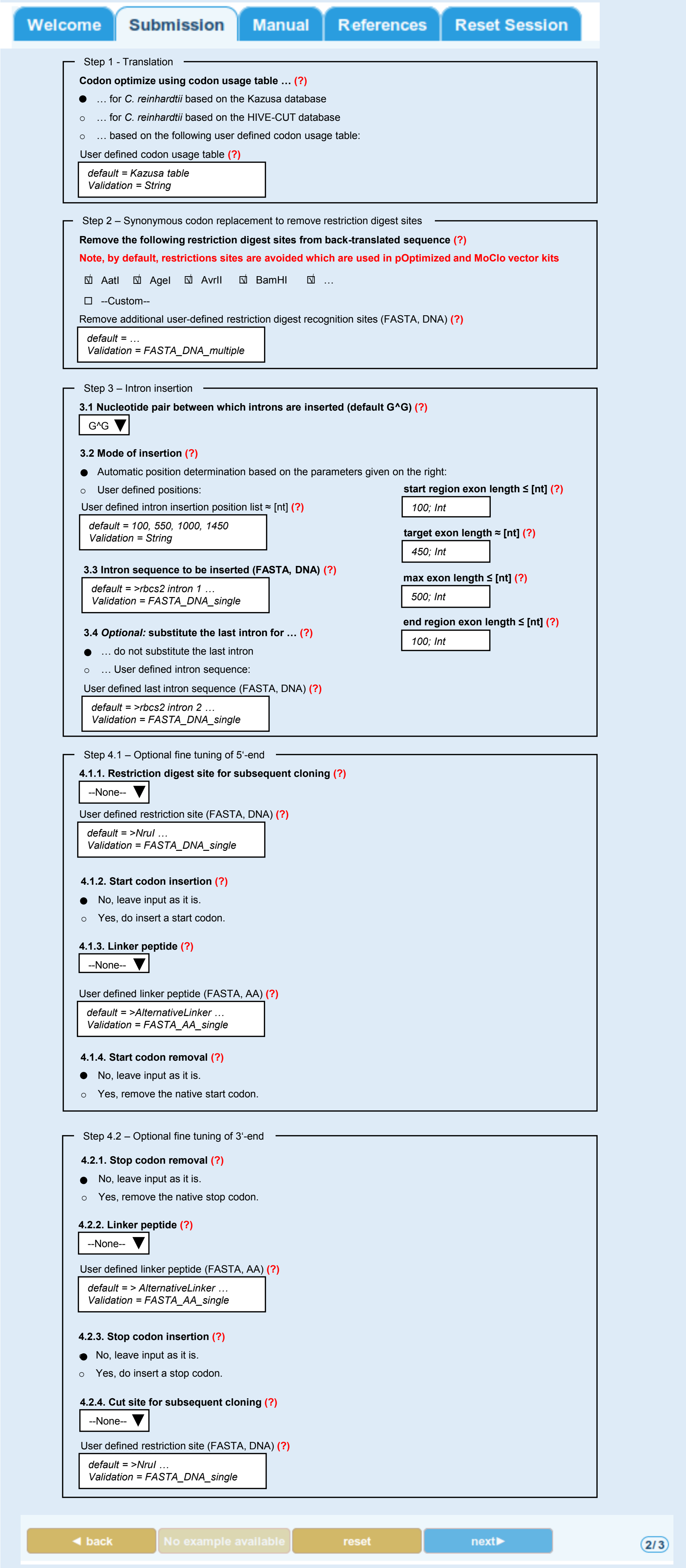

Step 1 - Translation

Codon optimize using codon usage table … (?)

● … for *C. reinhardtii* based on the Kazusa database

○ … for *C. reinhardtii* based on the HIVE-CUT database

○ … based on the following user defined codon usage table:

User defined codon usage table (?)

default = Kazusa table
Validation = String

Step 2 – Synonymous codon replacement to remove restriction digest sites

Remove the following restriction digest sites from back-translated sequence (?)

Note, by default, restrictions sites are avoided which are used in pOptimized and MoClo vector kits

☑ AatI ☑ AgeI ☑ AvrII ☑ BamHI ☑ …

☐ --Custom--

Remove additional user-defined restriction digest recognition sites (FASTA, DNA) (?)

default = …
Validation = FASTA_DNA_multiple

Step 3 – Intron insertion

3.1 Nucleotide pair between which introns are inserted (default G^G) (?)

G^G ▼

3.2 Mode of insertion (?)

● Automatic position determination based on the parameters given on the right:

○ User defined positions:

User defined intron insertion position list ≈ [nt] (?)

default = 100, 550, 1000, 1450
Validation = String

3.3 Intron sequence to be inserted (FASTA, DNA) (?)

default = >rbcs2 intron 1 …
Validation = FASTA_DNA_single

3.4 *Optional:* substitute the last intron for … (?)

● … do not substitute the last intron

○ … User defined intron sequence:

User defined last intron sequence (FASTA, DNA) (?)

default = >rbcs2 intron 2 …
Validation = FASTA_DNA_single

start region exon length ≤ [nt] (?)

100; Int

target exon length ≈ [nt] (?)

450; Int

max exon length ≤ [nt] (?)

500; Int

end region exon length ≤ [nt] (?)

100; Int

Step 4.1 – Optional fine tuning of 5'-end

4.1.1. Restriction digest site for subsequent cloning (?)

--None-- ▼

User defined restriction site (FASTA, DNA) (?)

default = >NruI …
Validation = FASTA_DNA_single

4.1.2. Start codon insertion (?)

● No, leave input as it is.

○ Yes, do insert a start codon.

4.1.3. Linker peptide (?)

--None-- ▼

User defined linker peptide (FASTA, AA) (?)

default = >AlternativeLinker …
Validation = FASTA_AA_single

4.1.4. Start codon removal (?)

● No, leave input as it is.

○ Yes, remove the native start codon.

Step 4.2 – Optional fine tuning of 3'-end

4.2.1. Stop codon removal (?)

● No, leave input as it is.

○ Yes, remove the native stop codon.

4.2.2. Linker peptide (?)

--None-- ▼

User defined linker peptide (FASTA, AA) (?)

default = > AlternativeLinker …
Validation = FASTA_AA_single

4.2.3. Stop codon insertion (?)

● No, leave input as it is.

○ Yes, do insert a stop codon.

4.2.4. Cut site for subsequent cloning (?)

--None-- ▼

User defined restriction site (FASTA, DNA) (?)

default = >NruI …
Validation = FASTA_DNA_single