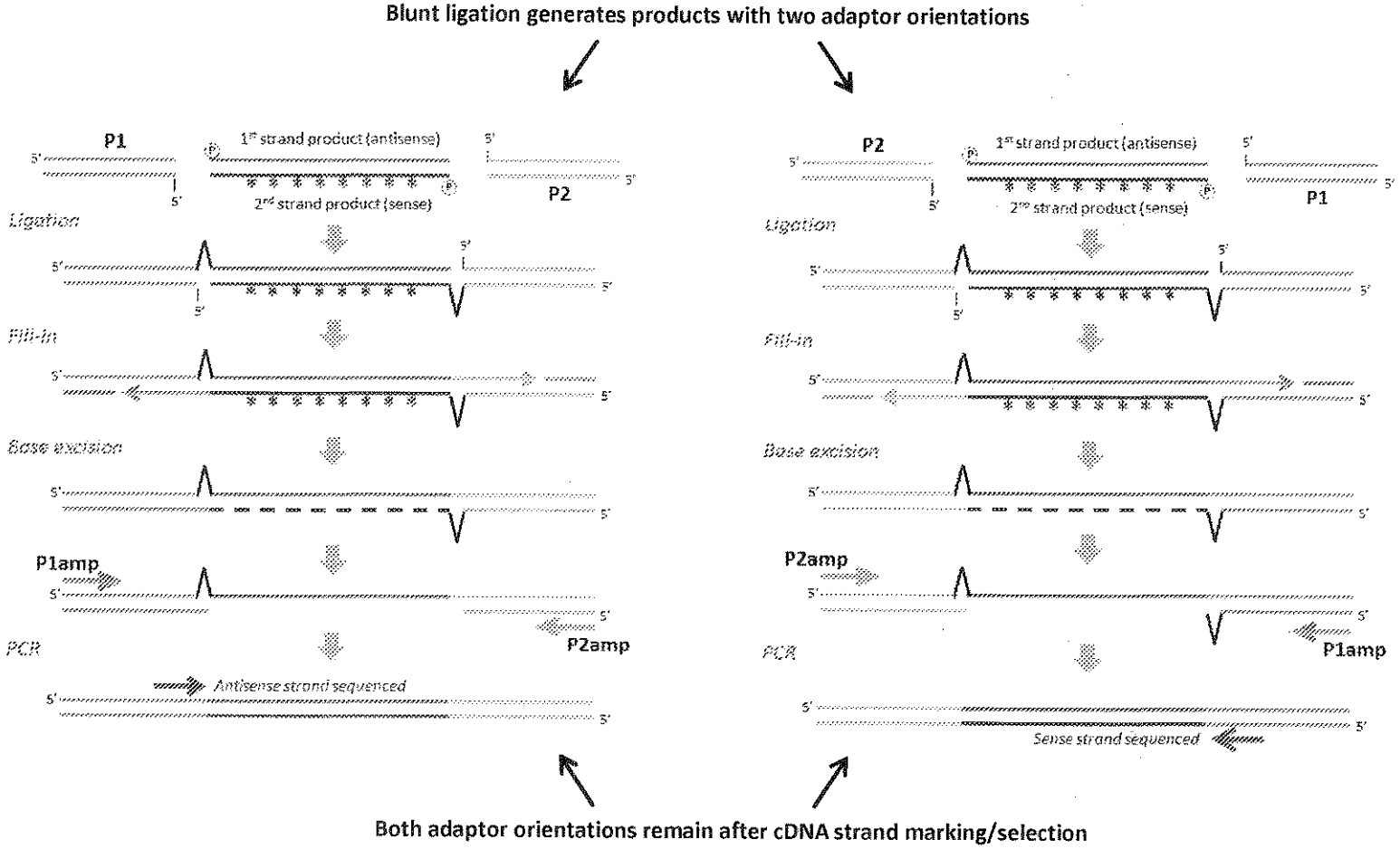
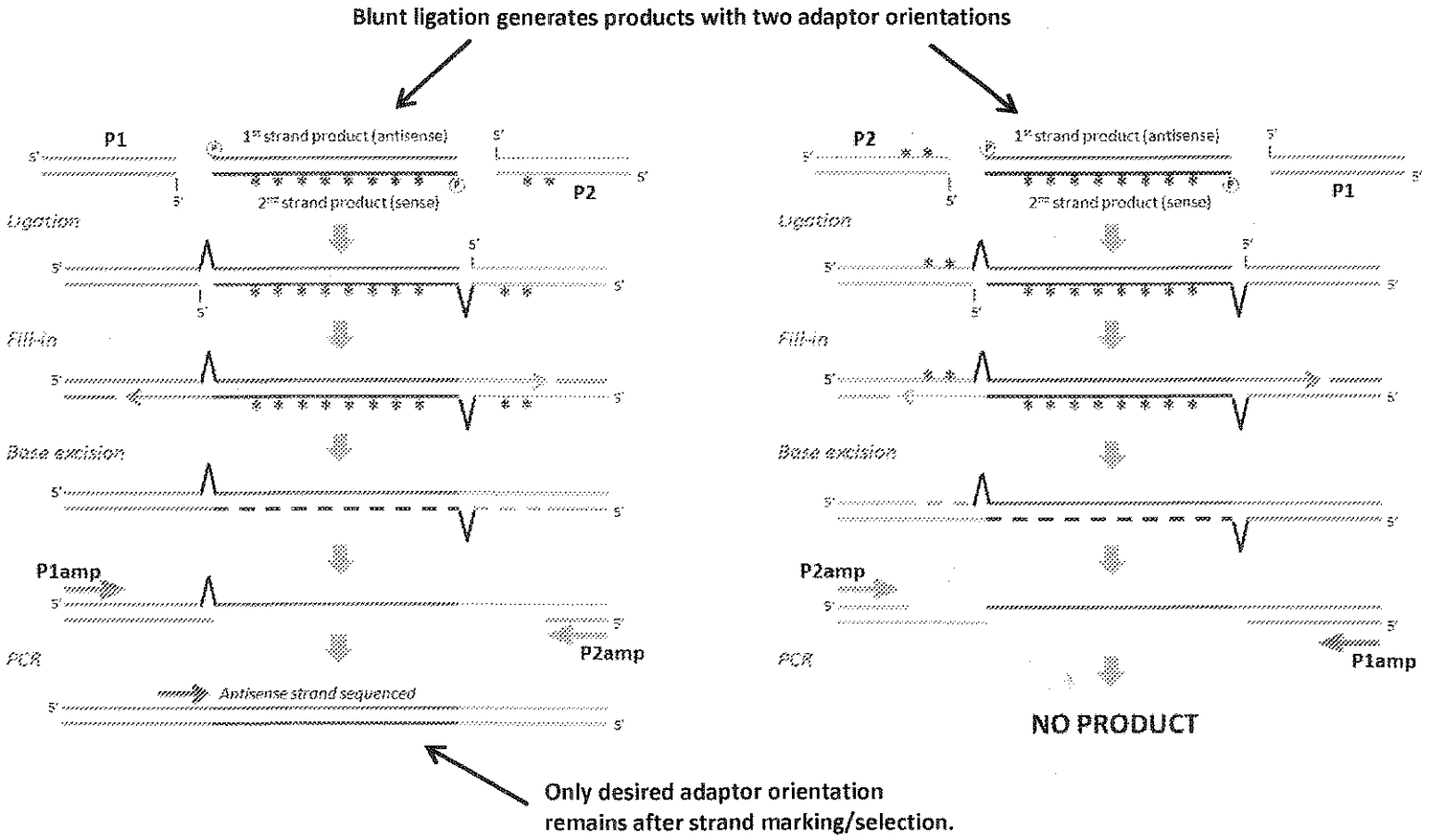
Figure 1. Conventional system: Strand marking of cDNA inserts is not sufficient for directional library construction.



The asterisks in the diagram (*) indicate dUTP incorporation.

Figure 2. Construction of directional (strand-specific) cDNA libraries using duplex adaptors: Strand marking of both cDNA and one adaptor results in directional library construction.



The asterisks in the diagram (*) indicate dUTP incorporation.

Figure 3. Summary of strand retention efficiency data for sequence reads mapping to coding exons of human mRNAs.

Library modifications				% of all reads			% of reads mapping to coding exons	
bases used in 2ss reaction	bases used in P2 adaptor	base excision	total reads	mapped	not mapped	strand orientation expected	strand orientation observed - sense*	strand orientation observed - antisense*
dA,dC,cG,dT	dA,dC,cG,dT	none	3261009	89.8	10.2	sense and antisense	52.3	47.7
dA,dC,cG,dT	dA,dC,cG,dT	UNG+APE	4111334	89.1	10.9	sense and antisense	51.1	48.9
dA,dC,cG,dU	dA,dC,cG,dU	UNG+APE	4829979	93.8	6.2	antisense only	2.1	97.9
dA,dC,cG,dT	dA,dC,cG,dT	none	3673934	89.6	10.4	sense and antisense	52.5	47.5
dA,dC,cG,dT	dA,dC,cG,dT	UNG+DMED	3977383	89.2	10.8	sense and antisense	51.2	48.8
dA,dC,cG,dU	dA,dC,cG,dU	UNG+DMED	4616560	93.8	6.2	antisense only	1.9	98.1

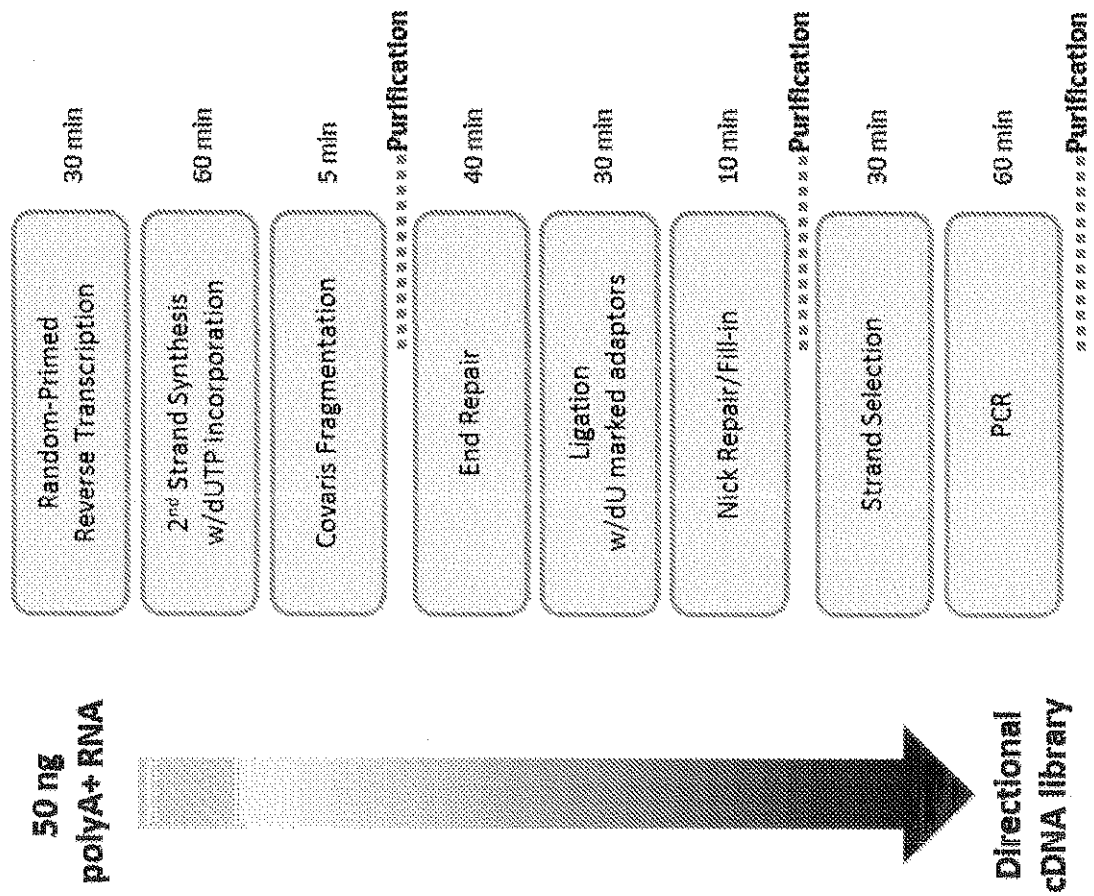


Figure 4. End-to-end workflow for construction of a directional cDNA library.