during transport.

**Q:** What is your normal turn around time?

**A:** We are averaging 5 business days for clinical samples. And research sample groups of 20-200 samples generally require 30 days for 98% of the types with resolution. This is largely based on the specimen quality.

**Frequently Asked Questions Regarding Sequence Based Typing (cont'd)**

**Q:** What is your resolution policy [class I]?

**A:** We've adopted a more aggressive resolution policy than required by the National Marrow Donor Program (NMDP) or the American Society for Histocompatibility and Immunogenetics (ASHI) by including exon 4. We include exon 4 for several reasons 1) exon 4 is exposed to a CD4 receptor 2) exon 4 has a growing number of ambiguities including many nulls 3) simplifies reporting of results. We do not regularly resolve ambiguities in the leader peptide coding exon 1 or transmembrane and cytoplasmic domains coded by exon 5-8. With the exception of nulls these ambiguities are simply biologically silent mutations to the immune system for which we are studying. These ambiguities can even be counter productive in the quest for knowledge. Ambiguities distract researchers and can reduce statistical power by creating subgroups with a data set that are biologically identical. The classical example is C\*0701/06/18 ambiguity. The C\*0701 is the dominant allele, but C\*0706 has a reasonable frequency due to its LD associations to B\*44 and B\*58. The ambiguity triplet represents a functionally identical antigen.

**Q:** What is a Cis/Trans ambiguity and how to you handle them?

**A:** We follow the new policy adopted by the NMDP and ASHI review committees report. Where only cis/trans combinations with "common alleles" will be eliminated. These ambiguities are created within a sequencing region when the two alleles are overlaid in heterozygous sequencing such that a completely different allele pair matches the actual allele pair perfectly. Separation by using an allele specific primer yields a homozygous sequence where the cis/trans position(s) can then be assigned to the correct allele.

**Q:** Using your Pure Type kit, how many tubes does it take to get to the center of all the Class I types?

**A:** Our Pure Type kit only requires 8 tubes to type exon 2-4, because we use specialized universal sequencing primers.

**Q:** How many tubes do the other leading SBT kits use?

**A:** Competitors use as many as 17 or more tubes to sequence the same exons and directions.

**Frequently Asked Questions Regarding Sequence Based Typing (cont'd)**

**Q:** How does your protocol compare to that of other kits?

**A:** The total incubation times combined are an average of 1 hour shorter than the other kits on the market. Also, the number of reagent additions is significantly less for our kit than for others.

Step	Pure Type	Dynal	Attria	Genome Diagnostics	Protrans
PCR	1h 23m	55m	2h 28m	2h 32m	2h 20m
PCR Cleanup	35m	40m	30m	50m	30m
Sequencing	21m	39m	46m	58m	1h 47m
Dye Removal	35m	40m	40m	45m	2h 7m
<b>Total</b>	<b>2h 54m</b>	<b>2h 55m</b>	<b>4h 23m</b>	<b>5h 5m</b>	<b>6h 49m</b>
<b>Time Savings</b>	<b>0</b>	<b>1m</b>	<b>1h 29m</b>	<b>2h 9m</b>	<b>3h 55m</b>

Reagent per Reaction	Pure Type	Dynal	Attria	Genome Diagnostics	Protrans
PCR Reagents	3	4	3	7	3
PCR Cleanup	1	1	1	1	1
Sequencing Reagents	3	2	2	5	7
Dye Removal	2	5	4	6	6
<b>Total</b>	<b>9</b>	<b>12</b>	<b>10</b>	<b>19</b>	<b>17</b>

Reagents to produce ABCDRDQ Type	Pure Type	Dynal	Attria	Genome Diagnostics	Protrans
Sequencing Mixes	6	17	17	17	17
<b>Total</b>	<b>15</b>	<b>29</b>	<b>27</b>	<b>36</b>	<b>34</b>