```
┌─────────────┐        ┌──────────────┐
│  Annotation │        │ Transcriptome│
└──────┬──────┘        └──────┬───────┘
       └──────────┬───────────┘
                  ▼
     ┌──────────────────────────┐
     │ Cluster at 80% identity  │
     │ with USEARCH             │
     └────────────┬─────────────┘
                  ▼
     ┌──────────────────────────┐     <15k         ┌──────────────────────────┐
     │ Nonredundant             │ ──sequences──▶    │ Abies sachalinensis      │
     │ unigene set              │                   │ Juniperus ashei          │
     └────────────┬─────────────┘                   │ Juniperus excelsa        │
                  │                                  │ Juniperus pinchotii      │
              ≥15k                                   │ Juniperus virginiana     │
              sequences                              │ Picea sitchensis         │
                  ▼                                  │ Pinus flexilis           │
     ┌──────────────────────────┐                   │ Pinus sylvestris         │
     │ Assess completeness with │     <60%          │ Pinus pinaster           │
     │ BUSCO                    │ ──complete──▶      │ Wollemia nobilis         │
     └────────────┬─────────────┘                   └──────────────────────────┘
                  │
              ≥60%                                   ┌──────────────────────────┐
              complete                               │ Abies alba               │
                  ▼                                  │ Araucaria cunninghamii   │
     ┌──────────────────────────┐   <80% of         │ Picea abies              │
     │ Orthogroup assignment    │  sequences in     │ Picea glauca             │
     │ with OrthoFinder         │ ─orthogroups─▶     └──────────────────────────┘
     └────────────┬─────────────┘
                  │                                  ┌──────────────────────────┐
              ≥80% of                                │ Ginkgo biloba            │
              sequences in                           └──────────────────────────┘
              orthogroups
                  ▼
     ┌──────────────────────────┐
     │ Gene family analysis     │
     │ with CAFE                │
     └────────────┬─────────────┘
                  ▼
     ┌──────────────────────────┐
     │ Abies balsamea           │
     │ Gnetum gnemon            │
     │ Metasequoia              │
     │   glyptostroboides       │
     │ Picea mariana            │
     │ Pinus albicaulis         │
     │ Pinus banksiana          │
     │ Pinus cembra             │
     │ Pinus canariensis        │
     │ Pinus lambertiana        │
     │ Pinus massonia           │
     │ Pinus monticola          │
     │ Pinus patula             │
     │ Pinus strobus            │
     │ Pinus taeda              │
     │ Pinus tecunumanii        │
     │ Pseudotsuga menziesii    │
     │ Sequoia sempervirens     │
     │ Sequoiadendron giganteum │
     │ Thuja occidentalis       │
     │ Amborella trichopoda     │
     └──────────────────────────┘
```

Figure S2 Gene family evolution analysis pipeline

Protein sequences were concatenated from annotation and transcriptome sources, then clustered with USEARCH at 80% identity. Taxa with at least 15k unigene sequences were assessed for completeness with BUSCO v4.0.2. Taxa with at least 60% completeness were analyzed with OrthoFinder. Taxa with at least 80% of sequences in orthogroups were included in gene family analyses with CAFExp.